MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CARROT (*Daucus carota* L.) GENOTYPES UNDER TROPICAL REGION

CHAITRA A. POLESHI

DEPARTMENT OF BIOTECHNOLOGY AND CROP IMPROVEMENT
COLLEGE OF HORTICULTURE, BAGALKOT
UNIVERSITY OF HORTICULTURAL SCIENCES,
BAGALKOT – 587104

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MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CARROT (*Daucus carota* L.) GENOTYPES UNDER TROPICAL REGION

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CHAITRA A. POLESHI
UHS14PGM453

DEPARTMENT OF BIOTECHNOLOGY AND CROP IMPROVEMENT
COLLEGE OF HORTICULTURE, BAGALKOT
UNIVERSITY OF HORTICULTURAL SCIENCES,
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CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CARROT (*Daucus carota* L.) GENOTYPES UNDER TROPICAL REGION"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **BIOTECHNOLOGY AND CROP IMPROVEMENT** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by **Ms. CHAITRA A. POLESHI**., **ID.NO. UHS14PGM453** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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CHAIRMAN

Approved by :
Chairman : _____
(SARVAMANGALA CHOLIN)

Members : 1. _____
(SATISH D.)

2. _____
(PALLAVI H. M.)

3. _____
(AMBIKA D. S.)

4. _____
(RATNAKAR M.SHET)

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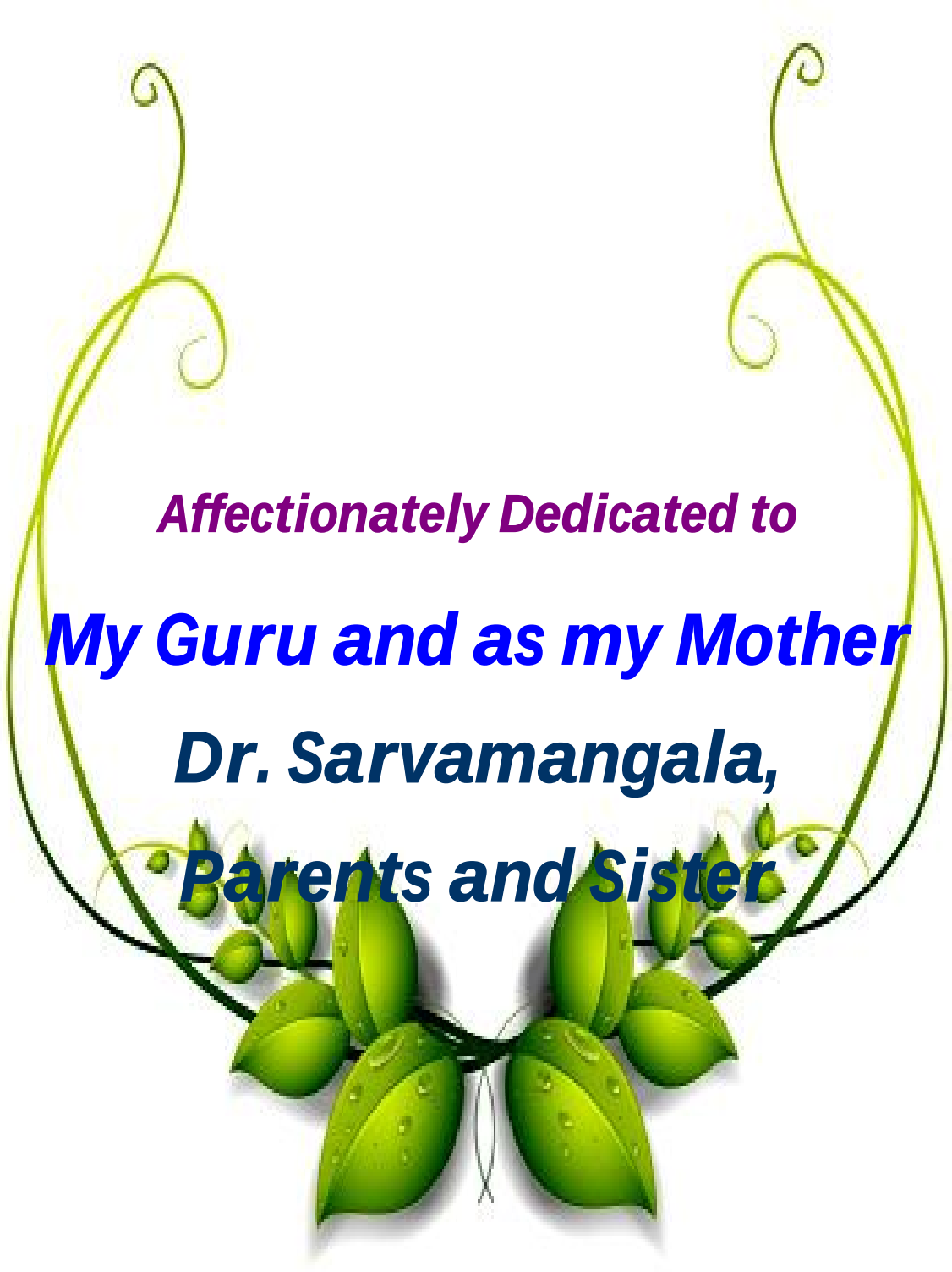

Affectionately Dedicated to

My Guru and as my Mother

Dr. Sarvamangala,

Parents and Sister

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1. INTRODUCTION

Carrots (*Daucus carota* L.) are among the top-ten most economically important vegetable crops in the world, in terms of both area of production and market value. In 2005, world production of carrot approached 24 Mt with an area of 1.1 million hectares. The total global market value of the more widely traded carrot seed crop has been estimated to be in the range of $100 million (FAOSTAT data, 2014).

Carrot, a member of the Apiaceae family is a diploid species (2n = 2x =18) with a relatively small genome size of 480 Mb (Iorizzo *et al.*, 2016). This includes about 2,500 species such as dill, caraway, cumin, chervil, coriander, fennel, anise, parsley, parsnip, and celery. Carrots contain not less than 89 percent water. It is a root vegetable, usually orange in colour, though black, purple, red, yellow and white varieties also exist and it has a crisp texture when fresh. The crop is native to Europe and South-Western Asia. Carrots are called the “nutritional heroes” and store goldmine of nutrients and is an excellent source of antioxidants, vitamins, minerals and microelements with zero fat, zero cholesterol and considered as a single source of vegetable for provitamin A carotenoid. Hence, it is widely consumed by all the age groups including infants, pregnant women, diet and health conscious people and widely used in many cuisines, especially in the preparation of salads. Among winter vegetables, carrot has the most vitamin A as beta carotene (3230 ug) followed by tomato (708 ug) (FAOSTAT data, 2014). It is believed that one medium sized carrot (60g) provides enough provitamin A carotene to fulfill adult vitamin A needs for one day.

A tap root is the economic part of carrot which is generally consumed; its propagation is mainly through seeds. Since, it is a biennial crop having vegetative and flowering phases in separate cycles, development of varieties through hybridization is challenging, as flowering is highly influenced by temperature conditions. But being a highly cross pollinated species, prolific seed producing nature and broad genetic base make this crop a great interest to breeder. 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 economic characters considered for improvement (Peterson and Simon, 1986; Rubatzky

et al., 1997). 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 high environmental influence, visual selection is less effective and more laborious.

Carrot is basically a cool season crop and its cultivation was earlier restricted to temperate regions only. Because of its health benefitting nature, farmers started growing varieties adaptable to warmer conditions there by identified cultivars suitable to warmer climates. Hence, carrot has become popular among all the regions of the world including tropical and subtropical regions but carrot cultivars of one class do not perform well in the regions outside the range of adaptation. Hence, broadening the genetic base of carrot to produce a high yielding superior quality marketable produce in the warmer regions is a major concern at the present context.

Among the nutritional quality of carrot, carotenoid is the most studied attribute as it is the main dietary sources of provitamin A. α and β-carotene account for both high provitamin A and familiar orange color to a carrot roots (Simon, 1992). 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.

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. Root flavor (sweet or harsh) is another most important quality trait which is determined by sugar content and volatile terpenoids. The sweet flavor associated with higher sugar content is polygenic in nature although single major gene, *Rs* determines whether the reducing sugars (glucose, fructose) or non-reducing sugars (sucrose) are the primary storage carbohydrates (Freeman and Simon, 1983; Stommel and Simon, 1989). Genetic

selection of carrot flavor needs a trained evaluator for effective selection in a breeding program (Simon *et al*., 1981).

Complete genome sequence of carrot is published recently (Iorizzo *et al.*, 2016) and hundreds of genic molecular markers as well as few of the gene specific markers related to carotenoid biosynthetic pathway are available in carrot. Understanding the genetic basis for various morphological, nutritional and adaptability traits at the molecular level using the crop specific markers such as simple sequence repeats (SSR) and other user-friendly PCR based marker systems (AFLP, SCAR) available in carrot so far is more useful in carrot breeding and improvement. The transferability of SSR markers across the *Daucas spp* and across the *Apiaceae* family has also been reported (Cavagnaro *et al.,* 2011) and hence, any sequence information available within and between *Apiaceae* can be utilized to study syntenic relationship and their association with economic traits is of great use.

Carrot has a very diverse global base of germplasm and breeders can access this base to develop varieties/hybrids with a wide range colors, shapes *etc*. For incorporation of many of these desired characters from wild germplasm into an individual variety or hybrids, evaluation of diverse germplasm and its characterization is the basic need for a plant breeder. Characterization of carrot germplasm using morphological markers requires collection of extensive field data and moreover, they are highly influenced by environment and the selection of low heritable traits is ineffective based on visual selection. Molecular characterization of crop plant diversity holds great potential for efficient crop improvement. Understanding how the individual cultivar and/or accessions are related can aid the plant breeder in selecting appropriate crosses, in identifying unique genes for nutritional and other desired traits and also in predicting the heterotic potential of a hybrid. Many important characters of tropical carrots such as their keeping quality, sweetness, disease resistance *etc* are unexplored even though lot of research is being done in temperate carrot both at the genomic level and in terms of development of new cultivars.

Molecular marker based selection along with the phenotype provides more efficient selection strategies in germplasm identification. To date no efforts have been made to characterize the available germplasm in carrot, their suitability to tropical

regions and their molecular basis of these economic traits of interest due to the problem related to flowering and production of seeds in this region. In this regard, the present study is more relevant in carrot to fulfill the present and basic requirements for carrot improvement with special reference to tropical regions. Hence, an attempt has been made to extensively characterize the tropical and temperate carrots in terms of morphological (qualitative, quantitative), biochemical and molecular markers mainly microsatellites and few gene specific markers.

The following objectives have been lay down with the aim of identifying the superior carrot cultivars suitable to tropical region.

Objectives

1. Phenotypic characterization for root morphology for the carrot genotypes under tropical region
2. Biochemical characterization for carotenoid and sugars for carrot genotypes under tropical region
3. Analysis for molecular marker based genetic and allelic diversity for carrot genotypes
4. Marker-trait association for the nutritional quality and root morphological traits with molecular markers
5. Identification of superior carrot genotypes adaptable/suitable to tropical region

2. REVIEW OF LITERATURE

Review of literature has been subdivided in to the following subheadings

2.1 Genetic diversity for root morphological traits in carrot

2.2 Genetic variation for nutritional quality traits

2.3 Genomics and molecular marker diversity in carrot

2.4 Marker-trait association for economic traits in carrot

2.1 Genetic diversity for root morphological traits in carrot

Carrot is the most economically important vegetable belonging to family Apiaceae is gaining much importance in recent decades worldwide due to the heightened awareness of health promoting attributes due to the rich source of Provitamin A carotenoid, development of fresh cut carrot products convenient for consumers and also adaptation of cultivars to warmer climates (Cavagnaro *et al*., 2011).

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 any defects, resistance to common diseases, non-bolting (Peterson and Simon, 1986).

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).

Rubatzky and Yamaguchi (1997) divided the cultivated carrots into two groups *viz.*, 1) Asian group that has traits such as yellow or purple root color, slightly soft texture, low sweet, pubescent leaves which give a green gray appearance, bolt easily, adapted to warm temperature; and (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.

Ramesh *et al.* (2011) conducted an experiment to characterize the European genotypes of carrot based on principle component and regression analysis for economic traits. Based on the root economic characters, genotypes were characterized in to 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. They also reported based that on the multiple linear regression model, average root weight can be predicted on the basis of basis of leaf length, shoulder thickness, crown diameter, marketable root yield per plot, forking and cracking percentage.

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 10cm) and TSS (4.4-14.7%) (Kasiri *et al*., 2013).

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).

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.

Santos *et al*. (2005), while working with two F2 carrot populations, studied the relationship between major root carotenes, root colour and several other root morphological traits based on correlation and path analyses. 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.

2.2 Genetic variation for biochemical traits

2.2.1 Carotenoids

Orange colour gives the attractiveness to foods on a plate and makes it rich in carotene, a precursor of Vitamin A (Rashidi and Bahri, 2009). It is also a rich source of protein, carbohydrates, fiber, vitamin A, potassium, sodium, thiamin and riboflavin (Ahmad *et al.*, 2005; Rashidi and Bahiri, 2009; Hassan *et al*., 2005).

Among the nutritional quality of carrot, carotenoid is the most studied attribute as it is the main dietary sources of provitamin A. α and β-carotene account for both high provitamin A and familiar orange colour to carrot roots (Simon, 1992).

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).

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*) (Pistrick, K., 2001).

Majority of the carrot species belongs to carotene carrot cultivars 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 (Kammerer *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.

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.

Transcriptional regulation is thought to be the major factor in carotenoid accumulation in the organs. Jeremy *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), phytoene desaturase (PDS), z-carotene desaturase (ZDS1 and ZDS2), lycopene *e*-cyclase (LCYE), lycopene b-cyclase (LCYB1), and zeaxanthin epoxidase (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.

Anthocyanins are abundant in taproots of purple carrot cultivars, whereas cyanidin-based anthocyanins represent almost all of the anthocyanins in carrots (Montilla *et al.*, 2011). Xu *et al.*, 2014 studied the structural genes involved in cyanidin-based anthocyanin biosynthetic pathway and identified a total of 17 genes in 10 gene families involved in the cyanidin-based anthocyanin pathway and also identified the Chalcone Flavonone Isomerise (CHI), flavonoid 30-monooxygenase (F3'H) and UDP-galactose flavonoid 3-O-galactosyltransferase (UF3GaT) gene sequences were identified in carrot for the first time.

Orange carrots are highly revered as "good for the eyes" due to their high content of hydrocarbon carotenoids, a class of phyto chemicals that are often precursors to vitamin A. a- and ß-Carotene predominate in orange carrots (Arscott and Tanumihardjo, 2010).

Carotenoids are responsible for the yellow, orange, and red colors of carrots, while anthocyanins, a class of polyphenolic compounds, are responsible for the color of purple carrots. All of these pigments have been studied for their health benefits, including protection from certain cancers and cardiovascular disease, and consumer

interest in natural whole foods rich in these compounds, often referred to as “functional foods,” is growing (Hasler and Brown, 2009). Modern vegetable breeders have initiated development of some colored carrot breeding lines (Simon and Lindsay 1983) to increase the nutritional quality and visual appeal of the food supply.

2.2.2 Sugars and Total carbohydrates

In addition to the nutrients provided by carrots, flavor is also an important component of overall quality. The major flavor attributes of raw carrot include sweetness, harshness, and bitterness. Consumers generally prefer sweet without harsh, terpentine after taste or bitterness (Simon *et al*., 1980; Simon, 1985). Sugars and volatile terpenoids account for sweetness and harshness, respectively (Simon *et al.*, 1982).Levels of these two in raw carrots persist even after cooking or heat processing (Simon, 1985; Simon and Lindsay, 1983).

Sugar content ranges from 3 to 7% for carrots grown in organic soil (Stommel 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.

Many compounds contribute to carrot flavor and some of these may contribute to effects on human physiology. The characteristic “fresh carrot” flavor has been attributed to the volatile compounds mono- and sesquiterpenes, and also to sugars (Simon and Lindsay, 1983)

2.3 Genomics and Molecular markers in carrot

Iorizzo *et al.* (2016), reported a high quality chromosome-scale assembly and analysis of the carrot genome. They identified a candidate gene, DCAR_03255, that conditions carotenoid accumulation (Y) in carrot taproot and is co-expressed with

several isoprenoid biosynthetic genes. The primary mechanism regulating carotenoid accumulation in carrot taproot is not at the biosynthetic level. They hypothesized that DCAR_03255 regulates upstream photosystem development and functional processes, including photo-morphogenesis and root de-etiolation.

Daucus carota is a typical biannual diploid (2n = 2x = 18) and an out-crossing species with a relatively small genome estimated as 473Mb (Budahn *et al*., 2014).

Considering the importance of carrot to humans, Xu *et al.*, (2014) published an information on a database containing the genome and transcriptome information of *D. carota* called CarrotDB has been developed which provides the whole draft genome sequences, nucleotide sequence of putative genes and amino acid sequences of putative proteins and SSRs with the designed primers of DC-27 carrot and also an assembled transcriptomic sequences with FPKM information of 14 carrot genotypes. A total of 2826 transcription factor (TF) genes classified into 57 families have been identified in the entire genome sequences. A total of 65,942 SSR markers targeting from mononucleotide repeats to hexanucleotide repeats are developed.

Cavagnaro *et al.* (2009) developed 156 GSSR (Genomic SSR) and 144 BSSR (BAC-end SSR) including 202 perfect SSR and 41 compound SSRs and they were characterized in 7 carrot F2 mapping populations. A total of 196 SSR markers were polymorphic and 123 SSRs were polymorphic in two or more mapping populations suggesting that these common markers may serve as anchoring points across maps. The mean polymorphic index was significantly higher in for GSSRs (23.6%), compared BSSR (9.8%) regardless of the mapping population.

2.4 Molecular diversity

Clerc and Briard (2003) used 70 AFLP and SSR markers to study the intervarietal variability and genetic distance of carrot germplasm both in cultivated and wild types and 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.

Santos and Simon 2002 used two different F2 populations such as Brasilia x HCM and B493 x QAL and constructed linkage map using 287 and 250 molecular markers respectively including AFLP and SCAR markers.

Ruhlman *et al*. (2006) published complete plastid genome sequence of *D. carota*, which contains a number of dispersed direct and inverted repeats scattered throughout coding and non-coding regions. The complete plastid genome of carrot provides essential information required for genetic engineering. Additionally, the sequence data add to the rapidly growing database of plastid genomes for assessing phylogenetic relationships among angiosperms. The complete carrot plastid genome is 155,911 bp in length, with 115 unique genes and 21 duplicated genes within the IR. There are four ribosomal RNAs, 30 distinct tRNA genes and 18 intron containing genes. Repeat analysis reveals 12 direct and 2 inverted repeats ≥ 30 bp with a sequence identity ≥90%.

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).

Asima *et al*. (2010) studied genetic variability by 13 morphological markers, 4 biochemical markers and 20 RAPD primers for 48 genotypes 254 band (100%) polymorphic PIC for 20 primer range 0.52 in oligo 678 to 0.94 in OPS 13. The similarity co-efficient analysis revealed 5 clusters. These clusters were further classified in sub cluster. Cluster 1 has 4 sub-cluster. Cluster II has 2, cluster III has 3 and cluster IV has 1 sub cluster.

Iorizzo *et al*. (2011) worked on *de novo* assembly of transcriptome sequence from four genetic backgrounds and produced 58,751 contigs and more than 50 % of these assembled sequences were annotated which helped to detect the transposable elements and new carrot anthocyanin genes. They also identified 114 computationally polymorphic SSR and 20,058 SNP out of total amplified products; more than 80% were polymorphic.

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).

2.5 Marker-Trait Association

The first cultivated carrots were purple or yellow rooted and they were gradually replaced by white or orange rooted forms which appeared in 1600s and concomitantly, a red type appeared in Asia and Japan in the 1700s and finally since nineteenth century orange rooted carrots have become predominant commercially. Root pigmentation depends on the relative proportion of different carotenoids for the white, yellow, orange and red types but only internally for the purple one. The genetic control for root carotenoid content might be partially associated with carotenoid biosynthetic genes.

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 the 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 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 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.

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).

Budahn *et al.,* (2014) F2 population was used derived from an intial cross b/w a yellow leaf (*yel*) chlorophyll mutant and a compressed lamina (cola) mutant with the unique flowers defects of sporophytic parts of male and female organs map length 781 cM included 281 loci. Length of 9 linkage groups is range from 65 and 145cM. Mapping of flower development and fertility locus was done. Two MADS-box genes (*DcMADS3, DcMADS5*) with the prominent roles in flowering and reproduction as well as three additional genes (*DCAOX2a, DCAOX2b*) with further importance for male reproduction were identified (Linke *et al*.,2003).

Vivek and Simon (1999), used a population of B9304 x YC7262 identified a locus of *Y2* - Differential xylem/phloem carotene levels, *R*s-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). Iorizzo *et al*. (2011) by *de novo* assembling of transcripts and observed a range of

functional TE transcripts suggesting the members of many TE families are potentially be active in carrot and MITEs and DcMaster related transposable elements are highly polymorphic in carrot and MITEs in the carrot genome are mainly associated non-coding regions of genes.

Dong *et al.* (2013), developed SNP from EST sequences of carrot for hairless seed character. cDNA libraries were constructed from seeds of short-hair seed phenotype CT-SMR 616 OP 659-1 line, hairy-seed phenotype CT-SMR 616 OP 677-14 line and short-hair seed phenotype CT-ATR 615 OP 666-13 line, hairy-seed phenotype CT-ATR 615 OP 671-9, respectively. 9SNP sites and 14 SNP sites in each of 2 combinations were confirmed by analyzing the EST sequences from short-hair and hairy-seed lines. High resolution melting (HRM) primers were analyzed using hairy seed phenotype CT-SMR 616 OP 1040 line and short-hair seed phenotype CT-SMR 616 OP 1024, 1025, 1026 lines. One set of HRM primers showed specific difference between the melting curves of hairy and short-hair seed phenotype lines. Based on this result, allele-specific (AS) PCR primers were designed for easier selection between hairy-seed carrot and hairless seed carrot and are expected to be useful in breeding of hairless seed carrot cultivar as a molecular marker.

3. MATERIAL AND METHODS

3.1 Plant material

For the present study, forty eight diverse germplasm lines of carrot representing both tropical and temperate regions of India were selected including released varieties, local collection and germplasm accessions. The details of the selected genotypes and for convenience the nomenclature was given as UHSBC series (University of Horticultural Sciences, Bagalkot Carrot) are given in Table 1. These genotypes were subjected to phenotypic evaluation for plant and root morphological traits, biochemical traits such as carotenoids, sugars and also subjected to molecular profiling using microsatellites and few gene specific functional markers.

3.2 The details of the methods followed to fulfill the objectives of the present study are divided into following sub-headings

3.2.1 Phenotypic characterization

3.2.2 Biochemical analyses

3.2.3 Molecular and Allelic diversity

3.2.4 Marker-Trait Association

3.2.5 Selection of superior genotypes

3.2.1 Phenotypic evaluation

Phenotyping was carried out in two seasons *viz.*, Summer season (S1) at Haveli farm and winter season (S2) at Udyanagiri Campus of University of Horticultural Sciences, Bagalkot, Karnataka, India during 2015. All the 48 diverse carrot genotypes were subjected to plant and root morphological characterization for a total of 39 characters. These 39 characters consisted of 18 qualitative traits recorded based on the standard IPGRI descriptor (IPGRI. 1998), 17 quantitative traits were recorded in SI units and 4 biochemical parameters were estimated using the standard protocol as given below. So, the total 39 traits were grouped in to morphological consisting of qualitative

Table 1: List of genotypes, source of collection and their UHSBC nomenclature of carrot germplasm lines utilized in the present study

Sl. No	Genotype Name	Selections	Source of Collection
1	VANNUR LOCAL 2	UHSBC-02	LOCAL COLLECTION
2	CENTURY SUPER KURUDA	UHSBC-06	OOTY COLLECTIONS
3	CENTURY EARLY NANTES	UHSBC-07	OOTY COLLECTIONS
4	CENTURY SHIN KURODA	UHSBC-08	OOTY COLLECTIONS
5	NUFIELD CARROT NANTES IMPROVED	UHSBC-11	OOTY COLLECTIONS
6	SUTTIND EARLY NANTES	UHSBC-13	OOTY COLLECTIONS
7	KANKANAKOPPA LOCAL-1	UHSBC-14	LOCAL COLLECTION
8	GHATAPRABHA LOCAL-2	UHSBC-17	LOCAL COLLECTION
9	BLACK WONDER	UHSBC-19	PRIVATE SECTOR
10	BAGALKOT LOCAL	UHSBC-20	LOCAL COLLECTION
11	MAHARASHTRA LOCAL	UHSBC-21	LOCAL COLLECTION
12	JATT LOCAL	UHSBC-22	LOCAL COLLECTION
13	VRCAR-1	UHSBC-23	IIVR, VARANASI
14	VRCAR-2	UHSBC-24	IIVR, VARANASI
15	VRCAR-5	UHSBC-25	IIVR, VARANASI
16	VRCAR-7	UHSBC-26	IIVR, VARANASI
17	VRCAR-8	UHSBC-27	IIVR, VARANASI
18	VRCAR-9	UHSBC-28	IIVR, VARANASI
19	VRCAR-11	UHSBC-29	IIVR, VARANASI
20	VRCAR-17	UHSBC-31	IIVR, VARANASI
21	VRCAR-20	UHSBC-32	IIVR, VARANASI
22	VRCAR-22	UHSBC-33	IIVR, VARANASI
23	VRCAR-25	UHSBC-34	IIVR, VARANASI
24	VRCAR-25	UHSBC-34-1	IIVR, VARANASI
25	VRCAR-26	UHSBC-35	IIVR, VARANASI
26	VRCAR-29	UHSBC-36	IIVR, VARANASI
27	VRCAR-32	UHSBC-37	IIVR, VARANASI
28	VRCAR-35	UHSBC-38	IIVR, VARANASI
29	VRCAR-40	UHSBC-39	IIVR, VARANASI
30	VRCAR-42	UHSBC-40	IIVR, VARANASI
31	VRCAR-45	UHSBC-41	IIVR, VARANASI
32	VRCAR-45	UHSBC-41-1	IIVR, VARANASI
33	VRCAR-54-1	UHSBC-42	IIVR, VARANASI
34	VRCAR-59	UHSBC-43	IIVR, VARANASI
35	VRCAR-59	UHSBC-43-1	IIVR, VARANASI
36	VRCAR-62	UHSBC-44	IIVR, VARANASI
37	VRCAR-63	UHSBC-45	IIVR, VARANASI
38	VRCAR-66	UHSBC-46	IIVR, VARANASI
39	VRCAR-68	UHSBC-47	IIVR, VARANASI
40	VRCAR-70	UHSBC-48	IIVR, VARANASI
41	VRCAR-74	UHSBC-49	IIVR, VARANASI
42	VRCAR-81	UHSBC-52	IIVR, VARANASI
43	VRCAR-85	UHSBC-53	IIVR, VARANASI
44	VAISHALI SEEDS	UHSBC-59	PRIVATE SECTOR
45	PUSA MEGHALI	UHSBC-65	IARI, NEW DELHI
46	PUSA ASITA	UHSBC-66	IARI, NEW DELHI
47	AKSHAY-1	UHSBC-68	PRIVATE SECTOR
48	NEW KURUDA	UHSBC-69	PRIVATE SECTOR

Table 2a: List of observations recorded for qualitative characters for plant and root characters in carrot

S. No	Characters	Details
Qualitative Traits		
X1	Root position in soil (score)	3-Shallow, 5-Medium, 7- Deep, 9-Very deep
X2	Shoot Attachment (score)	1-Single, 2-Multiple
X3	Leaf type (score)	1-Celery, 2-Normal, 3-Fern
X4	Root branching (score)	1-Absent, 3-Sparsely, 5-Intermediate, 7-Dense
X5	Root Hairiness (score)	1-Absent, 2-Very Low, 3-Low, 4-Moderate, 5-High, 6-Very high
X6	Root cracking (score)	1-Absent, 2-P, 3-Low, Intermediate-4
X7	Root tip (score)	1-Absent, 2-Present
X8	Root tapering (score)	1-Blunt, 2-Pointed
X9	Root texture (score)	1-Smooth, 2-Course, 3-Dimpled, 4-Ridged
X10	Root shape (score)	1-Round, 2-obovate, 3-Obstrangular, 4-oblong, 5-tapering, 6-others
X11	Root Shoulder shape (score)	1-Flat, 2-Flat to rounded, 3-Rounded, 4-Rounded to conical, 5-conical, 6-others
X12	White lines (score)	1-Absent, 2-Present
X13	Petiole pubescence (score)	1-Absent, 2-Present
X14	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.
X15	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
X16	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-Pinnk, 9-Red/dark red, 10-Purple, 11-Black/Dark Purple.
X17	Phloem colour (score)	
X18	Cambium colour (score)	

*scores were given based on the IPGRI Descriptor (IPGRI 1998, Enclosed).

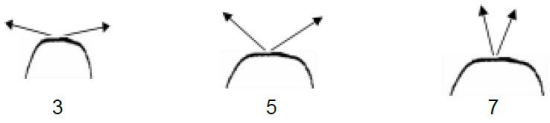
Table 2b: List of observations recorded for quantitative and biochemical characters for plant and root characters in carrot

Quantitative traits (Morphological)		
X19	No of petioles	Petioles Counted
X20	Root width (cm)	Digital Vernier Caliper
X21	Shoulder width (m)	Digital Vernier Caliper
X22	Xylem width (cm)	Measuring scale
X23	Phloem width (cm)	Measuring scale
X24	Cambium width (cm)	Measuring scale
X25	Petiole length (cm)	Measuring scale
X26	Root length (cm)	Measuring scale
X27	Shoot length (cm)	Measuring scale
X28	Shoulder length (cm)	Measuring scale
X29	Root yield(gms)	Weighing balance
X30	Shoot weight (gms)	Weighing balance
X31	Five roots weight (gms)	Weighing balance
X32	Five shoot weight (gms)	Weighing balance
X33	Root/shoot ratio	Five Root weight/Five Shoot Weight
X34	Harvest index (%)	Economic yield/Biological yield
X35	Phloem to Xylem ratio	Phloem width/xylem width
Biochemical Parameters		
X36	Beta carotenoid (ppm)	Standard beta carotene graph (Y=mx+C)-453nm
X37	Total Sugars (%)	Standard Glucose Graph (Y=mx+C)-490nm
X38	Reducing Sugars (%)	Standard Glucose Graph (Y=mx+C)-510nm
X39	Non-reducing Sugars (%)	Total sugars-Reducing Sugars)

Appendix II. Minimum characterization descriptors for carrot

Agreed at the Second Meeting of the Umbellifer Crops Working Group, 26-28 June 2013, St. Petersburg, Russian Federation.

Note: the "IPGRI numbers" refer to the *Descriptors for wild and cultivated Carrots* published by IPGRI (now Bioversity)[7].

Number	IPGRI number	Descriptor name	Descriptor state	Notes
1	7.1.12	**Leaf growth habit (attitude)**	3 Prostrate 5 Semi-erect 7 Erect	See Fig. 1
 3 5 7 **Fig. 1. Leaf growth habit (attitude)**				
2	7.1.14	**Leaf type**	1 Celery 2 Normal 3 Parsley or Fern	See Fig. 2
 1 2 3 **Fig. 2. Leaf type**				
3	7.1.16	**Leaf colour**	1 Yellow green 2 Green 3 Grey-green 4 Purple green 99 Other	
4	7.2.1	**Bolting tendency**	3 Low 5 Intermediate 7 High	

[7] IPGRI. 1998. Descriptors for wild and cultivated Carrots. International Plant Genetic Resources Institute, Rome, Italy.

Number	IPGRI number	Descriptor name	Descriptor state	Notes
5	**7.4.2**	**Root position in soil**	3 Shallow 5 Medium 7 Deep	(at maturity) See Fig. 3

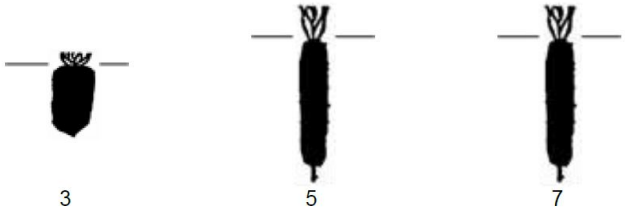


Fig. 3. Root position in soil

Number	IPGRI number	Descriptor name	Descriptor state	Notes
6	**7.4.11**	**Root surface**	1 Smooth 2 Coarse 3 Dimpled 4 Ridged 99 Other	See Fig. 4

Fig. 4. Root surface

Number	IPGRI number	Descriptor name	Descriptor state	Notes
7	**7.4.12**	**Root branching**	0 Absent 3 Sparse 5 Intermediate 7 Dense	See Fig. 5

Fig. 5. Root branching

Number	IPGRI number	Descriptor name	Descriptor state	Notes
8	**7.4.14**	**Root shape**	1 Round 2 Obovate 3 Obtriangular 4 Oblong 5 Tapering 99 Other	See Fig. 6

Fig. 6. Root shape

Number	IPGRI number	Descriptor name	Descriptor state	Notes
9	**7.4.16**	**Root shoulder shape**	1 Flat 2 Flat to rounded 3 Rounded 4 Rounded to conical 5 Conical 99 Other	See Fig. 7

Fig. 7. Root shoulder shape

Number	IPGRI number	Descriptor name	Descriptor state	Notes
10	**7.4.17** (modified)	**Colour of skin on shoulder**	0 No difference 3 Green 5 Violet	See Fig. 8

Fig. 8. Colour of skin on shoulder

Number	IPGRI number	Descriptor name	Descriptor state	Notes
11	**7.4.21**	**Root tip/end shape**	1 Blunt 2 Rounded 3 Pointed	See Fig. 9

Fig. 9. Root tip/end shape

Number	IPGRI number	Descriptor name	Descriptor state	Notes
12	**7.4.22**	**Root skin pigmentation colour**	1 White 2 Yellow 3 Orange 4 Red 5 Purple 99 Other	See Fig. 10

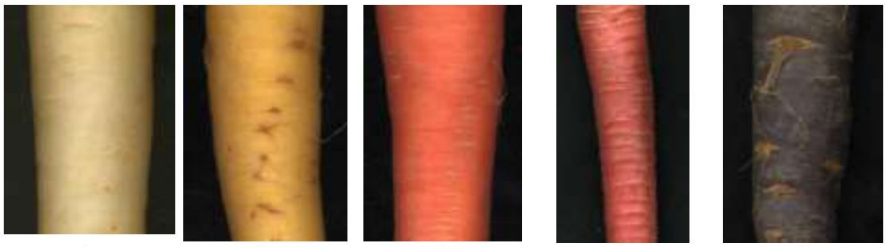


Fig. 10. Root skin pigmentation colour

Number	IPGRI number	Descriptor name	Descriptor state	Notes
13	**7.5.5**	**Outer core pigmentation/colour**	1 White 2 Yellow 3 Orange 4 Red 5 Purple 99 Other	(Observation at maximum diameter) See Fig. 11

Fig. 11. Outer core pigmentation/colour

Number	IPGRI number	Descriptor name	Descriptor state	Notes
14	**7.5.7**	**Inner core pigmentation/colour**	1 White 2 Yellow 3 Orange 4 Red 5 Purple 99 Other	(Observation at maximum diameter) See Fig. 12
15	**7.7.1**	**Accession longevity (lifespan)**	1 Annual 2 Biennial 3 Both	

1 2 3 4 5

Fig. 12. Inner core pigmentation/colour

(18) and quantitative (17) and biochemical (beta carotenoid and sugars). Details of both the experiments are given below and the general view of experimental plot is given in Plate 1**.**

3.2.1.1a Experiment-I (S1): First experiment was conducted at Haveli farm of UHS Bagalkot during the month of April-June 2015 in randomized complete block design (RCBD) with two replications.

3.2.1.1b Experiment-II (S2): Second experiment was conducted at Udyanagiri main campus of UHS Bagalkot during the month of October-December 2015 in a RCBD with two replications.

In each replication, for respective genotype, ridge and furrow method (4m length) of sowing was followed in both the seasons and the seeds were sown in the opposite hills of a furrow. The field was divided into blocks to make replications and the spacing followed was 22.5 x 10 cm with minimum of 40 plants were maintained in each plot. The agronomic practices were followed as per the package of practices of UHS Bagalkot. No pest and disease incidence was observed during the crop growth period.

3.2.1.2 Climatic conditions:

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 318mm and belongs to semi arid region.

3.2.1.3 Phenotyping for plant and root morphological characters

Phenotypic evaluation was done for all the 48 genotypes (germplasm lines) in two experiments (S1 & S2) for plant and root morphological characters. All the traits were recorded as per the IPGRI descriptor. Quantitative characters were recorded as per the SI units. The list of characters recorded in two experiments and their description is given in the table 2a and 2b.

Plate 1: General view of experimental plot of carrot consisting of 48 genotypes

For internal and external root colours: Although standard descriptor is available from IPGRI/NBPGR for internal and external root colour, but due to limited number of colour variation in the descriptor and more variations for the colour expressed in the present study (in both experiments), we followed our own descriptor with the scores given below for external root and internal colours (Xylem, Phloem and Cambium colour).

3.2.2 Biochemical estimation

The roots of all the forty eight carrot genotypes were collected from each replications and the best five roots were selected for phenotypic evaluation in both the seasons were further subjected to biochemical estimation. 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 a airtight tetrapack (Aluminium foil) covers and was stored in -20^0 C deep freezer till the estimations were carried out before using for biochemical estimation (Nagata *et al*., 2008). In each replication, 3 biological replicates were made with a total of six samples for each genotype in both the seasons for all the biochemical parameters.

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

Beta carotene was expressed in ppm after the estimation for each replication in each genotype in the respective seasons.

A total of 3 biological replicates were made for each replication in each cultivar, so a total of six replicates were used for beta carotene estimation and the mean data of each replication was used for statistical analysis.

Estimation of Sugars: Both the total sugars and reducing sugars were estimated for all the genotypes selected for the present study in both the seasons and the standard graph was used for the estimation of sample values and converted it in percentage.

Hundred grams of representative root sample was homogenized in 5ml of 80 per cent ethanol. The homogenate was centrifuged at 40000 rpm for 10 min and the supernatant was collected and made the volume to 10 ml and this solvent was used for the estimation of total sugar and reducing sugars by the following methods.

Estimation of total sugars in carrot roots:

Total sugars were estimated as per the method given by Dubois *et al*., 1956 and Krishnaveni *et al*. (1984). The protocol is as follows

Calculation

Absorbance corresponds to 0.1 mL of the test = x mg of glucose 100 mL of the sample solution contains =0.1 x100 mg of glucose= % of total carbohydrate present.

Estimation of reducing sugars:

Reducing sugars were estimated by following the Dinitrosalicylic acid (DNS) method given by Miller (1972) as it is a simple, sensitive and adoptable during handling of a large number of samples at a time.

Calculation

Amount of reducing sugars in the sampled roots was calculated by using the standard curve of glucose with the conc of 100mg/100ml.

Estimation of non-reducing sugars: Non reducing sugar was calculated by subtracting the reducing sugar from total sugar for all the genotypes in three biological replicates in each replication and the mean value for each replication was considered for statistical analysis.

Non-reducing sugars= Total sugars-reducing sugars

Statistical Analyses

Analysis of variance

The analysis of variance (ANOVA) for all the 39 traits was carried out by using the mean phenotypic data for both the seasons individually in S-I and S-II by following the method suggested by Panse and Sukhatme (1964).

The structure of ANOVA is as follows

Source of variation	d.f.	MSS	Expected value of MSS	Cal F.
Replication	(r-1)	M_1	-	
Genotypes	(g-1)	M_2	$\sigma^2e + r\sigma^2g$	M_2/M_3
Error	(r-1) (g-1)	M_3	σ^2e	
Total	(rg-1)	$M_1+M_2+M_3$		

3.6.1.2 Mean and range

The mean and range of each character were calculated for all the 39 traits, based on replicated means of each cultivar in both the seasons.

i) Mean $(\overline{X})$ = $\dfrac{\text{Sum of the observations of all the plants}}{\text{Number of plants}}$

ii) Range = The minimum and maximum values for each trait

Frequency distribution

Eighteen qualitative traits were subjected to frequency distribution in both the seasons using SPSS ver.16 software.

Estimation of genetic variability components

In order to assess and quantify the genetic variability among the characters under the study, following parameters were estimated.

Phenotypic and genotypic variances were estimated using the following formula (Singh and Chaudhary, 1979).

$$\text{Genotypic variance } (\sigma_g^2) = \frac{\text{MSS (genotypes) - MSS (error)}}{\text{Number of replications}} = \frac{M_2 - M_3}{R}$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \text{MSS error} = \frac{M_2 - M_3}{r} + M_3$$

Coefficient of variability

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

i) Genotypic coefficient of variability (GCV)

$$GCV \equiv \frac{\sigma_g}{\bar{X}} \times 100$$

ii) Phenotypic coefficient of variability (PCV)

$$PCV = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviatio

$\bar{X}$ = General mean of the character

GCV and PCV values were categorized as low, moderate and high as indicated by Siva Subramanian and Menon (1973). As follows

0-10% : Low

10-20% : Moderate

20% and above : High

c) Heritability (h^2 b.s)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson *et al*. (1956) and expressed as percentage.

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

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

The heritability percentage was categorized as low, moderate and high as given by Robinson *et al.* (1949).

0-30% : Low

30-60% : Moderate

60% and above : High

d) Genetic advance (GA)

Genetic advance was calculated by using the formula given by Johnson *et al*. (1955).

$$GA = h^2 k \sigma_p$$

Where,

h^2 = Heritability in broad sense

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

σ_p = Phenotypic standard deviation

e) Genetic advance as per cent of mean (GAM)

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

Where,

GA= Genetic advance

$\bar{X}$ = General mean of the character

Genetic advance as per cent mean was categorized as low, moderate and high as given by Johnson *et al.* (1955).

It is as follows.

0-10%	: Low
10-20%	: Moderate
20% and above	: High

3.6.1.5 Principle component analysis (PCA)

Principal components analysis is a variable-reduction technique that shares many similarities to exploratory factor analysis. Its aim is to reduce a larger set of variables into a smaller set of 'articifial' variables, called 'principal components', which account for most of the variance in the original variables. A total of 21 quantitative characters including 4 biochemical traits (in S-I and S-II) were subjected to PCA

analysis using SPSS (version 16.0) software by Factor analysis. Principle components were obtained by extraction method of PCA using the option of data reduction and only the principle components showing Eigen value of >1.0 was considered. The linearity of the variables were checked using screen plot.

3.6.1.6 Correlation analysis

The correlation coefficients were worked out to determine the degree of association for a group of characters (characters scored from descriptors and quantitative characters and biochemical characters). The correlations were calculated for both the experiments (S1) and (S2) only for 21 quantitative characters including 4 biochemical traits.

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

$$r_p = \frac{\text{Cov } XY_p}{\sqrt{\sigma_p^2 x \text{ X } \sigma_p^2 y}}$$

Where,

r_p = Phenotypic correlation

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

$\sigma_p^2 x$ and $\sigma_p^2 y$ = 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).

33.6.1.7 Path analysis

Path coefficient analysis was carried out for 21 quantitative traits in both the seasons by using the correlation coefficients to know the direct and indirect effects of all the components on root yield/plant as suggested by Wright (1921) and illustrated by Dewey and Lu (1959).

Path coefficients were obtained by solving the simultaneous equations, which express the basic relationship between correlations and path coefficients. The equations were as follows

r1.y = P1y + r1.2 P2y + r1.3 P3y + ... + r1.k Pky

r2.y = r2.1 P1y + P2y + r2.3 P3y +. . . . . + r2.kPky

...

...

rk-1.y = rk-1.1P1y + r k-1.2 P2y + rk-1.3 P3y +. . . . . + Pk-1y

Where, r1.y to rk-1.y denote the correlation coefficients between independent characters 1 to k-1 and dependent character 'y', r1.2 to rk-2.k-1 denote the correlation coefficients between all possible combinations of independent characters. P1y to Pk-1y denote the direct effects of characters 1 to k-1 on character y.

3.6.1.8 Genetic diversity analysis

Multivariate analysis using D^2 statistics

Mahalanobis (1936) D^2 statistics was used for assessing the genetic divergence between carrot cultivars by using the software Indostat version 5.1.

The generalized distance between any two populations is defined as,

$$D = \Sigma \lambda ij \, \delta i \, \delta j$$

Where,

λij = The reciprocal matrix to the common dispersion matrix

δi = The difference between the two mean values of the two populations for ith character ($\mu i1 - \mu i2$)

δj = The difference between the mean values of the two populations for the jth character ($\mu j1 - \mu j2$)

μ = Vector mean values for all the characters

The formula for the estimation of distance, D2 from samples:

$$D^2p = d1\ (S\text{-}1)\ d$$

Where,

D2p = Square of the distance considering P variables.

d1 = (Xi1 – Xi2)

X = Vector of mean values of all the characters

S-1 = inverse of variance covariance matrix

Formula for computation of D^2 values, which requires inversion of the matrix, becomes complicated especially when the numbers of variables under consideration are more. Therefore, the original correlated unstandardized variables (Xi) were transformed to standardized uncorrelated variables (Yi) so that the computation of D^2 values reduce to simple summation of squares of the differences between values of transformed variables of the two population i.e., D^2i.

From the newly transformed uncorrelated variables, the square of the distance was computed using the following formula,

$$\overline{D2} = \Sigma\ (\overline{Y}i1\text{-}Yi2)\ 2$$

Where,

$\overline{Y}i1$ = Vector of transformed mean values, for first genotype

$\overline{Y}i2$ = Vector of transformed mean values, for second genotype

The square root of the D^2 values gives the generalized distance (D) between the two populations. The D2 values were arranged in a matrix form. The significance of D^2 values between any two clusters was tested using the following formula,

$$F = \frac{(n1 + n2 - p - 1)}{(n1 + n2 - 2)\ P} \times \frac{(n1\ n2)\ D2}{(n1 + n2)}$$

This computed F was compared with table F value at 5 percent and 1 percent levels of significance with P(number of characters) and (n1 + n2 – p – 1) degrees of freedom.

Determination of population constellation

All the n(n –1)/2 D^2 values were considered for determining the population constellation. This was realized by using Tocher's method. The criterion used in clustering by this is that any two varieties belonging to the same cluster, should at least, on an average, show a smaller D^2 value than those belonging to different clusters. As per the device it was to start with two closely associated population and find a third population, which had the smallest average D^2 from these two. Similarly, the fourth was chosen to have a smallest average D^2 from the first three and so on. The permissible increase in D^2 values for clustering into the same group was fixed approximately nearer the maximum D^2 value shown by a population to the nearest population. This procedure was continued till D^2 values of all the pairs of genotypes were exhausted. After the formation of the clusters inter and intra group distances were calculated. The square root of the average D^2 values obtained from the above represents the distance (D) between and within clusters.

3.2.3 Molecular marker and allelic diversity

3.2.3.1 DNA Extraction methodology

Young leaves and tissues of all the 48 cultivars were collected from four weeks old plants grown in the main campus 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 immediate 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 dry 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.

3.2.3.2 Quantification of DNA

The stock DNA was checked for its quality and quantity in 0.8 % Agarose and nanospectophotometer respectively.

3.2.3.3 Genotyping and Amplification

Twenty four microsatellites markers including ten gene specific markers which were polymorphic out of 49 markers screened (selected from publications) as listed in Table 3.

1. Based on the quantification (280nm/260nm) in nanospectophotometer.
2. 50ng of working DNA was prepared for PCR for the 48 cultivars
3. For polymerase chain reaction, 50 microsatellite markers (listed in table 2 of Appendix) were diluted (10 picomoles) from the stock.
4. PCR was done for 10µl reaction, the components used for PCR with the respective concentration and the protocol is presented in the table

3.2.3.4 Electrophoresis of Microsatellites

After confirming the PCR amplification on 1.5% Agarose gel, the amplified products were size separated first in 4.0 % Agarose gel electrophoresis. The bands were scored for each allele in the respective markers as presence or absence. Plate 13a showing the allelic diversity and presence/absence pattern for the various molecular markers.

Table 4: PCR reactions for microsatellite primers

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 5: PCR protocol followed for microsatellite primers for 48 carrot cultivars

S. 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	∞	

3.2.3.5 Molecular marker diversity

A total of 49 markers including gene specific and microsatellite markers available in the public database were selected and out of these, 24 markers showed polymorphism across the 48 diverse carrot genotypes with a total of 62 alleles in the present study (Table 3). Scoring was done primer wise based on the presence/absence polymorphism for the respective alleles across 48 carrot germplasm lines were taken for consideration. Score was used for calculation of the following parameters

Each polymorphic marker was characterized for number of alleles per locus and gene diversity using 48 accessions of carrot

Size differences of the fragments in other genotypes were considered to be the result of alterations in the repeat number of the simple sequences at the corresponding site(s). Allelic polymorphic information content (PIC) was calculated using the following formula.

PIC = 1- å (Pi)2

Where, Pi is the proportion of the population carrying ith allele, calculated for each microsatellite locus (Botstein *et al.* 1980).

Table 3: List of sequence information of 24 carrot specific molecular markers screened across 48 carrot genotypes

S. No	Marker Name	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
1	GSSR-4	CAATCTTGCCACTAAAAGAGCA	CAGATACAATAGACAGGAAACATCG	Cavagnaro *et al.* 2011
2	GSSR-6	TCTCCTCTTGATTCTTCTTCGC	CCAATAAGCGTAAGCGTTTCTC	
3	GSSR-9	TTGACGCTGTAGTCGCACTTAT	CAGCAAATCAGAGGAAGGGTAG	
4	GSSR-16	ATGCAAACGACAATATCCACAG	GCCCAGCCACTTCCTAGAT	
5	GSSR-17	GGTCTCTTCCACACTCATGGAT	CCAGCATTCACTATGTCCACTC	
6	GSSR-44	AACTTCACCCCAGCTCACC	CAAAGCAAGTAAAGAGACAGCG	
7	GSSR-85	TGACTCGGTGGATGAATTAAGA	CACTGCTTTGCCATTGTTTT	
8	5'UTR - Exon1	AATCACCTTCCTCCCCAAAG	TCACTGAAGCCAAACATCACA	Soufflet-Freslon *et al.*, 2013
9	Exon3 - Exon5	TGCCTTGAACTCATTGGAAC	TGCGTTGATCATTGGTGTCT	
10	Exon4 - Exon7	CTCAAAATGCTGGAGACATAGC	TCCCATCTGGTAGCATTTGA	
11	Exon7 - Exon9	CTGGCGAATGGAAATGAGAT	TCCCTTCTGGAGCTAATGATG	
12	Exon9 - Exon11	TTGGTCAAATTTAGAGGTTCCA	GGCATTCCTAGTAAACCCTTTG	
13	DCM-2	CGACGAATAAGATGCGAGAGA	CACTCTTGAGCCACCACCTATAC	Niemann *et al.*, 2001
14	DCM-17	GCCTTCACTGAAATACATAAAA	TACTGACAATTACTTCAGCATA	
15	DCM-32	TACTCCATGGTGGTAGTGAGG	AGAGAGGGAGAGCGGAAGA	
16	GSSR-14	CCACCTTGGACAAAGCAAAC	GCCCAGTTCTTCTTAATTGCAG	
17	GSSR-19	CCGAGTTGGATTCGGAGAG	GTAAATTGAGGATTGCGAGTTG	
18	GSSR-63	ACACTTTTCATCCTCCAACTCC	TGCGACCATGACTATACGAAAC	
19	GSSR-138	CGCTCGAGTTTCGTAGAGT	CCTCCCCAACTCAATCCAAT	
20	GSSR-149	TGAAGCAACTCGTGATACAGAGA	TTCTCTTGTCCTGGTTAGCTC	
21	GSSR-111	GAGGAAGGGTAGATCCAGTCA	ATGGGATGTCTTTCCCCTCTAT	
22	Y2Mark	TAAAGTCGTATAGGAAGAACAT	TGGATCATCAGAACTCAACT	Cavagnaro *et al* 2009
23	DCOR	GAGATGCAAATACTGTCTAGGAACTG	GTACAGACAAGTAGGGCACA	
24	DCELF1α	GATCCCGCCAAAGAGGCTGCC	CCACCGCCTGTCAAGCACCC	Kawahara *et al* 1992

The marker index (MI) was calculated using the following formula (Powell *et al*. 1996).

MI = Average polymorphic information content (PIC) × Proportion of polymorphic bands × Average number of loci per assay unit

For the purpose of assessing genetic diversity leading to the preparation of a dendrogram, gels were scored in binary format, with the presence of a band scored as unity and its absence scored as zero. The binary data were used to compute pair-wise dissimilarity Coefficients (Jaccard, 1908), with 1000 bootstrap value and the dissimilarity matrix thus obtained was subjected to cluster analysis using the UPGMA (unweighted pair-group method with arithmetic average) algorithm on Darwin version 5.0 software. The diagonal matrix was then submitted to cluster analysis using the maximum likelihood method and a genetic distance of dendrogram was built with the help of radial method of graph rather than usual bar diagram.

He- expected heterozygosity for a genetic marker was calculated as:

He = 1 - pi2, where pi is the allele frequency of the i^{th} allele. The arithmetic mean heterozygosity (Hav) for each marker class was calculated as Hav = He/n, where n = number of markers or loci analyzed

The expected heterozygosity of each loci was defined as He= 1–Spi2, where pi is the frequency of the ith allele (Nei 1973). The observed heterozygosity was calculated according to Nei (1978), i.e., it was given as Ho = No. of accessions harbouring heterozygous genotypes at the ith allele/No. of total accessions.

3.2.4 Marker-trait association:

3.2.5 Single marker analysis (SMA)

Single marker analysis was performed to know the potential markers linked to the phenotypic data of root morphological and biochemical traits in the diverse carrot genotypes. The genotypic data of 24 markers having a total of 62 alleles and the phenotypic data of all the 39 morphological and biochemical traits recorded in the

present study in S-I and S-II for the carrot genotypes were subjected to stepwise linear regression analysis (Haley and Knott, 1992) using SPSS Version 16.0 software.

3.2.6 Selection of superior genotypes for tropical region

Since the main goal of the present study was to identify the carrot genotypes suitable for tropical region, hence the two experiments were evaluated in the Bagalkot region representing the tropical areas of Karnataka. Some of the important characters such as root colour (external and internal uniformity), root weight, biochemical parameters such as sugars and carotenoids were taken in to consideration along with its flowering ability in these regions (data not shown). Based on the mean values and respective critical difference (CD at 5%) values for the above traits, best three to four superior genotypes were selected.

4. EXPERIMENTAL RESULTS

Present study was conducted with an objective of identifying the carrot genotypes suitable for tropical region. The forty eight carrot genotypes were collected from different geographical regions and characterized using thirty five morphological, four biochemical and 24 carrot specific markers. Genotypes comprised of multi-colored and multiple types (*Asiatic* and *European*). They were screened in two seasons (summer season as S-I and winter season as S-II) at Bagalkot representing the tropical region of Karnataka, India during 2015-16. The details of the results of the experiments are presented in following subheadings.

4.1 Phenotypic characterization (Morphological and Biochemical)

4.2 Molecular characterization

4.3 Marker-trait association

4.4 Selection of superior carrot germplasm

4.1 Phenotypic characterization

A total of 39 characters including 35 morphological (18 qualitative traits based on descriptors, 18 quantitative traits) and 4 biochemical traits were recorded in two seasons (S-I and S-II). Out of 39 characters 21 traits (17 morphological and 4 biochemical) were considered as quantitative traits in the present study. The replicated data from S-I and S-II seasons were subjected to various statistical analysis such as analysis of variance (ANOVA), analysis for genetic variability and heritability components, frequency distribution (only for 18 qualitative traits), principle component analysis PCA (only for 21 quantitative traits), correlation analysis and path coefficient analysis (only for 21 quantitative traits), Mahalanobi's D^2 analysis ((21 quantitative traits and external and internal root colours including xylem and phloem colour) and the details of the results are discussed accordingly.

4.1.1 Analysis of variance (ANOVA)

All the thirty nine traits were subjected to ANOVA separately for individual seasons (S-I and S-II) and the respective coefficient of variation for various characters and the critical difference (CD) values at 1% level of significance are given in the tables 6 and 7. The source of variation was partitioned as between genotypes and between replications (within genotypes).

Analysis of variance revealed significant variation between genotypes for all the qualitative and quantitative traits during S-I except for root tapering, root shape, root width and cambium width characters (Table 6). In S-II also, almost all the characters showed significant variation for the genotypes except shoot attachment, root branching, root texture, root shape, root shoulder shape and petiole pubescence (Table 7).

Among the biochemical parameters such as beta carotenoids, total sugars, reducing sugars and non-reducing sugars, ANOVA revealed significant variation among the carrot genotypes in both the seasons (Table 6 and 7).

4.1.3 Mean, range, CV and CD

The mean, range, variability components in S-I and S-II seasons is presented in Tables 8 and 9. The mean values for each genotype for the 39 traits in S-I and S-II is given in Appendix 1 and 2 respectively.

For few qualitative characters such as, type of shoot attachment (single/ multiple), leaf type (celery, normal and fern), root cracking (absent, intermediate, sparsely), root tip (present or absent), root tapering (blunt or pointed), petiole pubescence (present or absent) and white lines on petioles (presence or absence), only 2-3 types were observed for the respective traits. Hence, the range was narrow for these qualitative characters in both the seasons among the 48 carrot germplasm selected for the study. Other qualitative characters, especially for external root colour and internal root colour (xylem, phloem and cambium), wider range (2.0 to 15.0) of colours were found among the genotypes selected for the study ranging from white to black.

The mean value for external root colour (5.65) indicates more of orange coloured carrots in the selected genotypes in S-I. The internal colour (xylem, phloem

Table 6: Analysis of Variance (ANOVA), Coefficient of variation (CV) and Critical Difference (CD at 1%) for morphological and biochemical traits in Season-I for 48 carrot genotypes

S. No.	Characters	Source of Variation			CV	CD 1%
		Replication (1)	Genotypes (47)	Error (47)		
Morphological (Qualitative Traits)						
X1	Root position in soil (score)	0.877	2.38**	0.599	12.29	0.76
X2	Shoot Attachment (score)	0.068	0.07	0.060	22.16	0.24
X3	Leaf type (score)	0.342	0.38**	0.088	16.39	0.29
X4	Root branching (score)	0.223	1.62**	0.604	37.17	0.77
X5	Root Hairiness (score)	0.889	1.901**	0.679	33.40	0.82
X6	Root cracking (score)	0.104	0.12**	0.022	13.89	0.15
X7	Root tip (score)	0.004	0.31**	0.050	13.30	0.22
X8	Root tapering (score)	0.458	0.13	0.113	19.23	0.33
X9	Root texture (score)	0.953	0.43*	0.259	29.21	0.51
X10	Root shape (score)	0.022	0.22	0.162	8.42	0.40
X11	Root Shoulder shape (score)	1.031	0.53*	0.316	26.05	0.56
X12	White lines (score)	0.115	0.14*	0.072	22.15	0.27
X13	Petiole pubescence (score)	0.025	0.12**	0.043	17.25	0.21
X14	Root colour (score)	3.267	10.64**	3.377	32.53	1.82
X15	Shoulder colour (score)	0.031	1.43**	1.332	68.26	1.15
X16	Xylem colour (score)	0.552	1.94**	0.726	36.17	0.85
X17	Phloem colour (score)	0.624	5.17**	1.271	28.93	1.12
X18	Cambium colour (score)	0.173	0.17**	0.868	32.02	0.92
Morphological (Quantitative Traits)						
X19	No of petioles	22.336	66.281*	35.537	44.46	5.91
X20	Root width (cm)	0.000	0.19	0.151	22.34	0.39
X21	Shoulder width (Cm)	0.150	2.21**	0.296	25.36	0.54
X22	Xylem width (cm)	0.028	0.09**	0.041	20.89	0.20
X23	Phloem width (cm)	0.001	0.02**	0.008	26.47	0.09
X24	Cambium width (cm)	0.034	0.10	0.129	84.05	0.36
X25	Petiole length (cm)	4.891	26.10**	11.436	17.63	3.36
X26	Root length (cm)	53.325	48.27	53.730	37.93	7.27
X27	Shoot length (cm)	27.108	156.46**	45.317	15.08	6.68
X28	Shoulder length (cm)	0.111	0.53**	0.102	41.19	0.32
X29	Root yield(gms)	2551.387	496.50	522.897	55.82	22.69
X30	Shoot weight (gms)	5717.843	2957.66**	1082.004	45.06	32.63
X31	Five roots weight (gms)	75120.487	12719.89	13170.975	56.62	113.85
X32	Five shoot weight (gms)	160797.021	75455.33**	26847.953	44.89	162.55
X33	Root/shoot ratio	0.001	0.581**	0.179	55.62	0.42
X34	Harvest index (%)	31.240	355.702**	74.529	22.27	8.56
X35	Phloem to Xylem ratio	0.001	0.02**	0.007	23.02	0.083
Biochemical Traits						
X36	Beta carotenoid (ppm)	44.605	6628.04**	266.115	5.14	16.18
X37	Total Sugars (%)	0.362	17.45**	0.920	10.79	0.95
X38	Reducing Sugars (%)	1.309	8.47**	0.691	15.72	0.83
X39	Non-reducing Sugars (%)	0.294	7.55**	1.272	31.38	1.12

*Probability at 5%, ** probability at 1%, CV-Coefficient of Variation, CD-Critical Difference, Sed-Standard error difference.

Table 7: Analysis of Variance (ANOVA), CV and CD for morphological and biochemical traits in Season-II for 48 carrot genotypes

S. No.	Characters	Replication (1)	Genotypes (47)	Error (47)	CV	CD 1%	Sed
Morphological (Qualitative Traits)							
X1	Root position in soil (score)	1.31	3.509**	0.557	10.234	0.740	0.191
X2	Shoot Attachment (score)	0.01	0.03	0.029	15.743	0.168	0.016
X3	Leaf type (score)	0.00	0.268**	0.020	6.941	0.139	0.053
X4	Root branching (score)	0.02	0.26	0.253	40.578	0.499	0.052
X5	Root Hairiness (score)	0.49	1.207**	0.315	27.358	0.557	0.112
X6	Root cracking (score)	0.05	0.129**	0.047	19.298	0.215	0.037
X7	Root tip (score)	0.00	0.120*	0.003	2.601	0.050	0.035
X8	Root tapering (score)	0.00	0.060*	0.022	7.529	0.146	0.025
X9	Root texture (score)	0.04	0.67	0.486	29.145	0.692	0.084
X10	Root shape (score)	0.06	0.13	0.106	6.718	0.324	0.036
X11	Root Shoulder shape (score)	0.13	0.44	0.359	33.295	0.595	0.068
X12	White lines (score)	0.03	0.164**	0.060	19.910	0.244	0.041
X13	Petiole pubescence (score)	0.01	0.17	0.037	15.847	0.192	0.042
X14	Root colour (score)	0.39	11.574**	1.997	21.068	1.402	0.347
X15	Shoulder colour (score)	0.59	4.944**	1.097	39.331	1.039	0.227
X16	Xylem colour (score)	0.35	5.616**	0.578	31.423	0.754	0.242
X17	Phloem colour (score)	0.63	7.333**	0.900	14.905	0.941	0.276
X18	Cambium colour (score)	0.44	6.485**	1.051	27.943	1.017	0.260
Morphological (Quantitative Traits)							
X19	No of petioles	6.01	10.707*	5.750	22.509	2.379	0.334
X20	Root width (cm)	0.00	0.196**	0.052	13.229	0.226	0.045
X21	Shoulder width (Cm)	0.01	0.514**	0.137	15.770	0.367	0.073
X22	Xylem width (cm)	0.00	0.060**	0.028	17.145	0.167	0.025
X23	Phloem width (cm)	0.01	0.020**	0.008	26.270	0.088	0.014
X24	Cambium width (cm)	0.00	0.122**	0.045	52.461	0.210	0.036
X25	Petiole length (cm)	0.02	34.332**	9.973	16.148	3.133	0.598
X26	Root length (cm)	45.20	22.924**	12.427	17.936	3.497	0.489
X27	Shoot length (cm)	19.68	172.531**	36.161	13.616	5.966	1.341
X28	Shoulder length (cm)	0.10	0.233**	0.107	29.888	0.325	0.049
X29	Root yield(gms)	104.67	295.132**	68.622	23.191	8.218	1.753
X30	Shoot weight (gms)	910.57	772.101**	145.282	26.227	11.958	2.836
X31	Five roots weight (gms)	8676.74	7714.994**	2152.390	27.117	46.026	8.965
X32	5 shoot weight (gms)	20925.30	20963.055**	4592.870	30.239	67.233	14.777
X33	Root/shoot ratio	0.08	0.746**	0.078	29.994	0.278	0.088
X34	Harvest index (%)	19.22	229.837**	24.760	10.959	4.936	1.547
X35	Phloem to Xylem ratio	0.01	0.024**	0.011	29.758	0.105	0.016
Biochemical Traits							
X36	Beta carotenoid (ppm)	707.91	3063.384**	581.337	6.935	23.920	5.649
X37	Total Sugars(%)	2.48	24.305**	2.507	14.268	1.571	0.503
X38	Reducing Sugars (%)	0.02	16.766**	1.302	17.750	1.132	0.418
X39	Non-reducing Sugars (%)	2.10	19.952**	3.142	37.987	1.759	0.456

*Probability at 5%, ** probability at 1%, CV-Coefficient of Variation, CD-critical Difference, Sed-Standard error difference.

and cambium colour) ranged from white to black but the mean values (2.36, 3.9 and 2.90) for respective traits indicated more of white to yellow colours. Root position in soil ranged from shallow (3.0) to very deep (9.0), for root shape and shoulder shape, oblong to tapering (3.77 to 5.00) and flat to rounded types (1.0 to 3.0) were found respectively in both the seasons.

Similar findings were also seen in S-II for most of these qualitative traits. But with respect to mean values for internal root colours, although xylem (2.42) and cambium (3.67) showed nearer to white or yellow colour, but the mean for phloem colour (6.37) showed the colour nearer to orange.

When these traits were compared across the seasons, the root position in soil was medium as per the mean value (~7.0) and the range was shallow to very deep. Similarly, the branching, hairiness, cracking were less based on the mean values; however, these abnormalities were seen for few of the genotypes as shown by the range.

Root texture was smooth to ridged type with the mean value showing coarse type of texture. With respect to root colours (external and internal), the trend was same as that of individual seasons. With respect to root shape, more of tapering types were seen as depicted by its mean value near to 5.0 and the shoulder shape with flat to rounded types (Tables 8 and 9).

Among the quantitative characters, in general wider range of variation was observed during S-I for most of the characters compared to S-II. Very wide range of variation was observed for five root weight and five shoot weight in S-I (49.50 to 403.75 g and 32.75 to 1023.75 g) compared to S-II (61.0 to 387.0 g and 32 to 469 g). Number of petioles ranged from 7.0 to 42.0 in S-I and 6.6 to 18.0 for S-II with the average of 13.41 and 10.65 in both the seasons respectively.

Mean root width, xylem, phloem and cambium width were approximately same in both the seasons, but the maximum phloem width was observed in S-II (0.63cm). In general, the portion of xylem was more than the phloem in the present study in both the seasons.

Shoulder width was more than the root width in both the seasons and the maximum shoulder width were recorded in S-II (3.95 cm) but maximum root width was

Table 8: Mean, Range, and genetic variability estimates for morphological and biochemical traits in Season-I for 48 carrot genotypes

S. No.	Traits	Mean±Sed	Range	GCV	PCV	h^2 (b.s)	GAM (1%)	EMG *
Morphological (Qualitative Traits)								
X1	Root position in soil (score)	6.3±0.16	3.00- 9.00	14.98	19.42	0.60	30.50	7.80
X2	Shoot Attachment (score)	1.11±0.03	1.00-2.00	5.48	22.86	0.06	3.47	1.14
X3	Leaf type (score)	1.81±0.06	1.10-3.00	20.97	26.92	0.61	43.11	2.41
X4	Root branching (score)	2.09±0.13	1.00-4.00	34.17	50.30	0.46	61.28	3.09
X5	Root Hairiness (score)	2.47±0.14	1.00-5.00	31.65	46.09	0.47	57.36	3.57
X6	Root cracking (score)	1.08±0.03	1.00-2.50	19.69	24.39	0.65	41.97	1.43
X7	Root tip (score)	1.68±0.06	1.00-2.00	21.61	25.30	0.73	48.72	2.31
X8	Root tapering (score)	1.75±0.04	1.00-2.00	4.40	20.31	0.05	2.52	1.78
X9	Root texture (score)	1.74±0.07	1.00-3.30	16.02	34.03	0.22	19.91	2.01
X10	Root shape (score)	4.78±0.05	3.77-5.00	3.72	9.14	0.17	4.00	4.92
X11	Root Shoulder shape (score)	2.16±0.07	1.00-3.32	14.58	30.38	0.23	18.48	2.47
X12	White lines (score)	1.21±0.04	1.00-2.00	15.15	26.96	0.32	22.48	1.42
X13	Petiole pubescence (score)	1.20±0.04	1.00-2.00	16.26	23.65	0.47	29.53	1.47
X14	Root colour (score)	5.65±0.33	2.00-15.00	33.74	46.87	0.52	64.12	8.47
X15	Shoulder colour (score)	1.69±0.12	1.00-4.30	14.95	69.20	0.05	8.53	1.80
X16	Xylem colour (score)	2.36±0.14	1.00-7.00	33.12	48.98	0.46	59.12	3.44
X17	Phloem colour (score)	3.90±0.23	1.83-8.40	35.91	46.02	0.61	73.98	6.15
X18	Cambium colour (score)	2.97±0.17	1.00-6.00	36.58	43.36	0.71	81.48	4.86
Morphological (Quantitative Traits)								
X19	No of petioles	13.41±0.83	7.08-42.00	29.37	53.14	0.31	42.85	17.89
X20	Root width (cm)	1.74±0.04	0.99-3.03	8.68	23.75	0.13	8.37	1.85
X21	Shoulder width (Cm)	2.15±0.15	0.21-3.95	45.58	52.09	0.77	105.30	3.91
X22	Xylem width (cm)	0.97±0.03	0.53-1.53	15.78	26.10	0.37	25.20	1.16
X23	Phloem width (cm)	0.34±0.01	0.13-0.55	20.45	33.31	0.38	33.15	0.43
X24	Cambium width (cm)	0.43±0.03	0.07-1.44	26.26	79.14	0.11	23.00	0.35
X25	Petiole length (cm)	19.17±0.52	13.90-30.03	14.19	22.55	0.40	23.56	22.70
X26	Root length (cm)	19.33±0.71	14.19-41.54	8.54	36.95	0.05	5.21	18.54
X27	Shoot length (cm)	44.66±1.28	22.80-62.75	16.72	22.47	0.55	32.85	56.10
X28	Shoulder length (cm)	0.78±0.07	0.00-1.58	59.38	72.30	0.67	128.73	1.56
X29	Root yield (gms)	40.97±2.27	10.10-80.75	14.30	56.24	0.07	9.60	37.90
X30	Shoot weight (gms)	73.00±5.55	7.65-204.75	40.86	62.30	0.43	70.74	113.29
X31	Five roots weight (gms)	202.71±11.51	49.50-403.75	14.56	57.51	0.06	9.73	187.32
X32	Five shoot weight (gms)	365.00±28.04	32.75-1023.75	41.47	62.80	0.44	72.28	570.87
X33	Root/shoot ratio	0.76±0.08	0.24-2.68	59.45	81.02	0.54	115.17	1.44
X34	Harvest index (%)	38.77±1.92	19.25-72.72	30.63	37.79	0.66	65.55	58.60
X35	Phloem to Xylem ratio	0.36±0.02	0.20-0.64	24.53	33.68	0.53	47.16	0.50
Biochemical Traits								
X36	Beta carotenoid (ppm)	317.63±8.31	275.00-420.60	17.76	18.48	0.92	45.08	429.36
X37	Total Sugars (%)	8.88±0.43	6.53-16.45	32.39	34.12	0.90	81.16	14.51
X38	Reducing Sugars (%)	5.29±0.30	2.35-11.77	37.25	40.48	0.85	90.48	9.02
X39	Non-reducing Sugars (%)	3.59±0.28	2.01-9.61	49.36	58.36	0.72	110.23	6.69

Table 9: Mean, Range, and genetic variability estimates for morphological and biochemical traits in Season-II for 48 carrot genotypes

Sl. No.	Characters	Mean ±Sed	Range	PCV	GCV	h^2 (b.s.)	GAM (1 %)	EMG
Morphological (Qualitative Traits)								
X1	Root position in soil (score)	7.29 ± 0.19	3.00-9.00	19.55	16.66	0.73	37.21	9.41
X2	Shoot Attachment (score)	1.08±0.02	1.00-1.40	15.34	3.25	-0.05	-1.83	1.06
X3	Leaf type (score)	2.02±0.05	1.00-3.00	18.79	17.46	0.86	42.94	2.70
X4	Root branching (score)	1.24±0.05	1.00-2.60	40.92	5.27	0.02	2.81	1.27
X5	Root Hairiness (score)	2.05±0.11	1.00-5.15	42.54	32.57	0.59	65.51	3.10
X6	Root cracking (score)	1.12±0.04	1.00-2.50	26.45	18.08	0.47	32.59	1.41
X7	Root tip (score)	1.93 ±0.04	1.00-2.00	12.83	12.56	0.96	32.48	2.42
X8	Root tapering (score)	1.96 ±0.03	1.00-2.00	10.35	7.11	0.47	13.06	2.16
X9	Root texture (score)	2.39 ±0.08	1.00-3.60	31.81	12.74	0.16	14.19	2.66
X10	Root shape (score)	4.86 ±0.04	4.00-5.00	7.05	2.13	0.09	1.78	4.92
X11	Root Shoulder shape (score)	1.80 ±0.07	1.00-3.08	35.21	11.44	0.11	10.41	1.95
X12	White lines (score)	1.23 ±0.04	1.00--1.90	27.12	18.42	0.46	33.27	1.56
X13	Petiole pubescence (score)	1.22 ±0.04	1.00-2.00	26.26	20.94	0.64	44.30	1.64
X14	Root colour (score)	6.71 ±0.35	2.00-14.10	38.84	32.63	0.71	72.70	10.51
X15	Shoulder colour (score)	2.66 ±0.23	1.00-7.70	65.25	52.07	0.64	110.08	4.95
X16	Xylem colour (score)	2.42 ±0.24	1.00-7.00	72.76	65.62	0.81	156.45	5.37
X17	Phloem colour (score)	6.37 ±0.28	1.50-9.00	31.87	28.17	0.78	65.81	9.64
X18	Cambium colour (score)	3.67 ±0.26	1.00-6.70	52.91	44.93	0.72	101.04	6.56
Morphological (Quantitative Traits)								
X19	No of petioles	10.65 ±0.33	6.60-18.70	26.93	14.78	0.30	21.39	12.43
X20	Root width (cm)	1.72 ±0.05	1.14-3.07	20.47	15.62	0.58	31.76	2.15
X21	Shoulder width (cm)	2.35 ±0.07	1.19-3.35	24.29	18.48	0.58	37.46	3.04
X22	Xylem width (cm)	0.98 ±0.03	0.55-1.43	21.42	12.85	0.36	20.74	1.14
X23	Phloem width (cm)	0.34 ±0.01	0.19-0.63	34.77	22.77	0.43	39.55	0.44
X24	Cambium width (cm)	0.40 ±0.04	0.08-1.65	71.62	48.76	0.46	88.84	0.68
X25	Petiole length (cm)	19.56 ±0.60	10.97-31.70	24.07	17.85	0.55	35.31	24.95
X26	Root length (cm)	19.66 ±0.49	12.30-25.20	21.39	11.66	0.30	15.53	22.04
X27	Shoot length (cm)	44.16 ±1.34	17.24-60.70	23.13	18.70	0.65	40.04	57.96
X28	Shoulder length (cm)	1.10 ±0.05	0.48-2.02	37.62	22.85	0.37	36.69	1.41
X29	Root yield(gms)	35.72 ±1.75	14.60-73.02	37.76	29.79	0.62	61.80	52.94
X30	Shoot weight (gms)	45.96 ±2.84	10.00-93.60	46.60	38.52	0.68	81.22	75.09
X31	Five roots weight (gms)	171.09 ±8.97	61.00-387.00	41.05	30.82	0.56	59.20	250.13
X32	Five shoot weight (gms)	224.12 ±14.78	32.00-469.00	50.44	40.37	0.64	82.97	369.22
X33	Root/shoot ratio	0.93 ±0.09	0.40-3.41	68.77	61.89	0.81	147.00	2.01
X34	Harvest index (%)	45.40 ±1.55	27.21-70.11	24.85	22.30	0.81	52.88	64.14
X35	Phloem to Xylem ratio	0.35 ±0.02	0.17-0.66	37.16	22.25	0.36	35.54	0.45
Biochemical traits								
X36	Beta carotenoid (ppm)	347.65 ±5.65	293.98-474.77	12.28	10.13	0.68	22.05	407.45
X37	Total Sugars (%)	11.10 ±0.50	6.84-15.38	32.99	29.75	0.81	70.82	17.23
X38	Reducing Sugars (%)	6.43 ±0.42	4.29-11.77	46.75	43.25	0.86	105.88	11.74
X39	Non-reducing Sugars (%)	4.67 ±0.46	2.04-15.04	72.82	62.13	0.73	140.19	9.77

EMG: Expected mean to next generation

recorded in S-I (3.07cm). Maximum petiole length was recorded in S-II (31.70cm) with the approximately same mean petiole length in both the seasons (~19.0cm).

Highest root length was seen in S-I (41.5 cm) and the mean value for this trait in both the seasons (~19.0cm) was approximately same. For shoot length, S-I recorded maximum (62.75 cm), but again the mean value was on par with each other in both the seasons (44.0 cm). Shoulder length is generally less or absent in temperate (European carrot) carrots than Asiatic/tropical carrots and in our study, absence of shoulder was seen in S-I (0.0 cm), but not in S-II (0.48cm) and the highest shoulder length was recorded in S-II (2.02cm) and the average shoulder length 0.78 cm and 1.10 cm in S-I and S-II respectively.

With respect to the weight of the root and shoots of carrot, in the present study, individual plants root and shoot weight as well as five roots and five shoots weight were recorded. Root to shoot weight ratio, harvest index were calculated from five root/shoot ratio and phloem to xylem ratio was calculated from the width of xylem and phloem again in both the seasons.

Individual root weight was highest in S-I (80.75 g) than S-II (73.02 g) and the average root weight was also high in S-I (40.97g). The trend was same for five roots weight with its average of 202.71g and 171.09 g in S-I and S-II respectively with the highest of five roots weight of 403.75 g in S-I.

Wide range was observed for the weight of root as well as shoot in both the seasons. Phloem to xylem ratio is an important parameter, as phloem contains the reserved food material like carbohydrates, rich in carotenoids and important for consumer point of view. The information about the mean performance of 48 genotypes for all the morphological and biochemical traits is given in table 1 and 2 of Appendix.

4.1.3.2 Biochemical parameters

Among the biochemical parameters, beta carotenoids and sugars were estimated across 48 carrots genotypes in both the seasons. As high as 420.60 ppm of beta carotenoids was shown in S-I (Table 8 and 9) and the highest beta carotenoids content recorded in S-II was 293.98 ppm. The average beta carotenoids were 317.63 ppm and 347.65 ppm respectively in S-I and II with the range of 275.00 ppm to 420.60ppm and

293.98 to 474.77ppm in two seasons respectively. With respect to total sugars, highest % of total sugar was 16.45 % with a minimum of 6.53 % in both the seasons and an average of 8.88% and 11.10 % in S-I and II respectively.

4.1.4 Analysis for genetic variability and heritability

All the thirty nine characters in both S-I and S-II were subjected to analysis for genetic variability components such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) as well as heritability components such as heritability (h^2 in broad sense) and genetic advance (GA). To compare the GA among various traits, genetic advance as percent mean was calculated from GA with the population mean for respective traits (Tables 8 and 9).

During the first season, among the qualitative characters, all most all traits explained moderate to high PCV and GCV except for root shape where the variability components showed lower GCV and PCV.

Although high PCV was recorded among sixteen qualitative characters out of eighteen, but the GCV was moderate or low in most of these characters as shown in table 8. Higher PCV and GCV were observed for some of the qualitative characters such as leaf type, root branching, root hairiness, root tip, xylem, phloem and cambium colour.

High PCV but low GCV was recorded for shoot attachment character. The heritability was moderate for most of these qualitative characters except shoot attachment, root tapering, root shoulder shape and shoulder colour, for them, lower heritability was observed. Only for leaf type, root cracking, root tip and phloem colour, the heritability was high in S-I.

When the genetic advance as percent mean (GAM) was compared among the traits studied and the expected mean to the next generation for important traits, the highest GAM was shown by shoulder length followed by root to shoot ratio. The expected mean of root yield to the next generation for root yield was 37.90 g although its heritability and GAM were lower. Due to higher heritability and GAM for

biochemical traits, the mean improvement to the next generation was also very high for the respective traits (Table 8).

In second season (S-II), with respect to genetic variability or heritability parameters among these eighteen qualitative characters, the trend was not same for few of the characters. For example, for external root colour and internal root colours (xylem, phloem and cambium), and petiole pubescence, both genetic variability parameters (PCV, GCV) as well as heritability components were high in this season.

For other twelve characters, PCV and GCV was moderate to high except for root shape, root tapering, and root branching, wherein, GCV was low for these characters. Among these traits studied, GAM was highest for xylem colour followed by root to shoot ratio. Similar to S-I, the expected mean to the next generation for biochemical traits, leaf type, root colour, root to shoot ratio, harvest index was high as the heritability and GAM was high for these traits in S-II (Table 9).

The analysis for genetic variability and heritability was also done for other twenty one quantitative characters including four biochemical parameters in both the seasons. Most of these morphological characters showed moderate to higher PCV and GCV, except, root width for which although PCB was high but GCV was lower.

With respect to heritable components, most of the morphological characters explained moderate heritability below 60.0% (0.60). Higher heritability was recorded for shoulder width, shoulder length and harvest index. During S-I, out of thirty five morphological characters including 18 qualitative and 17 quantitative characters, highest heritability was recorded for shoulder width (0.763) followed by root tip (0.724) with higher GAM for these traits.

With respect to genetic variability for quantitative characters of morphological data in S-II, all the 17 characters showed moderate to high PCV and GCV as well as heritability. Very high heritability of above 80.0% (0.80) was recorded for root to shoot ratio and harvest index. Overall, the highest heritability among the 35 morphological characters during S-II was for root tip (0.96) followed by shoot attachment (0.86).

4.1.5 Frequency distribution

The present investigation was consisting of eighteen qualitative characters which were recorded based on the scores available in the descriptor and hence, it would be appropriate to subject the data to frequency distribution to know the frequency of various types available in the carrot germplasm selected in the study and their way of expression in S-I and S-II is shown in the Fig. 1 and 2.

The root position in soil in both the seasons ranged from shallow (3.0) to very deep (9.0), but the most of the genotypes were having deeper position (7.0) in the soil observed in both the seasons as shown by the frequency distribution analysis. The number of shoots attached to the root was evaluated as shoot attachment character with either single or multiple attachments and the single type of shoot attachment (1.0) were commonly found in the germplasm in both the seasons (Plate 2). However, few of the genotypes were also having multiple type of shoot attachment.

With respect to leaf type, majority of the genotypes were showing normal leaves of carrot type with the score 2.0. However, the distribution graph indicates both celery (1.0) and fern types (3.0) are also available in the selected carrot genotypes in the study. The three different types of leaf screened in the present study is presented in Plate 3.

In carrot, root branching, root cracking and root hairiness are the undesirable traits which make the carrot genotypes unsuitable for market and consumption, hence, the level of branching, cracking as well as hairiness were analyzed in both the seasons (Plate 4). When compared to S-I, in the second season, very few genotypes showed branching, hairiness and cracking of the roots as shown in the frequency distribution graph. But these three abnormalities were comparatively high in the first season may be due to the soil type, although majority of the genotypes were having normal types.

Type of root tip and tapering may have direct influence on weight of the root and in turn on productivity. Hence, for these characters scoring was done as presence (2.0) or absence (1.0) of root tip and pointed (2.0) or blunt type (1.0) with respect to root tapering.

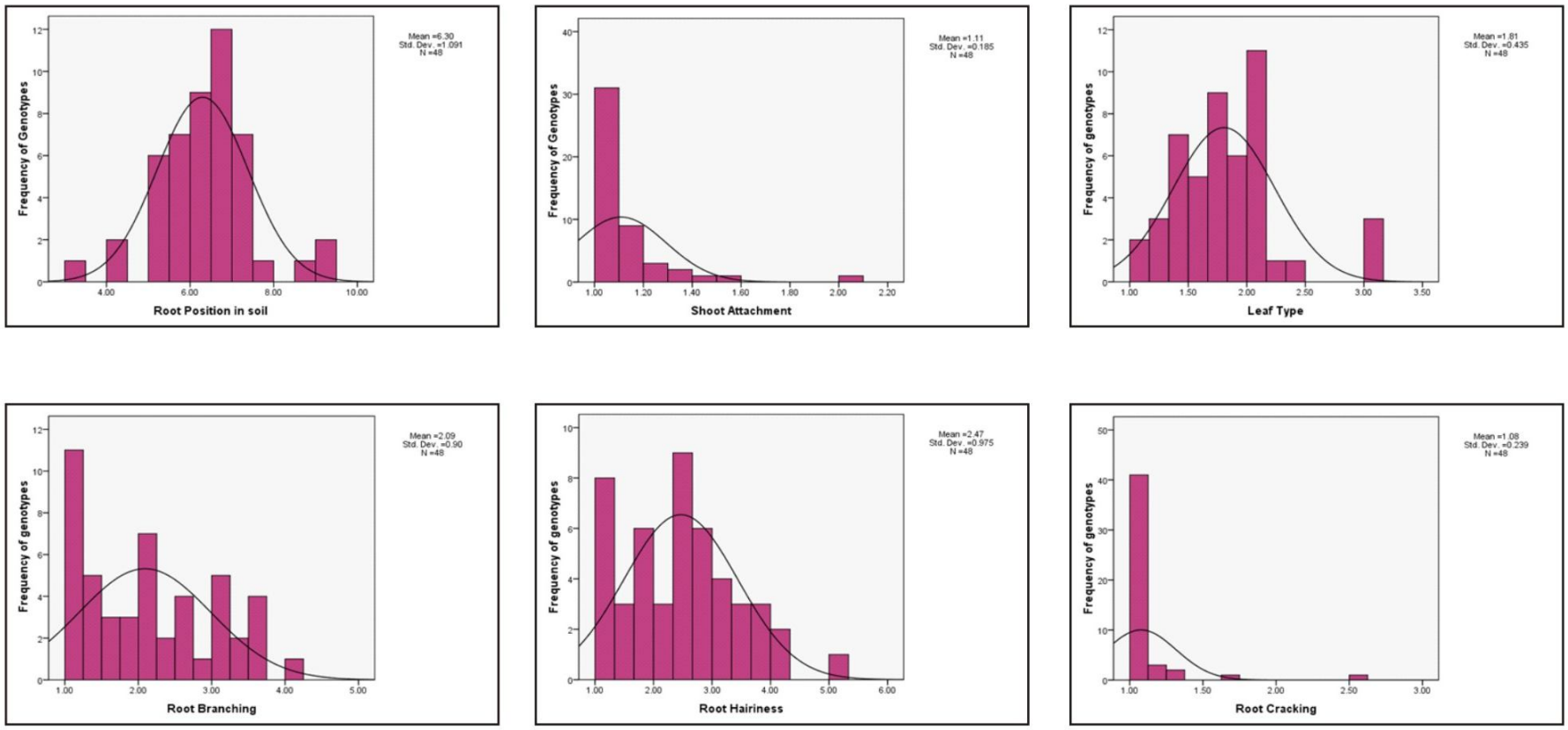


Fig. 1: Frequency distribution for twenty one qualititative characters for root morphology during season-I among the 48 carrot genotypes

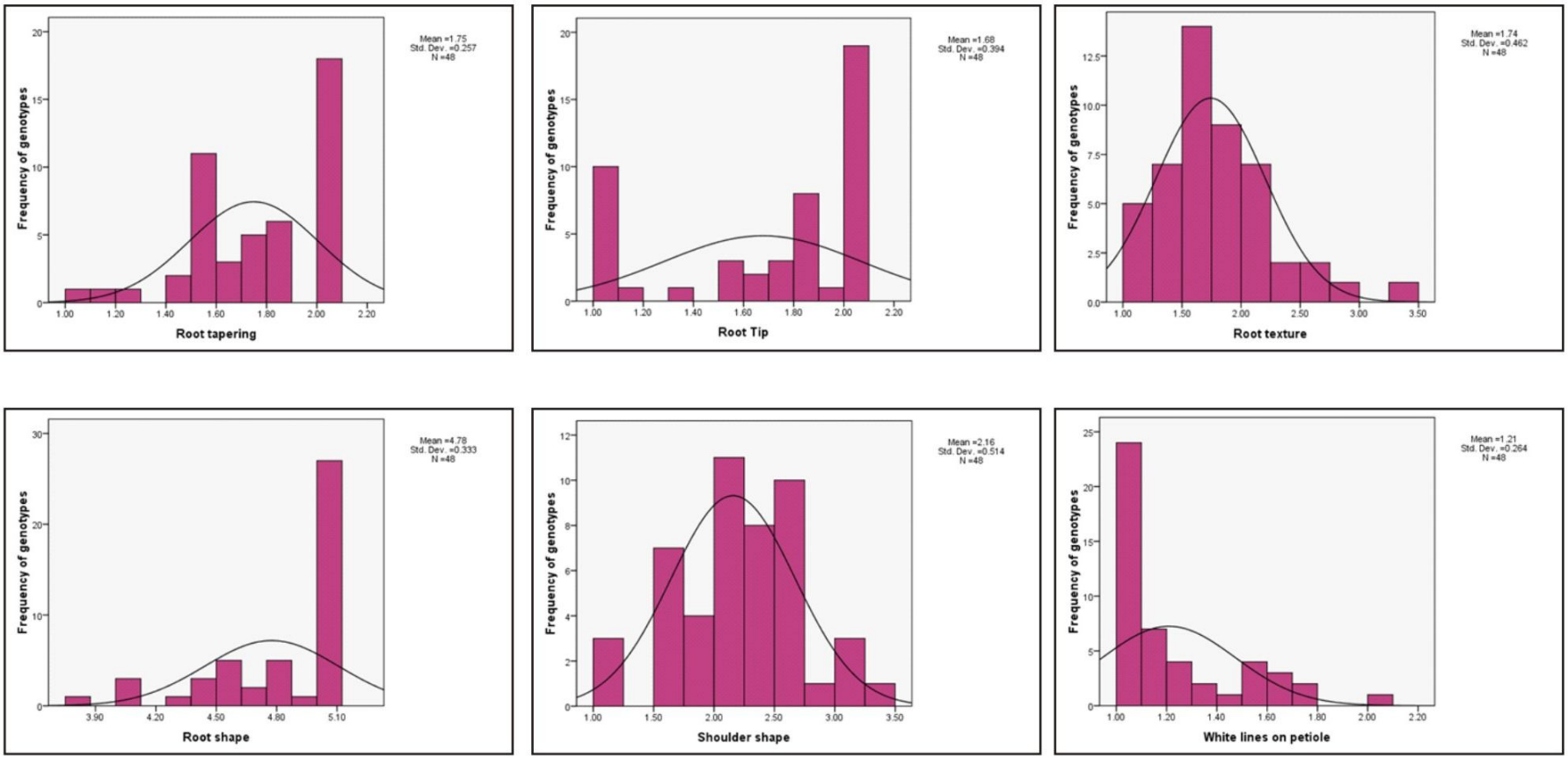

Mean =1.75
Std. Dev. =0.257
N =48
Frequency of genotypes
Root tapering
Mean =1.68
Std. Dev. =0.394
N =48
Frequency of genotypes
Root Tip
Mean =1.74
Std. Dev. =0.462
N =48
Frequency of genotypes
Root texture
Mean =4.78
Std. Dev. =0.333
N =48
Frequency of genotypes
Root shape
Mean =2.16
Std. Dev. =0.514
N =48
Frequency of genotypes
Shoulder shape
Mean =1.21
Std. Dev. =0.264
N =48
Frequency of genotypes
White lines on petiole

Fig. 1: Contd...

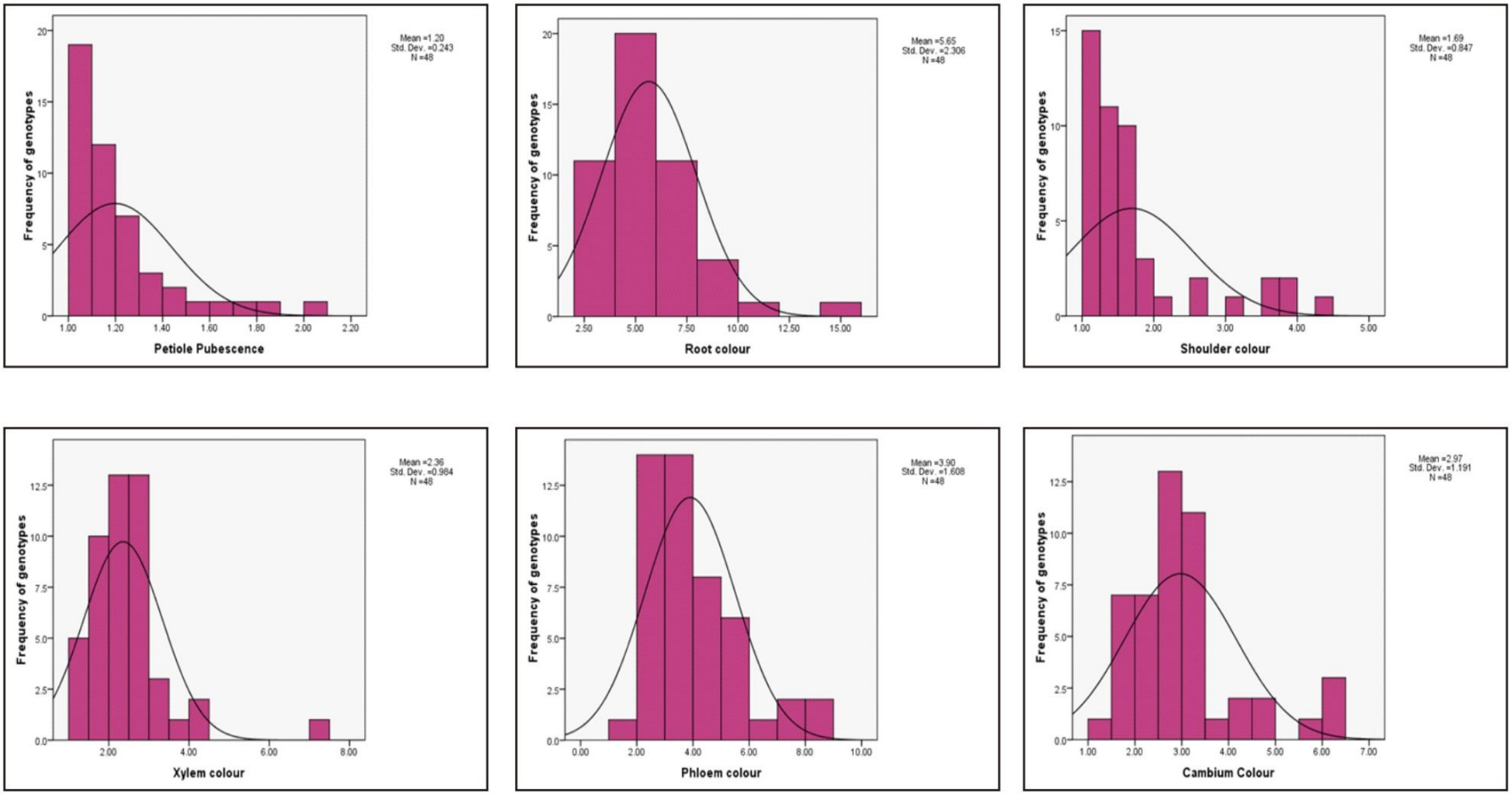
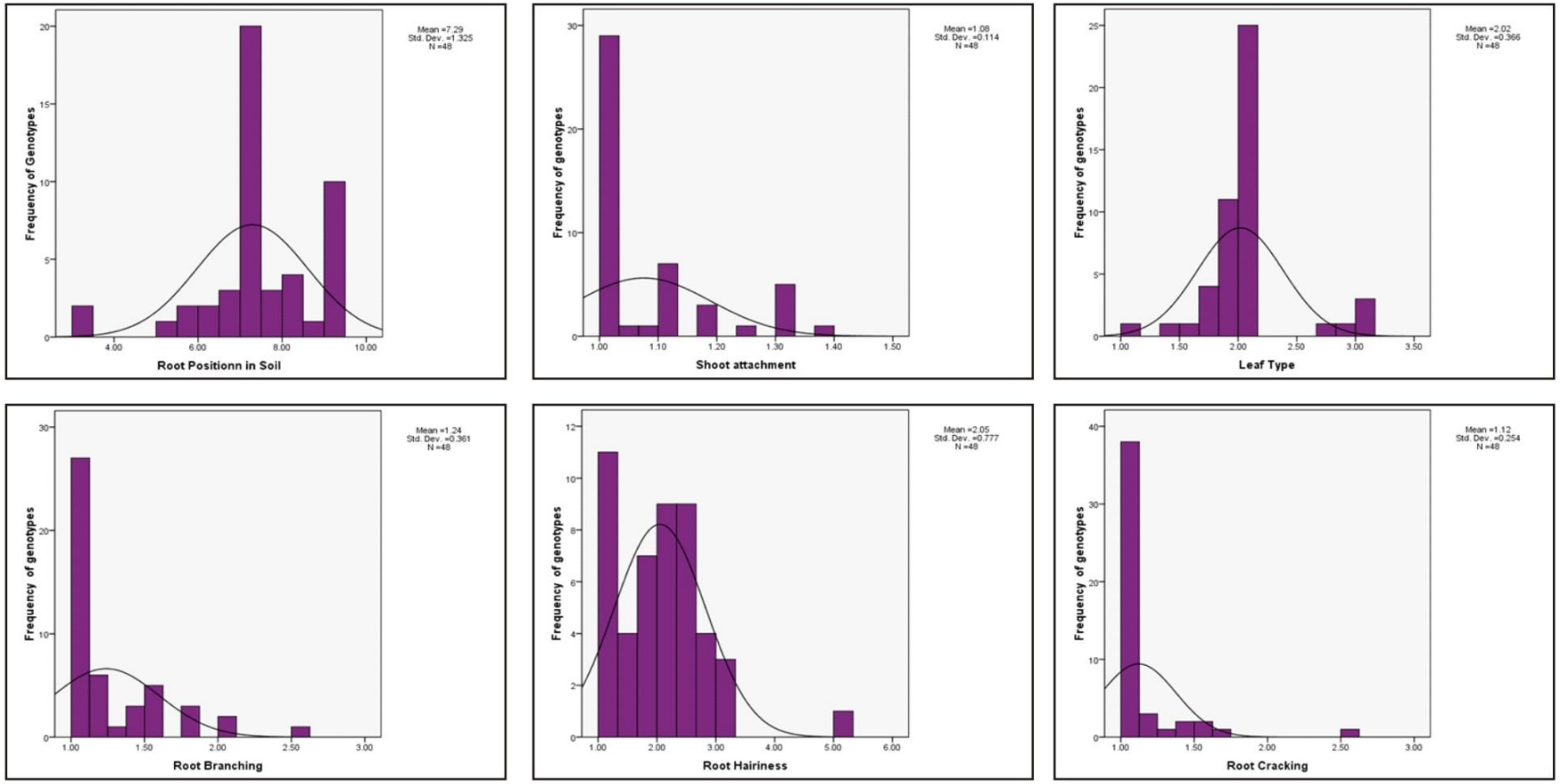

Mean =1.20
Std. Dev. =0.243
N =48
Frequency of genotypes
Petiole Pubescence
Mean =5.65
Std. Dev. =2.306
N =48
Frequency of genotypes
Root colour
Mean =1.69
Std. Dev. =0.847
N =48
Frequency of genotypes
Shoulder colour
Mean =2.36
Std. Dev. =0.984
N =48
Frequency of genotypes
Xylem colour
Mean =3.90
Std. Dev. =1.608
N =48
Frequency of genotypes
Phloem colour
Mean =2.97
Std. Dev. =1.191
N =48
Frequency of genotypes
Cambium Colour

Fig. 1: Contd...



2: Frequency distribution of eighteen qualitative traits in season-II among 48 genotypes of carrot

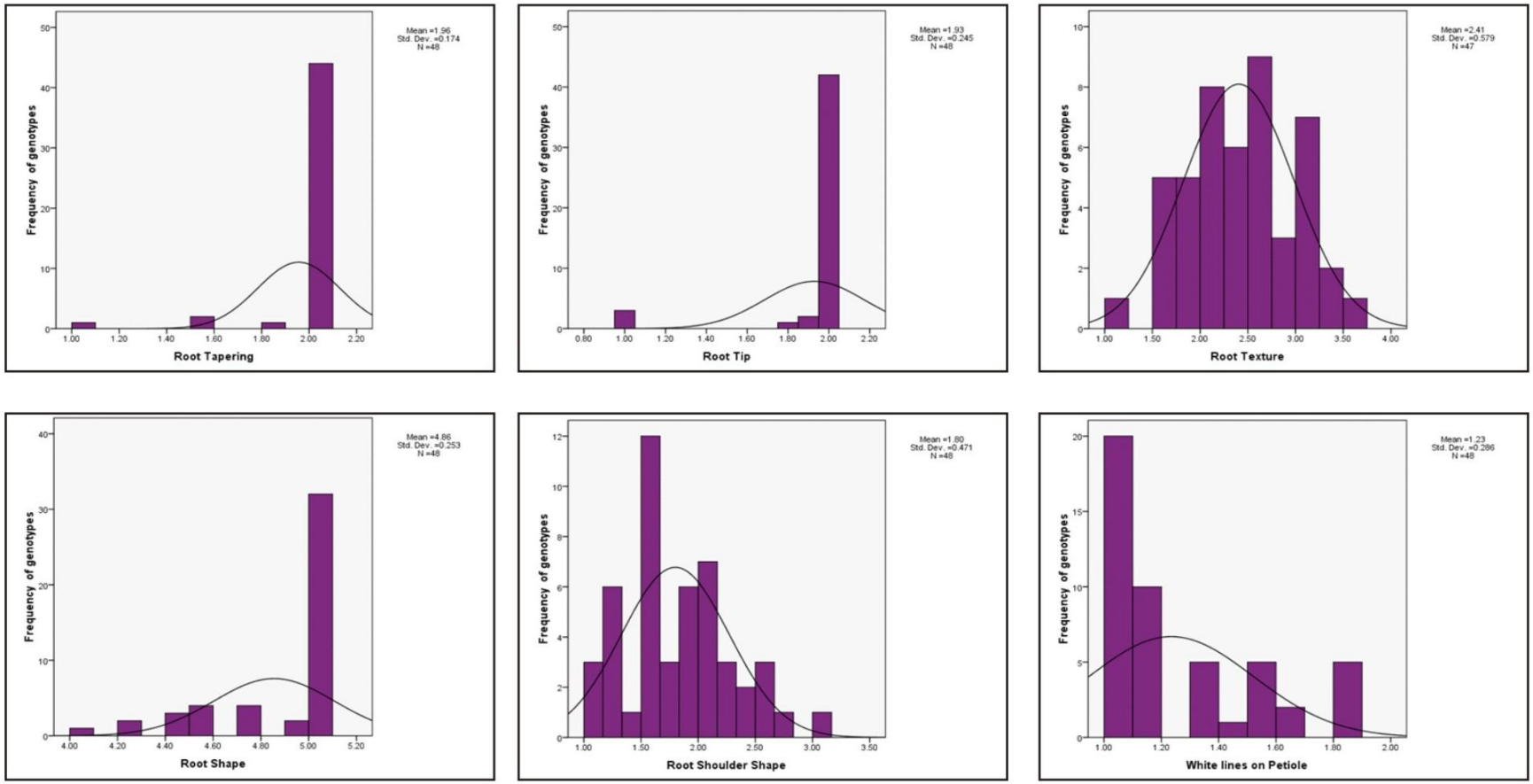

Mean =1.96
Std. Dev. =0.174
N =48
Frequency of genotypes
Root Tapering
Mean =1.93
Std. Dev. =0.245
N =48
Frequency of genotypes
Root Tip
Mean =2.41
Std. Dev. =0.579
N =47
Frequency of genotypes
Root Texture
Mean =4.86
Std. Dev. =0.253
N =48
Frequency of genotypes
Root Shape
Mean =1.80
Std. Dev. =0.471
N =48
Frequency of genotypes
Root Shoulder Shape
Mean =1.23
Std. Dev. =0.286
N =48
Frequency of genotypes
White lines on Petiole

Fig. 2: Contd...

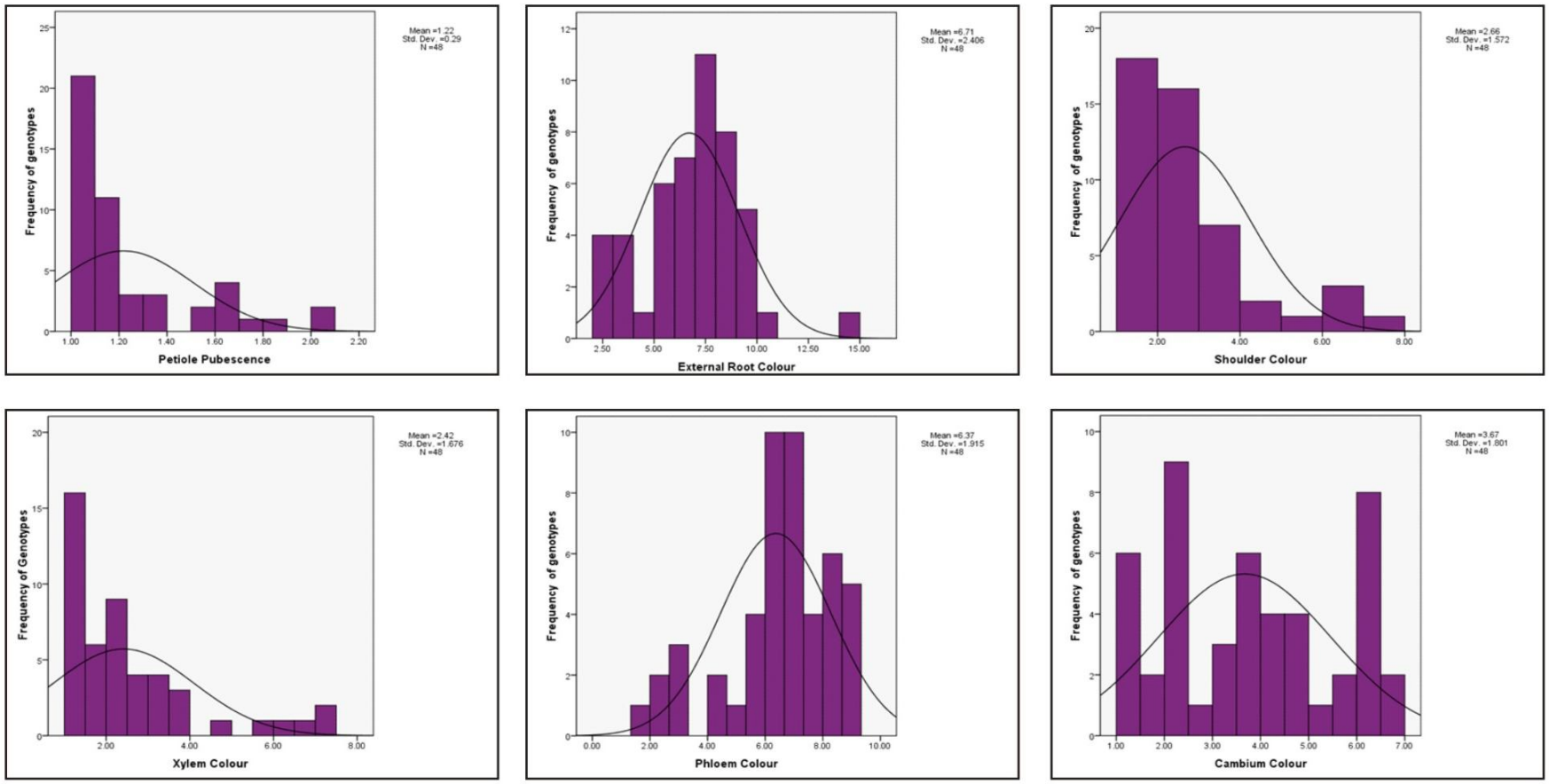

Mean =1.22
Std. Dev. =0.29
N =48
Frequency of genotypes
Petiole Pubescence
Mean =6.71
Std. Dev. =2.406
N =48
Frequency of genotypes
External Root Colour
Mean =2.66
Std. Dev. =1.572
N =48
Frequency of genotypes
Shoulder Colour
Mean =2.42
Std. Dev. =1.676
N =48
Frequency of Genotypes
Xylem Colour
Mean =6.37
Std. Dev. =1.915
N =48
Frequency of genotypes
Phloem Colour
Mean =3.67
Std. Dev. =1.801
N =48
Frequency of genotypes
Cambium Colour

Fig. 2: Contd...

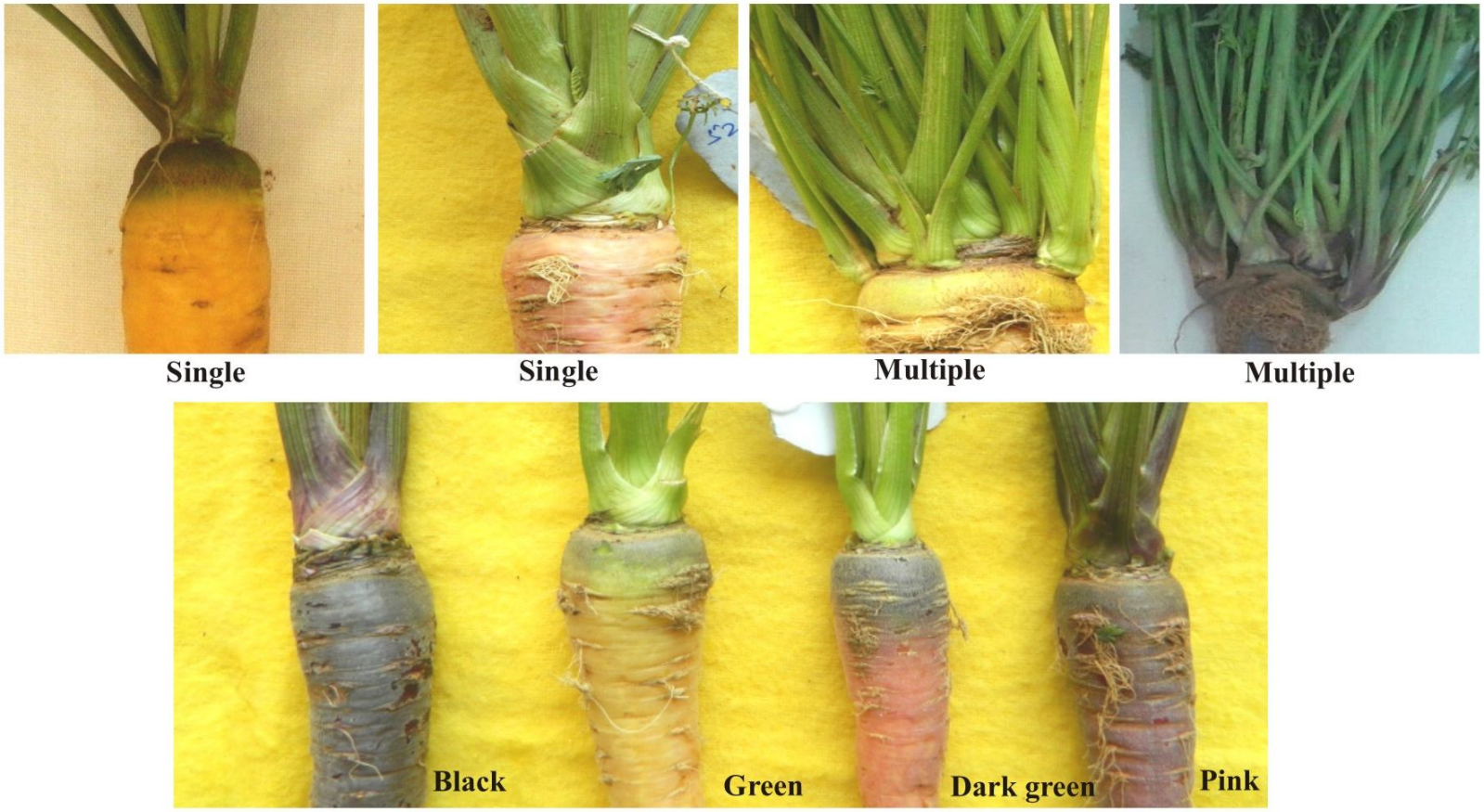


Plate 2: Genetic variation for shoot attachment and shoulder colour in carrot for 48 genotypes

Normal

Celery

Fern

White lines on petiole

Pubesence on petiole

Plate 3: Genetic variation for leaf type, white lines and pubesence on leaf petiole in carrot for 48 genotypes

Hairiness

Hairiness

Branching

Cracking

Plate 4: Abnormalities like root hairiness, branching and cracking in carrot for 48 genotypes

Variation in shape

Variation in root and shoot length

Plate 5: Genetic variation for root shape, root and shoot length in carrot for 48 genotypes

Maximum genotypes showed presence of root tip and pointed tapering, although both the types were present in the present study in both the seasons. Maximum genotypes showed pointed type of roots in S-II compared to S-I. The type and hardiness of the soil may have influence on the pointed or blunt type of roots with presence or absence of root tip as they will be lost during harvesting if, the soil is hard leading to more number of broken carrots than complete ones though the character is controlled genetically.

Root texture indicating the surface of the root, is another important quality character which influences the palatability of carrot for consumption. But, coarse textured roots are not preferred for consumption. In the present study, ranging from smooth (score 1.0) and ridged (4.0) types, coarse (2.0) and dimpled (3.0) were observed in the genotypes selected for the study in both the seasons. Very few extreme types (smooth or ridged) for the root texture were found in both the seasons, but most commonly found were coarse textured (2.0) to dimpled (3.0) types as shown by the frequency distribution (Plate 5).

In carrot although, many root shapes are available *viz.*, round, obovate, obtriangular, oblong, tapering *etc* and for shoulder shapes we can find flat, flat to rounded, rounded, rounded to conical, conical *etc* in descriptors, but we did not find any of the conical shaped shoulders, but other shapes were available in genotypes selected for the study (Plate 2).

Majority of the root shapes were of tapering types (score 5.0) in both the seasons as shown by its skewed distribution towards the tapering side. However, the variation was present for both root shape and shoulder shape in the genotypes as shown by the distribution curve for different shapes (Plate 6).

When the petioles were observed carefully, we could find the white lines on them in few genotypes but not in others, hence, to know the influence of this on the economic traits, the observation was recorded as presence (2.0) or absence (1.0) of white line on the petioles in both the seasons. Majority of the genotypes showed absence of white lines although the lines were present in few genotypes in both the seasons (Plate 2).

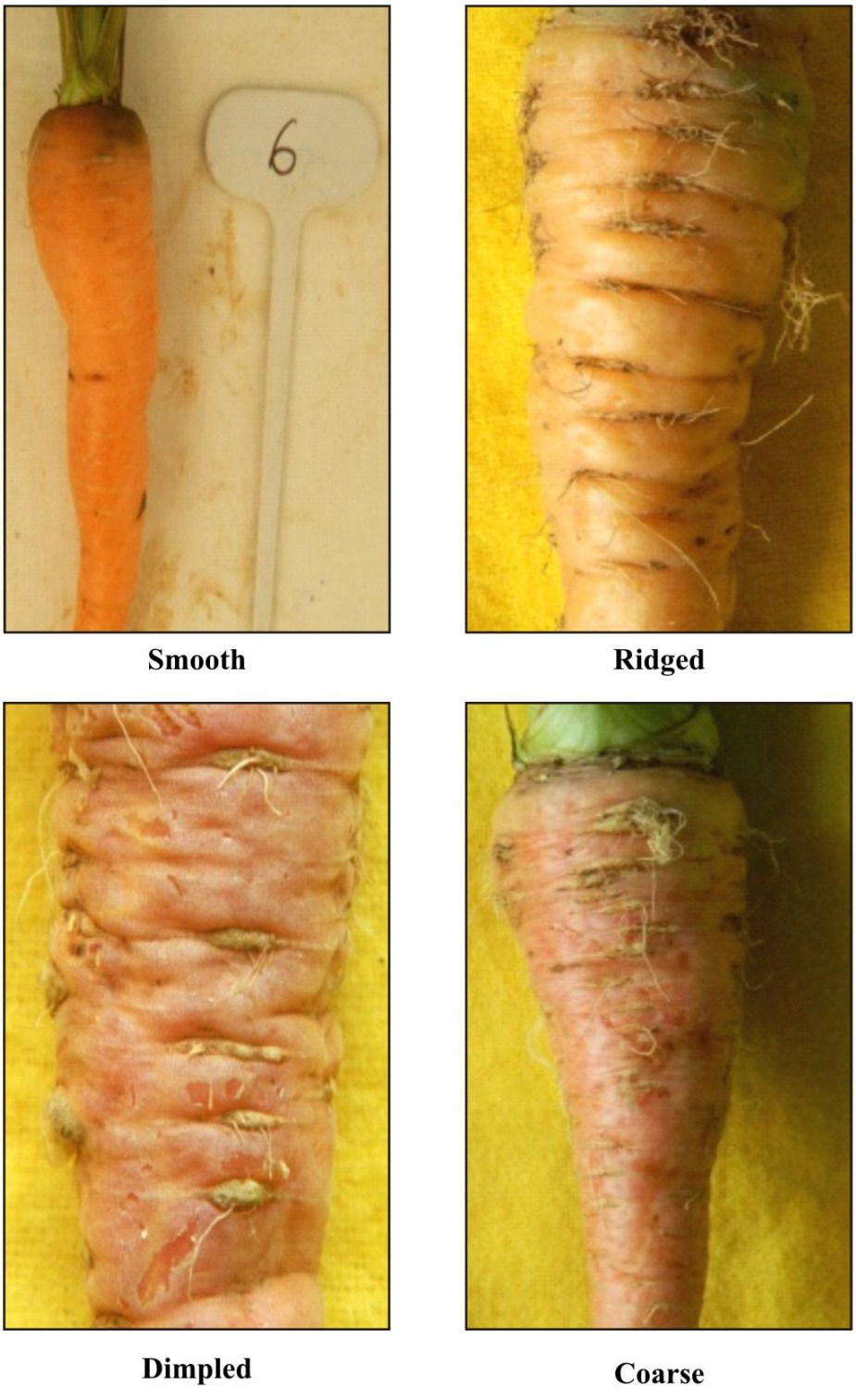


Plate 5: Genetic variation for root texture, hairiness and root branching in carrot

Pubescence on the leaf and petioles is another interesting character which could play an indirect role on the insect resistance. Hence, the observations were recorded for the 48 genotypes utilized for the present investigation and scoring was given as presence or absence in both the seasons. Very few genotypes were showing presence of pubescence but majority of the genotypes were not having the pubescence on leaf and petioles.

Orange coloured carrots are most commonly grown and consumed in many of regions of the world, though various colours such as white, yellow, red, purple, black, pink etc are also available. In the present study, detailed evaluation was carried out among 48 genotypes for internal root colour (xylem, phloem, and cambium), external root colour as well as shoulder colour across the seasons (Plate 7 and 8).

Though, in descriptor, only 4-5 types of colours are available, but in the present study, there was larger variation and many different colours were expressed in both the seasons. Hence, with respect to the root colours, we followed our own descriptor and the details are presented in material and methods chapter.

Majority of the carrots selected for the present study were more of orange types, but the germplasm also comprised of white, yellow, red, pink, purple, black and in different combinations of these colours like yellowish orange, deep or light orange/red/purple/black etc. The distribution for external root colour across the genotypes was normal and hence found all the different coloured carrots in both the seasons.

Uniformity in external and internal roots is an important breeding objective in carrot; hence, the observations of vascular tissues such as xylem, phloem and cambium colours were also recorded in both the seasons. In S-I, xylem and cambium colour were showing skewed distribution towards white colour (with score 1.0-3.0) but in phloem majority of the genotypes shown orange colour. Very few genotypes, both external and internal root colours were same like white-white, red-red, orange-orange, black-black *etc* were shown. In S-II, except cambium colour, the distribution of xylem and phloem were skewed, but for cambium colour it was showing normal distribution ranging from white (1.0) to black (7.0) (Plate 9).

Plate 7: Genetic variation for external root colour in carrot for 48 genotypes

Plate 8: Genetic variation for external root colour in carrot for 48 genotypes

Plate 9: Genetic variation for internal root xylem and phloem colour in carrot for 48 genotypes

4.1.6 Principle component analysis (PCA)

As per the rules of PCA, only quantitative data can be subjected to principle component analysis. Hence, in the present investigation, only twenty characters including four biochemical parameters and seventeen morphological characters for both the seasons.

During S-I, a total variation was partitioned in to seven principle components with the cumulative variation of 83.20%. The first component explained 28.27 % of variation. The first two components explained the variation with >3.0 Eigen values and other 4 components were showing >1.0 Eigen value. The eigen values for the respective principle components in each season is presented in screen plot (Figures 3 and 4).

The components with Eigen value (<1.0) were ignored as per Guttmann's lower bound principle (Kaiser, 1958). First three principle components explained up to >50.0 of the total variation. All the seven components were retained in the analysis because of the substantial amount of variation explained by these six components (Table 10 and 11).

Further, based on the component matrix of PCA analysis from the extraction method, the first component had the combination of 14 characters (Tables 12 and 13) out of which 10 characters had positive loadings, with the maximum positive loading was observed for five shoot weight and individual plant shoot weight with >0.90 loadings.

Second principle component was the combination of 10 characters with all the characters showing positive loadings, out of which characters such as shoot length, shoulder width root weight, five root weight, root width and xylem width were common for first two components with positive loadings. In the third principle components, it was consisting of all the four biochemical parameters with positive loadings.

In the second season also, there was a common trend of the total principle components extracted, with Eigen values and the combination of different characters in first three components. Here, first principle components explained up to 34.31 % of the total variation and the total of 83.60% of cumulative variation from six components.

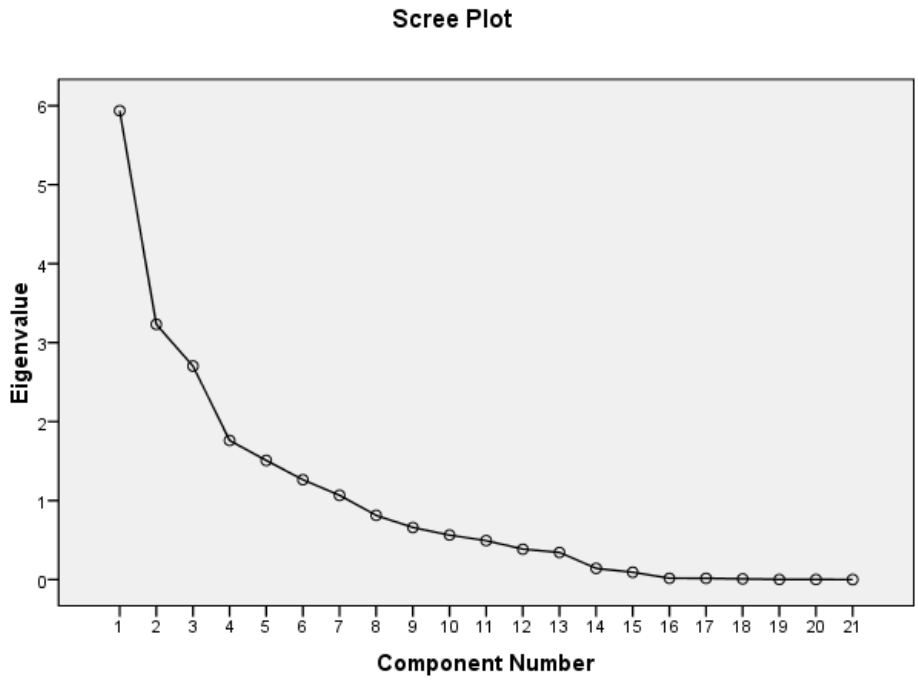


Fig. 3: Screen plot showing number of principle components and respective Eigen Values for the 21 quantitative traits by PCA analysis in S-I for 48 genotypes of carrot

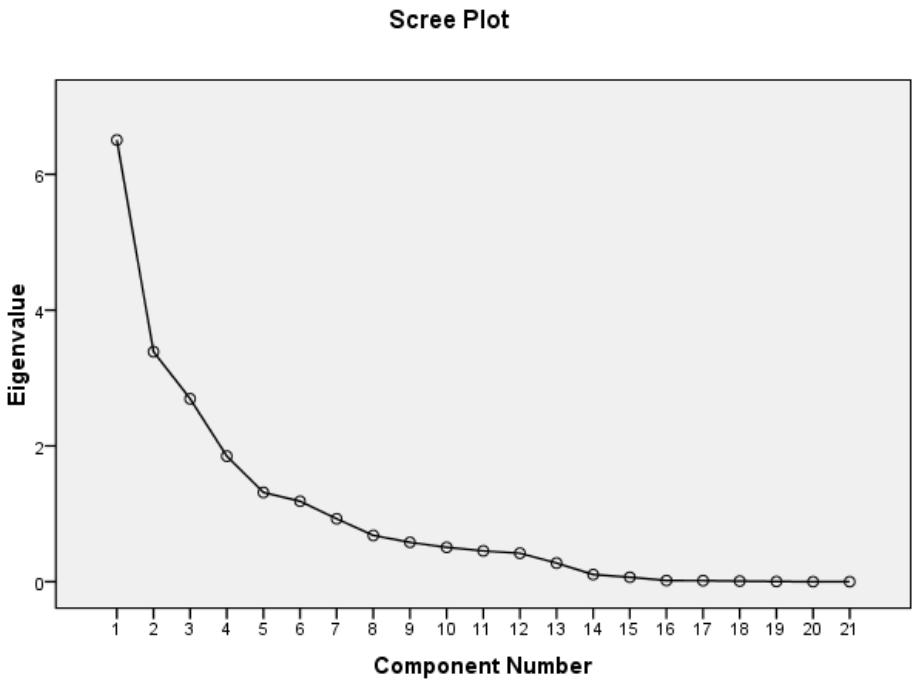


Fig. 4: Screen plot showing number of principle components and respective Eigen Values for the 21 quantitative traits by PCA analysis pooled over seasons for 48 genotypes of carrot

Table 10: Principle component analysis (PCA) for 21 quantitative traits in Season-I for 48 carrot genotypes

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	5.94	28.27	28.27	5.94	28.27	28.27	4.82	22.95	22.95
2	3.23	15.39	43.66	3.23	15.39	43.66	2.47	11.76	34.71
3	2.70	12.87	56.54	2.70	12.87	56.54	2.33	11.10	45.81
4	1.76	8.39	64.92	1.76	8.39	64.92	2.24	10.68	56.49
5	1.51	7.18	72.10	1.51	7.18	72.10	2.16	10.27	66.77
6	1.26	6.02	78.12	1.26	6.02	78.12	1.77	8.44	75.20
7	1.07	5.08	83.20	1.07	5.08	83.20	1.68	8.00	83.20
8	0.81	3.87	87.07						
9	0.66	3.13	90.20						
10	0.56	2.68	92.88						
11	0.49	2.34	95.23						
12	0.38	1.83	97.06						
13	0.34	1.63	98.70						
14	0.14	0.67	99.37						
15	0.09	0.44	99.81						
16	0.02	0.08	99.88						
17	0.02	0.07	99.96						
18	0.01	0.04	100.00						
19	0.00	0.00	100.00						
20	0.00	0.00	100.00						
21	0.00	0.00	100.00						

Table 11: Principle component analysis (PCA) for 21 quantitative traits in Season-II for 48 carrot genotypes

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	7.21	34.31	34.31	7.21	34.31	34.31	6.23	29.65	22.95
2	3.35	15.96	50.28	3.35	15.96	50.28	3.71	17.69	40.64
3	2.58	12.28	62.56	2.58	12.28	62.56	2.10	9.98	50.62
4	1.82	8.65	71.21	1.82	8.65	71.21	1.97	9.38	60.00
5	1.56	7.43	78.63	1.56	7.43	78.63	1.78	8.50	68.50
6	1.04	4.96	83.60	1.04	4.96	83.60	1.76	8.40	76.90
7	0.89	4.26	87.85						
8	0.76	3.62	91.48						
9	0.51	2.45	93.93						
10	0.32	1.52	95.45						
11	0.28	1.32	96.77						
12	0.25	1.18	97.95						
13	0.15	0.69	98.64						
14	0.12	0.55	99.19						
15	0.10	0.46	99.66						
16	0.04	0.19	99.84						
17	0.02	0.09	99.93						
18	0.01	0.05	99.98						
19	0.00	0.02	100.00						
20	0.00	0.00	100.00						
21	0.00	0.00	100.00						

Table 12: Component Matrix in PCA analysis for 21 quantitative traits in Season-II for 48 carrot genotypes

Component Matrix							
Traits	**Components**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Five shoot weight	0.929						
Shoot weight	0.926						
Root to Shoot ratio	-0.749	0.401					
No. of Petioles	0.723						
Harvest index	-0.72	0.574					
Shoot length	0.677	0.301			-0.474		
Shoulder width	0.582	0.433	-0.404				
Petiole length	0.554				-0.421		0.511
Phloem to Xylem Ratio	-0.452	0.415	-0.359	0.339		0.352	0.403
Phloem Width		0.805				0.302	
Root weight	0.53	0.591			0.348	-0.338	
Five root weight	0.558	0.585			0.363	-0.324	
Root width	0.394	0.573		-0.475		0.41	
Xylem Width	0.519	0.521			-0.326		-0.441
Total sugars			0.904				
Reducing sugars	-0.314		0.739	-0.304			
Beta carotenoid			0.428		0.39		
Cambium Width				-0.676	0.373	0.468	
Non-reducing sugars			0.591	0.601			
Root length			0.469	0.488			-0.462
Shoulder length	-0.33	0.427			-0.433		

Table 13: Component Matrix (Loadings) in PCA analysis for 21 quantitative traits in Season-II for 48 carrot genotypes

Component Matrix						
	1	**2**	**3**	**4**	**5**	**6**
Five shoot weight	0.891					
Shoot weight	0.886					
Root weight	0.839	0.42				
Shoulder Width	0.839					
Five root weight	0.837	0.401				
Shoot length	0.834					
Petiole Length	0.788					
Xylem Width	0.708	0.359				-0.356
Root width	0.678	0.309	0.432			0.32
Root length	0.613			0.44		
Harvest Index	-0.416	0.836				
Root to shoot ratio	-0.517	0.655				
Beta carotenoids	-0.372	0.559	0.417			
Cambium width			0.7			0.545
Phloem to xylem ratio	-0.309	0.379	-0.635			0.481
Phloem width		0.566	-0.626		0.317	
No of petioles			0.557	-0.475	-0.325	
Reducing sugars		-0.52		0.659		
Shoulder length		0.444		0.469		
Total sugars		-0.421	0.389		0.791	
Non reducing sugars			0.462	-0.578	0.614	

The first two components showed Eigen value of >3.0 and all the six components extracted showed >1.0 initial Eigen value hence, all the six components were considered, with respect to the combination of characters contributed with positive or negative loadings, the results were same in both the seasons with few exceptions like, root length in S-II with positive loading and beta carotenoids with negative loading, instead of reducing sugars in S-I having negative loading.

Five root weight, shoot weight, root weight, shoulder width, five root weight and shoot length had the loadings of >0.800 in second season for first principle component. For second component, the total variation was explained with 12 combinations of characters with nine characters showing positive loading and other three showed negative values. Root width was frequently observed in first three components with positive loadings. Beta carotenoids and phloem to xylem ratio were also contributed for variation in first three components.

4.1.7 Correlation analysis

In the present study, although a total of 39 characters were evaluated for the 48 genotypes in two seasons, but only the 21 quantitative traits which include 17 morphological and four biochemical traits were subjected for detailed correlation studies in S-I and S-II seasons. The tables from 14 and 15 represent the phenotypic correlation for 21 quantitative characters in S-I and S-II seasons respectively.

In general, the pattern of correlation (positive or negative) was consistently same across two seasons, but, there was a variation for the strength of correlation between two seasons. Among the morphological characters, strong positive correlation was seen for number of petioles with petiole length, shoot weight (single and five shoot weight) and root weight as well, but there was a negative correlation for this trait (no of petioles) with harvest index, root to shoot ratio.

Root weight or five root weight are the important yield parameter of carrot, which were positively correlated with root width, shoulder width, xylem width, shoot length, shoot weight in both o of petiole, five shoot weight and root length.

There was no significant negative correlation was found for root weight with any of the characters. Harvest index is another important trait which decides the economic yield of carrot, showed strong negative correlation with number of petioles (S-I), shoot length, shoot weight, five shoot weight (both S-I ad S-II) and a strong positive correlation was seen with root to shoot ratio and shoulder length in both the seasons.

There was a strong positive correlation between root length and shoot length, total sugars and reducing or non reducing sugars, petiole length and shoot length, root width with shoulder width, cambium width, xylem width and phloem width.

Similarly, strong negative correlation was also found between root width and xylem phloem cambium width, xylem width and shoots length, petiole length and shoots length, root weight, shoot weights etc. The correlation between other characters is presented in Tables 14 and 15.

In general, harvest index showed negative correlation with number of petioles, petiole length, shoot length as well as five shoots weight and strong positive correlation with phloem to xylem ratio and shoulder length. Root yield is positively correlated with five roots weight, root width, shoulder width, xylem width, shoot weight, shoot length, five shoot weight in S-I. Along with these characters, root length (S-II), phloem width, and petiole length in S-II.

Among the biochemical parameters, no significant correlation was found between carotenoids and sugars, but among the sugars, total sugars was strong positive correlation with reducing sugars and non-reducing sugars, but there was no correlation between reducing and non-reducing sugars (SII) and negative correlation in S-II (Plate 10).

4.1.8 Path coefficient analysis

The same twenty one quantitative characters which were utilized for correlation analyses were also used for phenotypic path coefficient analyses and presented in tables 16 and 17. The root weight was selected as dependent character to know the direct and

Table 14: Pearson's Correlation Coefficient among 21 quantitative (morphological and biochemical) traits in Season I for 48 carrot genotypes

Traits	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39
X19	1.000																				
X20	0.094^{NS}	1.000																			
X21	0.242^{NS}	0.393**	1.000																		
X22	0.170^{NS}	0.685**	0.398**	1.000																	
X23	-0.168^{NS}	0.406**	0.433**	0.372**	1.000																
X24	0.041^{NS}	0.574**	-0.010^{NS}	-0.136^{NS}	-0.204^{NS}	1.000															
X25	0.362*	0.134^{NS}	0.320*	0.302*	0.070^{NS}	-0.123^{NS}	1.000														
X26	0.200^{NS}	0.055^{NS}	-0.052^{NS}	0.205^{NS}	0.021^{NS}	-0.119^{NS}	-0.015^{NS}	1.000													
X27	0.200^{NS}	0.397**	0.458**	0.543**	0.148^{NS}	-0.016^{NS}	0.659**	-0.034^{NS}	1.000												
X28	-0.335*	0.062^{NS}	-0.084^{NS}	0.122^{NS}	0.224^{NS}	-0.120^{NS}	-0.039^{NS}	-0.071^{NS}	0.087^{NS}	1.000											
X29	0.328*	0.418**	0.416**	0.421**	0.253^{NS}	0.079^{NS}	0.233^{NS}	0.141^{NS}	0.445**	-0.000^{NS}	1.000										
X30	0.829**	0.184^{NS}	0.470**	0.296*	-0.154^{NS}	0.040^{NS}	0.446**	0.207^{NS}	0.466**	-0.375**	0.375**	1.000									
X31	0.331*	0.416**	0.467**	0.426**	0.276^{NS}	0.063^{NS}	0.232^{NS}	0.131^{NS}	0.445**	-0.031^{NS}	0.987**	0.399**	1.000								
X32	0.830**	0.181^{NS}	0.470**	0.293*	-0.143^{NS}	0.034^{NS}	0.438**	0.205^{NS}	0.463**	-0.382**	0.373**	0.995**	0.408**	1.000							
X33	-0.440**	-0.172^{NS}	-0.229^{NS}	-0.208^{NS}	0.264^{NS}	-0.154^{NS}	-0.331*	-0.167^{NS}	-0.518**	0.343*	-0.080^{NS}	-0.709**	-0.103^{NS}	-0.713**	1.000						
X34	-0.503**	-0.008^{NS}	-0.257^{NS}	-0.091^{NS}	0.371**	-0.080^{NS}	-0.332*	-0.096^{NS}	-0.383**	0.414**	0.093^{NS}	-0.777**	0.056^{NS}	-0.776**	0.918**	1.000					
X35	-0.308*	-0.102^{NS}	0.131^{NS}	-0.371**	0.695**	-0.090^{NS}	-0.177^{NS}	-0.169^{NS}	-0.263^{NS}	0.148^{NS}	-0.106^{NS}	-0.399**	-0.085^{NS}	-0.387**	0.468**	0.471**	1.000				
X36	0.173^{NS}	0.044^{NS}	-0.155^{NS}	-0.032^{NS}	-0.139^{NS}	0.150^{NS}	0.040^{NS}	0.108^{NS}	-0.128^{NS}	-0.053^{NS}	0.131^{NS}	0.038^{NS}	0.091^{NS}	0.033^{NS}	0.057^{NS}	0.076^{NS}	-0.170^{NS}	1.000			
X37	0.000^{NS}	0.090^{NS}	-0.351*	-0.003^{NS}	-0.053^{NS}	0.149^{NS}	0.044^{NS}	0.345*	-0.074^{NS}	0.192^{NS}	0.041^{NS}	-0.112^{NS}	-0.059^{NS}	-0.166^{NS}	0.107^{NS}	0.200^{NS}	-0.075^{NS}	0.215^{NS}	1.000		
X38	-0.190^{NS}	0.101^{NS}	-0.467**	-0.078^{NS}	-0.167^{NS}	0.281^{NS}	0.021^{NS}	0.070^{NS}	-0.101^{NS}	0.211^{NS}	-0.033^{NS}	-0.294*	-0.120^{NS}	-0.338*	0.194^{NS}	0.270^{NS}	-0.147^{NS}	0.216^{NS}	0.756**	1.000	
X39	0.202^{NS}	0.030^{NS}	-0.040^{NS}	0.078^{NS}	0.097^{NS}	-0.070^{NS}	0.045^{NS}	0.451**	-0.005^{NS}	0.069^{NS}	0.097^{NS}	0.142^{NS}	0.038^{NS}	0.106^{NS}	-0.042^{NS}	0.018^{NS}	0.041^{NS}	0.098^{NS}	0.720**	0.090^{NS}	1.000

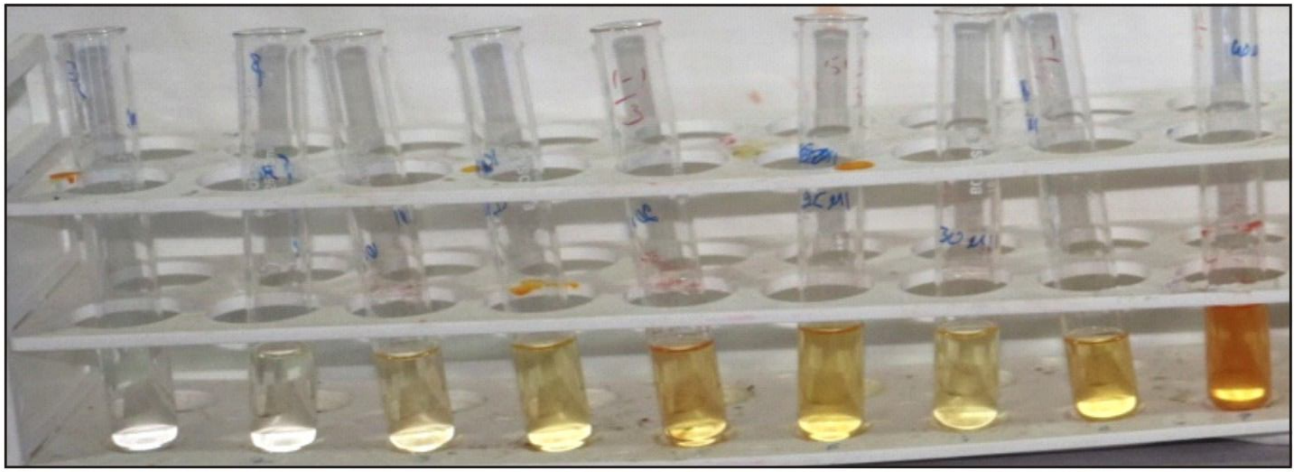
Ns: Non significant * Probability at 5%, **1% level of probability, * The descriptions for traits name of X19 to X39 is presented in table 2 of the materials and methods chapter.

Table 15: Pearson's Correlation Coefficient among 21 quantitative traits in Season II for 48 carrot genotypes

Traits	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39
X19	1.000																				
X20	-0.039^{NS}	1.000																			
X21	-0.142^{NS}	0.446**	1.000																		
X22	-0.200^{NS}	0.693**	0.638**	1.000																	
X23	-0.409**	0.162^{NS}	0.377**	0.237^{NS}	1.000																
X24	0.254^{NS}	0.716**	-0.033^{NS}	0.080^{NS}	-0.364*	1.000															
X25	-0.339*	0.435**	0.744**	0.546**	0.188^{NS}	0.093^{NS}	1.000														
X26	-0.098^{NS}	0.501**	0.479**	0.414**	-0.011^{NS}	0.351*	0.561**	1.000													
X27	-0.064^{NS}	0.389**	0.728**	0.393**	0.084^{NS}	0.182^{NS}	0.761**	0.534**	1.000												
X28	-0.083^{NS}	0.313*	0.270^{NS}	0.297*	0.081^{NS}	0.157^{NS}	0.305*	0.309*	-0.031^{NS}	1.000											
X29	-0.054^{NS}	0.687**	0.784**	0.718**	0.387**	0.210^{NS}	0.569**	0.474**	0.554**	0.353*	1.000										
X30	-0.025^{NS}	0.472**	0.673**	0.515**	0.102^{NS}	0.194^{NS}	0.561**	0.347*	0.748**	0.005^{NS}	0.694**	1.000									
X31	-0.031^{NS}	0.654**	0.750**	0.667**	0.387**	0.204^{NS}	0.530**	0.506**	0.549**	0.328*	0.956**	0.718**	1.000								
X32	-0.013^{NS}	0.456**	0.667**	0.494**	0.127^{NS}	0.178^{NS}	0.589**	0.400**	0.744**	-0.013^{NS}	0.704**	0.964**	0.760**	1.000							
X33	-0.046^{NS}	-0.106^{NS}	-0.346*	-0.029^{NS}	0.061^{NS}	-0.139^{NS}	-0.345*	-0.096^{NS}	-0.649**	0.231^{NS}	-0.172^{NS}	-0.631**	-0.161^{NS}	-0.628**	1.000						
X34	-0.027^{NS}	0.025^{NS}	-0.173^{NS}	0.023^{NS}	0.243^{NS}	-0.081^{NS}	-0.238^{NS}	-0.023^{NS}	-0.546**	0.306*	0.028^{NS}	-0.648**	-0.032^{NS}	-0.606**	0.851**	1.000					
X35	-0.257^{NS}	-0.244^{NS}	-0.085^{NS}	-0.376**	0.785**	-0.360*	-0.218^{NS}	-0.304*	-0.233^{NS}	-0.116^{NS}	-0.107^{NS}	-0.293*	-0.086^{NS}	-0.259^{NS}	0.164^{NS}	0.304*	1.000				
X36	0.380**	-0.000^{NS}	-0.190^{NS}	-0.137^{NS}	0.018^{NS}	0.089^{NS}	-0.350*	-0.133^{NS}	-0.366*	0.231^{NS}	-0.018^{NS}	-0.434**	-0.067^{NS}	-0.450**	0.443**	0.628**	0.135^{NS}	1.000			
X37	0.059^{NS}	0.070^{NS}	0.021^{NS}	-0.025^{NS}	-0.214^{NS}	0.195^{NS}	0.014^{NS}	-0.066^{NS}	0.070^{NS}	0.053^{NS}	-0.094^{NS}	0.013^{NS}	-0.194^{NS}	-0.091^{NS}	-0.233^{NS}	-0.235^{NS}	-0.217^{NS}	-0.112^{NS}	1.000		
X38	-0.218^{NS}	-0.091^{NS}	-0.075^{NS}	-0.181^{NS}	-0.202^{NS}	0.097^{NS}	0.105^{NS}	0.165^{NS}	0.073^{NS}	0.128^{NS}	-0.129^{NS}	0.059^{NS}	-0.165^{NS}	0.013^{NS}	-0.214^{NS}	-0.350*	-0.167^{NS}	-0.403**	0.523**	1.000	
X39	0.265^{NS}	0.160^{NS}	0.091^{NS}	0.139^{NS}	-0.051^{NS}	0.127^{NS}	-0.080^{NS}	-0.224^{NS}	0.010^{NS}	-0.058^{NS}	0.014^{NS}	-0.040^{NS}	-0.063^{NS}	-0.113^{NS}	-0.060^{NS}	0.061^{NS}	-0.087^{NS}	0.246^{NS}	0.624**	-0.340*	1.000

NS: Non-significant, * Probability at 5%, ** Probability at 1%, * The descriptions for traits name of X19 to X39 is presented in table 2 of the materials and methods chapter.

Sugar conten



Beta-carotene

Plate 10: Genetic variation for beta-carotenoid concentration and total sugar content in carrot for 48 genotypes

Table 16: Path coefficient analysis of 20 quantitative traits (root morphology and biochemical traits) on root yield among 48 genotypes of carrot in season-I

Traits	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39	r (root yield)
X19	**0.048**	0.075	-0.006	-0.101	0.033	-0.024	-0.005	0.000	0.010	-0.001	1.013	0.311	-0.994	0.055	-0.110	0.022	0.003	0.001	1.264	-1.269	0.328*
X20	0.005	**0.794**	-0.009	-0.407	-0.079	-0.331	-0.002	0.000	0.020	0.000	0.225	0.391	-0.216	0.021	-0.002	0.007	0.001	0.858	-0.669	-0.189	0.418**
X21	0.012	0.312	**-0.024**	-0.236	-0.084	0.006	-0.004	0.000	0.023	0.000	0.574	0.438	-0.562	0.029	-0.056	-0.009	-0.003	-3.354	3.106	0.250	0.416**
X22	0.008	0.544	-0.009	**-0.594**	-0.073	0.078	-0.004	0.000	0.028	0.000	0.361	0.400	-0.350	0.026	-0.020	0.026	-0.001	-0.029	0.517	-0.489	0.421**
X23	-0.008	0.323	-0.010	-0.221	**-0.195**	0.118	-0.001	0.000	0.008	0.000	-0.188	0.259	0.172	-0.033	0.081	-0.050	-0.002	-0.503	1.114	-0.609	0.253NS
X24	0.002	0.456	0.000	0.081	0.040	**-0.577**	0.002	0.000	-0.001	0.000	0.049	0.059	-0.041	0.019	-0.017	0.006	0.003	1.425	-1.869	0.443	0.079NS
X25	0.017	0.107	-0.008	-0.179	-0.014	0.071	**-0.013**	0.000	0.034	0.000	0.545	0.218	-0.525	0.041	-0.072	0.013	0.001	0.422	-0.140	-0.284	0.233NS
X26	0.010	0.044	0.001	-0.122	-0.004	0.069	0.000	**0.002**	-0.002	0.000	0.253	0.123	-0.246	0.021	-0.021	0.012	0.002	3.297	-0.466	-2.832	0.141NS
X27	0.010	0.316	-0.011	-0.322	-0.029	0.009	-0.009	0.000	**0.051**	0.000	0.570	0.418	-0.555	0.065	-0.084	0.019	-0.002	-0.707	0.672	0.034	0.445**
X28	-0.016	0.049	0.002	-0.072	-0.044	0.069	0.001	0.000	0.004	**0.002**	-0.458	-0.029	0.458	-0.043	0.090	-0.011	-0.001	1.836	-1.407	-0.431	-0.000NS
X30	0.040	0.146	-0.011	-0.176	0.030	-0.023	-0.006	0.000	0.024	-0.001	**1.222**	0.375	-1.191	0.089	-0.170	0.028	0.001	-1.067	1.958	-0.894	0.375**
X32	0.016	0.330	-0.011	-0.253	-0.054	-0.036	-0.003	0.000	0.023	0.000	0.488	**0.939**	-0.489	0.013	0.012	0.006	0.002	-0.559	0.799	-0.236	0.987**
X33	0.040	0.144	-0.011	-0.174	0.028	-0.020	-0.006	0.000	0.024	-0.001	1.216	0.383	**-1.197**	0.089	-0.169	0.028	0.001	-1.587	2.249	-0.664	0.373**
X34	-0.021	-0.136	0.005	0.123	-0.051	0.089	0.004	0.000	-0.026	0.001	-0.867	-0.096	0.854	**-0.125**	0.200	-0.033	0.001	1.024	-1.290	0.266	-0.080NS
X36	-0.024	-0.006	0.006	0.054	-0.072	0.046	0.004	0.000	-0.020	0.001	-0.950	0.053	0.929	-0.115	**0.218**	-0.034	0.001	1.909	-1.795	-0.113	0.093NS
X37	-0.015	-0.081	-0.003	0.220	-0.136	0.052	0.002	0.000	-0.013	0.000	-0.487	-0.080	0.463	-0.059	0.103	**-0.071**	-0.003	-0.718	0.977	-0.258	-0.106NS
X38	0.008	0.035	0.004	0.019	0.027	-0.086	-0.001	0.000	-0.007	0.000	0.047	0.086	-0.040	-0.007	0.017	0.012	**0.018**	2.052	-1.435	-0.617	0.131NS
X37	0.000	0.071	0.008	0.002	0.010	-0.086	-0.001	0.001	-0.004	0.000	-0.137	-0.055	0.199	-0.013	0.044	0.005	0.004	**9.543**	-5.029	-4.522	0.041NS
X38	-0.009	0.080	0.011	0.046	0.033	-0.162	0.000	0.000	-0.005	0.000	-0.360	-0.113	0.405	-0.024	0.059	0.010	0.004	7.211	-6.656	-0.563	-0.033NS
X39	0.010	0.024	0.001	-0.046	-0.019	0.041	-0.001	0.001	0.000	0.000	0.174	0.035	-0.127	0.005	0.004	-0.003	0.002	6.873	-0.597	-6.280	0.097NS

Residual effect: 0.00421

* The descriptions for traits name of X19 to X39 is presented in table 2 of the materials and methods chapter.

Table 17: Path coefficient analysis of 20 quantitative traits (root morphology and biochemical traits) on root yield among 48 genotypes of carrot in season-II

Traits	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39	r (root yield)
X19	**0.038**	-0.110	0.001	0.281	0.415	-0.567	-0.015	0.006	-0.002	0.002	-0.018	-0.020	0.005	0.018	-0.019	-0.023	-0.013	0.391	1.157	-1.579	-0.054^{NS}
X20	-0.002	**2.808**	-0.003	-0.972	-0.164	-1.599	0.019	-0.029	0.009	-0.007	0.341	0.435	-0.173	0.041	0.018	-0.022	0.000	0.458	0.485	-0.957	0.687**
X21	-0.005	1.253	**-0.006**	-0.894	-0.382	0.073	0.033	-0.028	0.017	-0.006	0.487	0.499	-0.253	0.134	-0.124	-0.008	0.006	0.136	0.397	-0.545	0.784**
X22	-0.008	1.946	-0.004	**-1.402**	-0.241	-0.178	0.024	-0.024	0.009	-0.007	0.373	0.443	-0.188	0.011	0.017	-0.034	0.005	-0.162	0.963	-0.827	0.718**
X23	-0.016	0.454	-0.002	-0.332	**-1.014**	0.812	0.008	0.001	0.002	-0.002	0.074	0.257	-0.048	-0.024	0.174	0.071	-0.001	-1.403	1.075	0.302	0.387**
X24	0.010	2.011	0.000	-0.112	0.369	**-2.232**	0.004	-0.021	0.004	-0.003	0.140	0.135	-0.068	0.054	-0.058	-0.033	-0.003	1.283	-0.516	-0.755	0.210^{NS}
X25	-0.013	1.222	-0.004	-0.765	-0.191	-0.207	**0.044**	-0.033	0.018	-0.007	0.406	0.352	-0.224	0.134	-0.171	-0.020	0.012	0.093	-0.558	0.479	0.569**
X26	-0.004	1.406	-0.003	-0.580	0.011	-0.784	0.025	**-0.059**	0.012	-0.007	0.251	0.336	-0.152	0.037	-0.016	-0.028	0.005	-0.435	-0.877	1.334	0.474**
X27	-0.002	1.093	-0.004	-0.552	-0.085	-0.406	0.034	-0.031	**0.023**	0.001	0.541	0.365	-0.283	0.252	-0.391	-0.021	0.012	0.459	-0.390	-0.061	0.554**
X28	-0.003	0.878	-0.002	-0.417	-0.083	-0.350	0.013	-0.018	-0.001	**-0.022**	0.003	0.218	0.005	-0.089	0.219	-0.011	-0.008	0.351	-0.679	0.347	0.353*
X30	-0.001	1.324	-0.004	-0.722	-0.103	-0.433	0.025	-0.020	0.017	0.000	**0.724**	0.477	-0.366	0.245	-0.464	-0.027	0.015	0.083	-0.312	0.237	0.694**
X31	-0.001	1.836	-0.004	-0.934	-0.393	-0.454	0.023	-0.030	0.013	-0.007	0.519	**0.665**	-0.288	0.062	-0.023	-0.008	0.002	-1.277	0.878	0.376	0.956**
X32	-0.001	1.280	-0.004	-0.693	-0.129	-0.397	0.026	-0.023	0.017	0.000	0.698	0.505	**-0.380**	0.244	-0.434	-0.024	0.015	-0.599	-0.069	0.671	0.704**
X33	-0.002	-0.298	0.002	0.041	-0.062	0.309	-0.015	0.006	-0.015	-0.005	-0.457	-0.107	0.239	**-0.388**	0.610	0.015	-0.015	-1.528	1.140	0.358	-0.172^{NS}
X34	-0.001	0.069	0.001	-0.032	-0.247	0.181	-0.010	0.001	-0.013	-0.007	-0.469	-0.021	0.230	-0.330	**0.717**	0.028	-0.021	-1.547	1.863	-0.365	0.028^{NS}
X35	-0.010	-0.686	0.000	0.528	-0.796	0.803	-0.010	0.018	-0.005	0.003	-0.212	-0.057	0.098	-0.064	0.218	**0.091**	-0.005	-1.424	0.885	0.516	-0.107^{NS}
X36	0.015	0.000	0.001	0.192	-0.018	-0.199	-0.015	0.008	-0.009	-0.005	-0.314	-0.045	0.171	-0.172	0.450	0.012	**-0.034**	-0.737	2.145	-1.464	-0.018^{NS}
X37	0.002	0.196	0.000	0.035	0.217	-0.436	0.001	0.004	0.002	-0.001	0.009	-0.129	0.035	0.090	-0.169	-0.020	0.004	**6.569**	-2.779	-3.722	-0.094^{NS}
X38	-0.008	-0.256	0.000	0.254	0.205	-0.217	0.005	-0.010	0.002	-0.003	0.043	-0.110	-0.005	0.083	-0.251	-0.015	0.014	3.433	**-5.318**	2.025	-0.129^{NS}
X39	0.010	0.451	-0.001	-0.194	0.051	-0.283	-0.004	0.013	0.000	0.001	-0.029	-0.042	0.043	0.023	0.044	-0.008	-0.008	4.101	1.807	**-5.961**	0.014^{NS}

Residual value: 0.01289,

* The descriptions for traits name of X19 to X39 is presented in table 2 of the materials and methods chapter

indirect effects of other 20 characters including 4 biochemical parameters as independent characters in both the seasons.

In both the seasons, the trend (positive or negative) was almost same with respect to direct or indirect effects with few exceptions.

Nine characters had a positive direct influence on root yield and remaining eleven characters showed negative influence on the root yield. All the internal colours showed positive indirect influence on root yield through petiole pubescence character. Root width, shoulder width and petiole length also influence root yield indirectly through shoulder length character positively.

Among the morphological quantitative characters, shoot weight and five roots weight showed highest positive effects and five shoots weight showed highest negative effect (S-I). Root to shoot ratio showed higher positive indirect effect on root yield through shoot weight. In S-II, Xylem and phloem width showed negative direct effects and root width showed positive direct effect.

Biochemical characters showed highest direct and indirect effects and the highest positive direct was shown by total sugars (S-I and S-II) and reducing and non-reducing sugars showed negative direct effect. From the biochemical traits, the highest direct positive effect on root yield was recorded by total sugars and higher negative direct influence on root yield was shown by reducing and non-reducing sugars. Although beta carotene has no much direct influence on root yield, but the indirect of this trait total sugars on root yield was high and positive.

In S-I, the positive indirect effect of root length through total sugars on root yield was highest followed by indirect effect of five shoot weight through reducing sugars. The indirect effect of beta carotene through total sugars was positive and high in both the seasons.

In S-II, the negative indirect of total sugars through many characters *viz.*., phloem width, five roots weight, harvest index, phloem to xylem ration etc was negative on root yield, in contrast, root to shoot ratio and harvest index, root length, shoulder

length, cambium width *etc* showed positive indirect effect through total sugars on root yield.

Shoulder length was least influenced the root yield directly or indirectly in S-I. Shoulder width and petiole length through shoot weight showed higher positive indirect effect among the morphological traits in S-I and the same two characters along with shoot length showed negative indirect effect through five shoots weight on root yield.

In S-II also, biochemical parameters had positive and negative direct or indirect effect through various morphological characters on root yield as presented in Table 17. Among the morphological characters, the trend was same as S-I as explained above. Root width had highest positive direct effect on root yield followed by harvest index and shoot weight.

4.1.9 Mahalanobi's Diversity analysis

In carrot, root colour (internal and external), productivity related characters and nutritional quality characters are considered to be economic traits both for consumer and a grower. In the present study, although, the carrot genotypes were screened for 39 characters, but among the qualitative characters, only external root colour, xylem and phloem colour were considered for Mahalanobi's diversity analysis (D^2 analysis). Other 21 quantitative characters (morphological and 4 biochemical) were also considered along with above 3 qualitative characters. Hence, 24 characters were subjected to diversity analysis in both the seasons.

In general, biochemical characters contributed maximum to the diversity in all the three seasons analyzed, especially, total sugars and reducing sugars contributed highest percentage to the diversity in both S-I and S-II. Among the morphological traits, shoulder length (S-I), five shoot weight and root colours (S-II) contributed more to the diversity (Table 18 and 19).

With respect to the number of clusters and composition of clusters, in general, the genotypes, UHSBC-36 and UHSBC-08 separated from other genotypes by forming separate clusters in both seasons (Tables 20 and 21). UHSBC-36 is a IIVR collection and UHSBC-08 is a Ooty collection which were present in two different clusters.

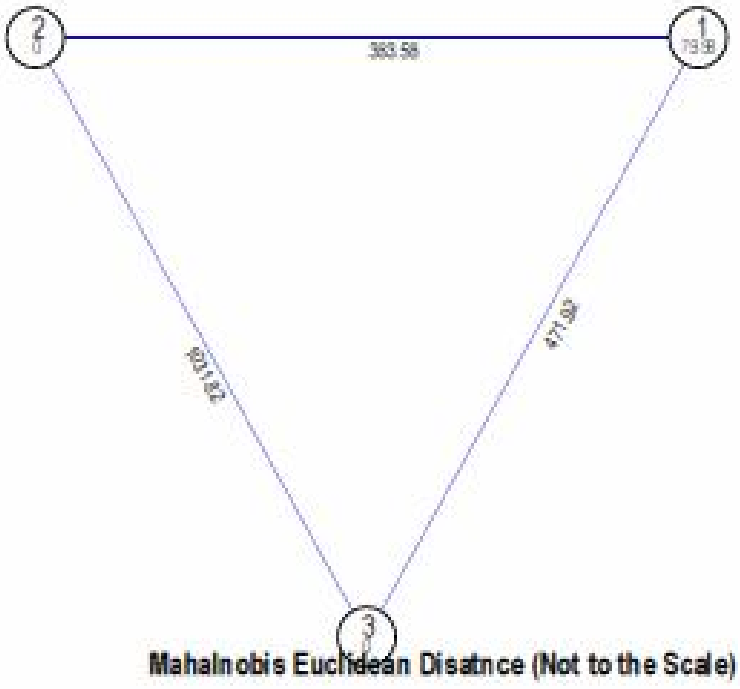


Fig. 5: Cluster diagram by Tocher method involving 3 clusters from 24 characters (17 quantitative, 4 biochemical parameters and external root colour, xylem and phloem colour) in D2 analysis in Season-I analyzed for 48 genotypes of carrot

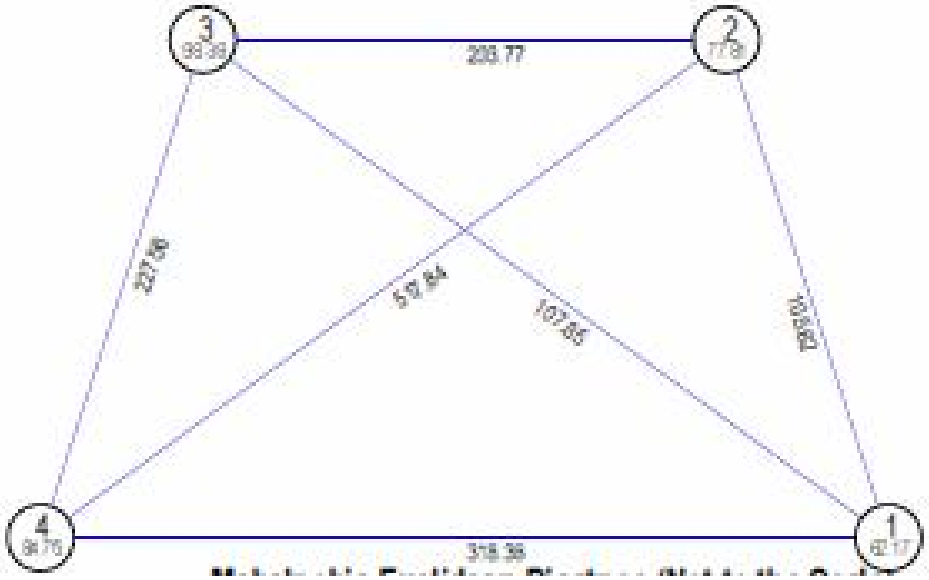


Fig. 6: Cluster diagram by Tocher method involving 3 clusters from 24 characters (17 quantitative, 4 biochemical parameters and external root colour, xylem and phloem colour) in D2 analysis in Season-II analyzed for 48 genotypes of carrot

Table 18: Percent contribution to diversity from 24 traits (quantitative, internal and external root colours) in D^2 analysis during Season-I among 48 genotypes of carrot

S. No	Source	Times ranked 1st	Contribution %
X14	Root colour (score)	28	2.48%
X16	Xylem colour (score)	10	0.89%
X17	Phloem colour (score)	18	1.60%
X19	No of petioles	7	0.62%
X20	Root width (cm)	3	0.27%
X21	Shoulder width (Cm)	61	5.41%
X22	Xylem width (cm)	16	1.42%
X23	Phloem width (cm)	13	1.15%
X24	Cambium width (cm)	3	0.27%
X25	Petiole length (cm)	38	3.37%
X26	Root length (cm)	1	0.09%
X27	Shoot length (cm)	3	0.27%
X28	Shoulder length (cm)	124	10.99%
X29	Root yield(gms)	6	0.53%
X30	Shoot weight (gms)	6	0.53%
X31	Five roots weight (gms)	-	0.0 %
X32	Five shoot weight	30	2.66%
X33	Root/shoot ratio	28	2.48%
X34	Harvest index (%)	45	3.99%
X35	Phloem to Xylem ratio	5	0.44%
X36	Beta carotenoid (ppm)	193	17.11%
X37	Total Sugars (%)	365	32.36%
X38	Reducing Sugars (%)	113	10.02%
X39	Non-reducing Sugars (%)	12	1.06%

Table 19: Percent contribution to diversity by 24 traits (internal and external root colour and quantitative traits) in D^2 analysis during Season-II for analyzed for 48 genotypes of carrot

S. No	Source	Times ranked 1st	% Contribution
X14	Root colour (score)	30	2.66%
X16	Xylem colour (score)	25	2.22%
X17	Phloem colour (score)	45	3.99%
X19	No of petioles	1	0.09%
X20	Root width (cm)	22	1.95%
X21	Shoulder width (Cm)	13	1.15%
X22	Xylem width (cm)	1	0.09%
X23	Phloem width (cm)	8	0.71%
X24	Cambium width (cm)	9	0.80%
X25	Petiole length (cm)	3	0.27%
X26	Root length (cm)	7	0.62%
X27	Shoot length (cm)	10	0.89%
X28	Shoulder length (cm)	24	2.13%
X29	Root yield(gms)	15	1.33%
X30	Shoot weight (gms)	28	2.48%
X31	Five roots weight (gms)	-	0.00 %
X32	Five shoot weight (gms)	47	4.17%
X33	Root/shoot ratio	26	2.30%
X34	Harvest index (%)	17	1.51%
X35	Phloem to Xylem ratio	25	2.22%
X36	Beta carotenoid (ppm)	52	4.61%
X37	Total Sugars (%)	150	13.30%
X38	Reducing Sugars (%)	527	46.72%
X39	Non-reducing Sugars (%)	43	3.81%

Table 20: Cluster composition of 48 genotypes based on 24 traits (internal and external root colour and quantitative traits) in D2 analysis in Season-I

S. No.	Cluster No.	No of genotypes	Genotypes Name
1	Cluster I	46	UHSBC02, UHSBC06, UHSBC07, UHSBC11, UHSBC13, UHSBC14, UHSBC17, UHSBC19, UHSBC21, UHSBC22, UHSBC23, UHSBC24, UHSBC25, UHSBC26, UHSBC27, UHSBC28, UHSBC29, UHSBC30, UHSBC31, UHSBC32, UHSBC33, UHSBC34, UHSBC35, , UHSBC37, UHSBC38, UHSBC39, UHSBC40, UHSBC41, UHSBC41-1, UHSBC42, UHSBC43, UHSBC43-1, UHSBC44, UHSBC45, UHSBC46, UHSBC47, UHSBC48, UHSBC49, UHSBC52, UHSBC53, UHSBC59, UHSBC64, UHSBC66, UHSBC68, UHSBC69
2	Cluster II	1	UHSBC36
3	Cluster III	1	UHSBC08

Table 21: Cluster composition of 48 genotypes based on 24 traits (internal and external root colour and quantitative traits) in D2 analysis in season-II

S. No.	Cluster No.	No of genotypes	Genotypes Name
1	Cluster I	31	UHSBC02, UHSBC14, UHSBC21, UHSBC22, UHSBC23, UHSBC24, UHSBC25, UHSBC26, UHSBC27, UHSBC28, UHSBC29, UHSBC30, UHSBC31, UHSBC32, UHSBC33, UHSBC34, UHSBC34-1, UHSBC35, UHSBC37, UHSBC38, UHSBC39, UHSBC41, UHSBC42, UHSBC43, UHSBC44, UHSBC44-1, UHSBC45, UHSBC46, UHSBC47, UHSBC48, UHSBC53
2	Cluster II	4	UHSBC17, UHSBC19, UHSBC 36, UHSBC41-1
3	Cluster III	7	UHSBC49, UHSBC44, UHSBC59, UHSBC64, , UHSBC13, UHSBC66, UHSBC40
4	Cluster IV	6	UHSBC 08, UHSBC69, UHSBC13, UHSBC11, UHSBC68, UHSBC 07

In season-I, only three clusters were obtained with cluster II and cluster III were solitary clusters consisting of single genotypes UHSBC-36 and UHSBC-08 respectively. Remaining 46 genotypes were grouped in to cluster I (Table 20).

When the inter cluster distances were estimated by D^2 method, the highest distance was obtained between cluster-II and cluster-III (1031.82) followed by cluster-I and cluster-III as presented in Table 22.

When the cluster means were observed for root colour, there was more uniformity in external root colour and phloem colour with a score nearer to 9.0 (dark purple pink) in cluster II. The root weight was highest in cluster-I followed by cluster-II but with respect to biochemical parameters, cluster II showed better performance compared to other two clusters. High harvest index of 51.37 % was recorded for cluster III followed by cluster II with 46.25% for other important yield parameters, such as five roots weight, cluster I performed well compared to other two clusters with a mean five root weight of 205.83 gms. Similarly, for root to shoot ratio cluster III performed better. The highest root length of 19.50cm was shown in cluster II followed by slight lesser root length of 19.37 in cluster I.

Diversity analysis in season-II resulted in four clusters with the number of genotypes ranged from four to thirty one and there were no solitary clusters (Table 21). The highest percent contribution to diversity was shown for reducing sugars (46.72%) followed by total sugars (13.30%). Among the morphological traits, the highest contribution to diversity was shown by five shoots weight (4.17%) followed by phloem colour (3.99%).

The inter-cluster distance (Table 23) was highest between cluster II and Cluster IV (517.84) followed by between cluster III and cluster IV (227.56). The intra-cluster distance was highest for cluster III (98.38) followed by cluster IV (84.75).

The cluster means for root weight was highest for cluster III (43.26gms) followed by cluster II (40.01g). With respect to uniformity in root colours all the clusters showed uniformity in external and phloem root colours except cluster III, but

Table 22: Inter, Intra-Cluster distance for 24 traits (internal and external root colour, quantitative traits) in D2 analysis in Season-I

Clusters	Cluster I	Cluster II	Cluster III
Cluster I	79.98	363.58	471.92
Cluster II		0.00	1031.82
Cluster III			0.00

Table 23: Inter, Intra (diagonal)-Cluster distance for 24 traits (internal and external root colour, quantitative traits) in D2 analysis in Season-II

Clusters No.s	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**62.17**	105.62	107.85	318.36
Cluster II		**77.81**	203.77	517.84
Cluster III			**98.38**	227.56
Cluster IV				**84.75**

Table 24: Cluster means for 24 traits (internal and external root colour, quantitative traits) from D^2 analysis in Season-I

S. No	Characters	Cluster I	Cluster II	Cluster III
X14	Root colour (score)	5.57	9.00	6.00
X16	Xylem colour (score)	2.35	1.90	3.00
X17	Phloem colour (score)	3.82	8.30	3.00
X19	No of petioles	13.52	12.40	9.17
X20	Root width (cm)	1.75	1.73	1.53
X21	Shoulder width (Cm)	2.18	0.23	2.39
X22	Xylem width (cm)	0.98	0.80	0.81
X23	Phloem width (cm)	0.34	0.21	0.45
X24	Cambium width (cm)	0.43	0.72	0.27
X25	Petiole length (cm)	19.29	19.38	13.9
X26	Root length (cm)	19.37	19.50	17.04
X27	Shoot length (cm)	44.76	48.26	36.25
X28	Shoulder length (cm)	0.77	1.15	0.90
X29	Root yield(gms)	41.3	39.80	27.00
X30	Shoot weight (gms)	74.42	46.40	34.25
X31	Five roots weight (gms)	205.83	127.00	135.00
X32	Five shoot weight (gms)	373.8	154.00	171.25
X33	Root/shoot ratio	0.75	0.76	1.19
X34	Harvest index (%)	38.34	46.25	51.37
X35	Phloem to Xylem ratio	0.36	0.27	0.58
X36	Beta carotenoid (ppm)	323.16	376.39	443
X37	Total Sugars (%)	8.65	21.38	7.3
X38	Reducing Sugars (%)	5.16	11.77	4.43
X39	Non-reducing Sugars (%)	3.48	9.61	2.87

Table 25: Cluster means for 24 traits (internal and external root colour, quantitative and biochemical) from D2 analysis in Season-II

S. No.	Traits	Cluster I	Cluster II	Cluster III	Cluster IV
X14	Root colour (score)	6.52	4.93	8.59	6.67
X16	Xylem colour (score)	1.93	1.23	2.29	5.88
X17	Phloem colour (score)	6.72	4.58	6.77	5.25
X19	No of petioles	10.57	10.06	11.43	10.56
X20	Root width (cm)	1.68	1.75	2.06	1.53
X21	Shoulder width (Cm)	2.44	2.59	2.32	1.76
X22	Xylem width (cm)	0.97	1.00	1.10	0.88
X23	Phloem width (cm)	0.34	0.30	0.32	0.36
X24	Cambium width (cm)	0.37	0.45	0.64	0.28
X25	Petiole length (cm)	20.35	22.06	18.72	14.75
X26	Root length (cm)	19.87	22.02	19.54	17.1
X27	Shoot length (cm)	46.43	51.79	44.1	27.47
X28	Shoulder length (cm)	1.05	1.21	1.18	1.15
X29	Root yield(gms)	35.45	40.01	43.26	25.44
X30	Shoot weight (gms)	50.03	52.86	53.11	11.99
X31	Five roots weight (gms)	171.4	176.5	213.14	116.83
X32	5 shoot weight (gms)	247.52	244	259.93	48.17
X33	Root/shoot ratio	0.71	0.72	0.85	2.32
X34	Harvest index (%)	41.59	41.47	45.51	67.64
X35	Phloem to Xylem ratio	0.35	0.30	0.30	0.45
X36	Beta carotenoid (ppm)	335.78	334.03	364.45	398.46
X37	Total Sugars (%)	11.09	15.40	10.72	8.71
X38	Reducing Sugars (%)	6.83	11.05	3.74	4.40
X39	Non-reducing Sugars (%)	4.26	4.35	6.97	4.31

the colour in respective clusters were different *viz.*, cluster-I has orange colour (~7.0), cluster II consisted of yellowish colours (~4.0) and orange types in cluster IV. With respect to biochemical parameters, cluster IV was superior for beta carotene and cluster II was good for sugars.

The composition of genotypes in each cluster is presented in tables 21. Here the first cluster composed of 31 genotypes, II cluster with 4 genotypes and III and IV clusters had 7 and six genotypes respectively. The diverse cluster showing II and IV consisted of local cultivars, some private sector cultivars and IIVR collections.

4.2 Molecular characterization

A total of 49 molecular markers were screened for polymorphism in the 48 genotypes of carrot, among them, only 24 markers including eight gene specific markers were polymorphic in the present study. A total of 62 alleles were obtained from these markers. Scoring was done based on allele sizing (for 19 markers and remaining 5 markers were scored as presence or absence as per the published data.

4.2.1 Molecular marker Diversity

The molecular diversity of the 48 genotypes for the 24 markers were studied by the banding patterns of 62 loci/alleles using Jaccard's dissimilarity coefficient followed by analysis for cluster distance giving 1000 bootstrap values and presented in the radial graph of dendogram (Figure No 7). Mainly two clusters were obtained in the cluster analysis (cluster I and cluster II) (Plate 11 and 12).

In the cluster I again there were four sub clusters, cluster II was further subdivided into 21 sub clusters out of which thirteen sub clusters were solitary cluster with single genotypes in them. The genotypes belonging to the respective clusters are depicted in the cluster diagram. The genotypic composition of each cluster is depicted in the figure.

Similar to the morphological diversity, UHSBC-36 and UHSBC-08 which were separated in to two clusters were also present in separate clusters as per the molecular diversity analysis. Along with these clusters, most of the local cultivars were separated

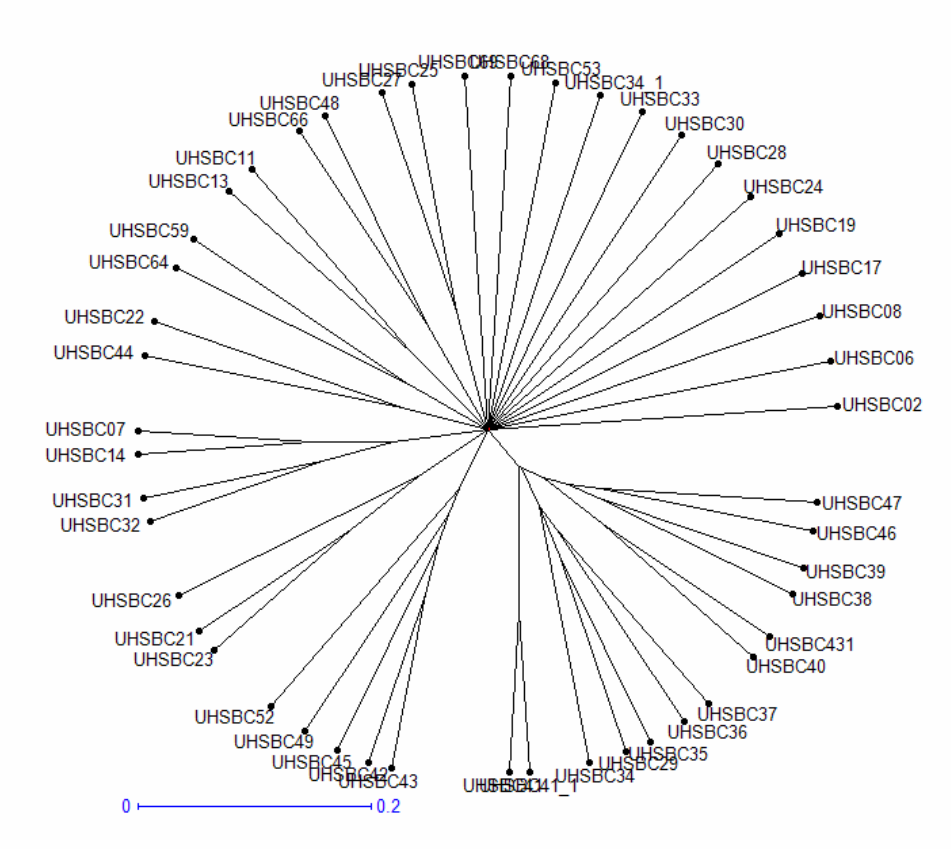


Fig. 7: Jaccard's dissimilarity coefficient for the genetic relationship among 48 carrot genotypes obtained from 24 molecular markers (62 allele)

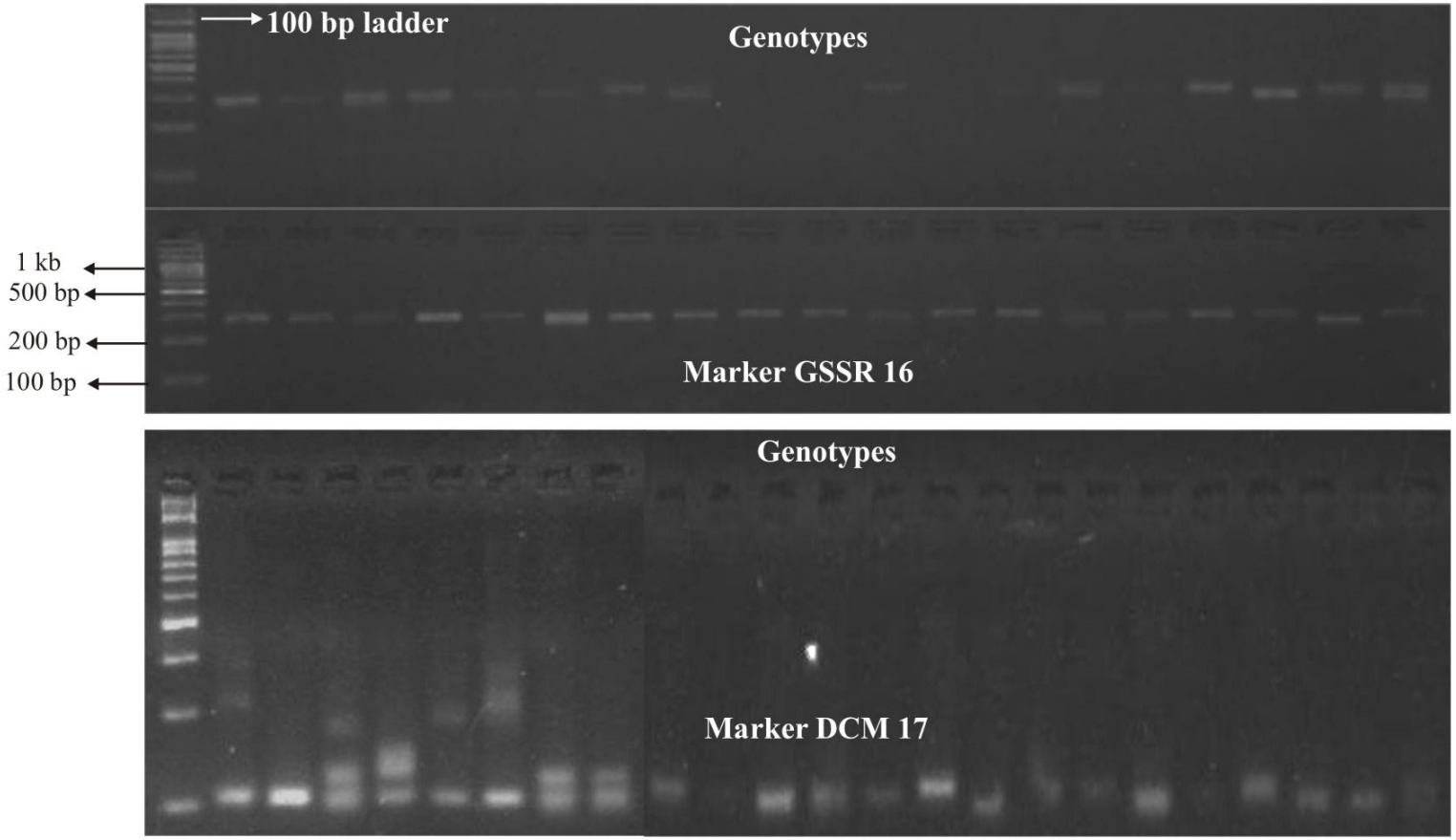


Plate 11: Molecular characterization and allele sizing in carrot for 48 genotypes using micro sattelite markers

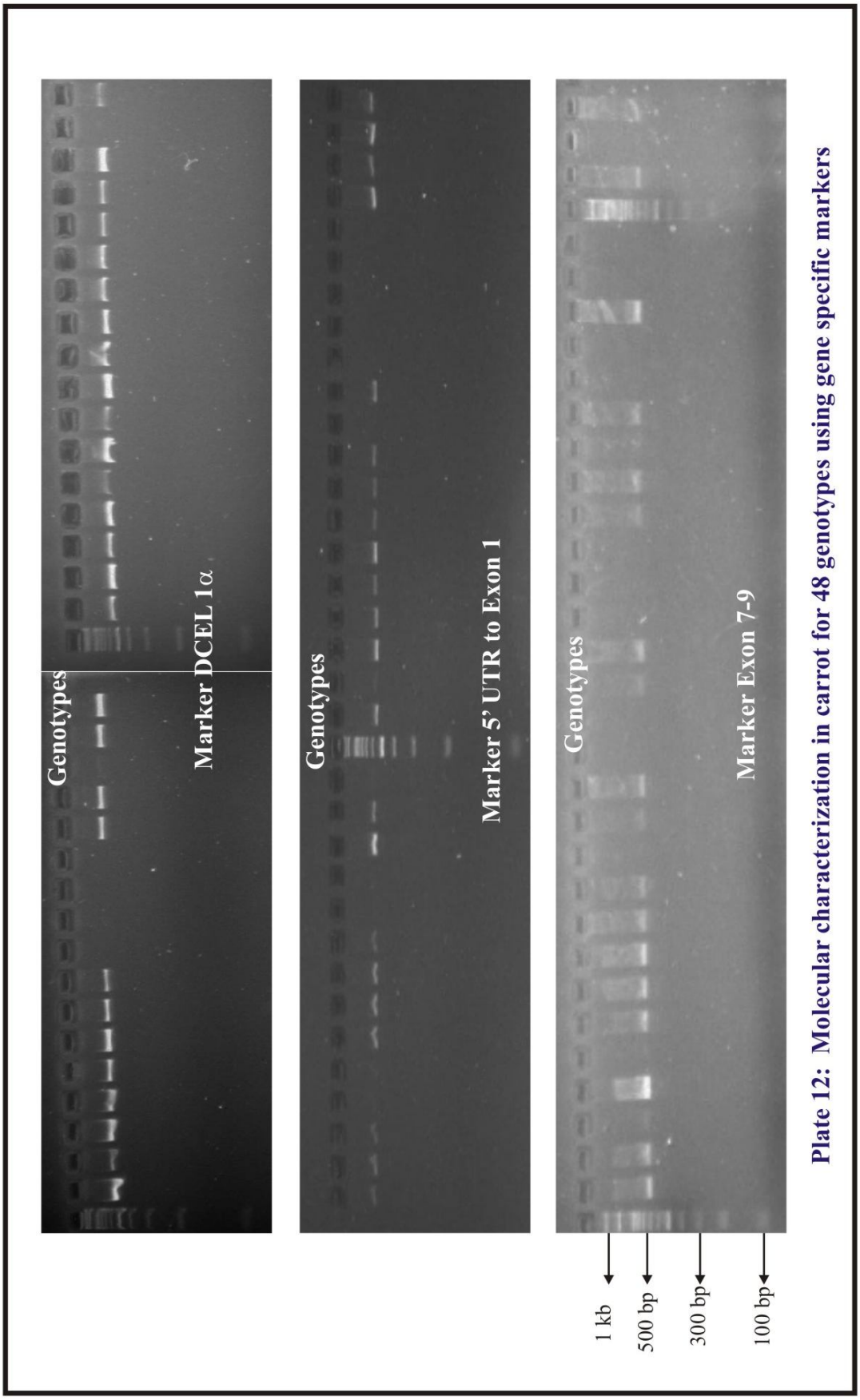


Plate 12: Molecular characterization in carrot for 48 genotypes using gene specific markers

from genotypes of Ooty collection (temperate types). Cluster I was mainly consisted of IIVR collections and few private sector genotypes, where as in cluster II, mainly the genotypes were from Ooty collections, local cultivars and few of the IIVR collections.

4.2.2 Microsatellite based allelic diversity

From the 24 markers, a total of 62 alleles were obtained in from the selected diverse carrot genotypes. The percent polymorphism (%P), polymorphic information content (PIC), number of alleles for the respective markers, marker index (MI), the level of heterozygosity (He) in terms of total number of polymorphic markers (Hav) are presented in table 26.

The number of alleles in microsatellite markers ranged from two to six with the highest number of alleles recorded in marker GSSR-19. The PIC value ranged from 0.13 (DCM-32) to 0.50 (EXON 4-EXON 7). The % polymorphism was highest in EXON 7-EXON 9 (85.42%) followed by Y2 marker (77.08%). The highest He value was observed for GSSR16 (0.90) with five alleles followed by GSSR19 (0.87) with highest number of alleles were six. The heterozygosity in comparison with the total number of markers studied was again for GSSR16 (0.038) followed by GSSR19 (0.036). From the present study, the markers with maximum of six alleles could be identified from a genic microsatellite markers in the Agarose electrophoresis itself.

4.3 Marker-Trait association

All the twenty four markers with their 62 loci were subjected to marker-trait association for 39 phenotypic characters recorded in the present study in S-I and S-II and presented in tables 27 and 28. Step wise linear regression method was followed to study the marker-trait association and only the markers showing significant association in terms of phenotypic variance (adjusted R^2 value) was considered. The significant markers for various characters ranged from one (reducing sugars) to eight (number of petioles). The cumulative effects of the molecular markers which explained the phenotypic variance for the 39 traits in each seasons is presented in Table 27.

In S-I, cumulative regression value (R^2) for root yield was 0.455 explained by 4 alleles. The highest phenotypic variance was obtained for shoulder length which was

together contributed by 7 alleles (6 makers) but that was an undesirable trait but present more in tropical type of carrots. Among the important root yield and yield components, for root yield/plan and five roots yield, the total R^2 explained was 0.455 from 4 molecular markers.

For harvest index, a total of 6 alleles (five markers) explained the phenotypic variance with as high as 0.632 R^2. Another important character for economic yield is phloem to xylem ratio where, five markers totally explained 0.484 R^2. Among the qualitative traits, like root colour (external), three markers (4 alleles) explained a total of 0.444 phenotypic variance with the highest explanation from GSSR-16_2 (0.208). Similarly, for phloem colour, only two markers explained their phenotypic variance with 0.228 R^2.

Among the biochemical traits, especially for beta carotene, five markers explained together the phenotypic variance of 0.432. Two markers were associated with total sugars, one for reducing sugar and two for non-reducing sugars were also influencing the respective traits with R^2 value ranged from 0.143, 0.088 and 0.155 respectively.

In the second season, among the qualitative traits of roots, for xylem colour total of as high as 0.723 R^2 was explained by nine makers and 10 alleles and for phloem colour, the R^2 value was 0.552 together by 6 markers.

For external root colour, the total phenotypic variance explained by the molecular markers was only 0.421 cumulatively by 4 markers (5 alleles). Among the quantitative traits of root morphology characters, the highest phenotypic variance of as high as 0.915 was explained for harvest index, together from fifteen markers with the highest contribution from GSSR85_4 (0.268). For root yield per plant seven molecular markers contributed R^2 value of as high as 0.631. Similarly, for root to shoot ratio, phloem to xylem ratio the respective R^2 explained was 0.478 and 0.425 respectively.

Among the biochemical traits, six markers explained R^2 of 0.485 for beta-carotene, seven markers with 0.596 R^2 for reducing sugars, five markers for non-reducing sugars with 0.377 R^2. No significant marker-trait association was recorded for total sugars in second season.

Table 26: Molecular marker analysis for the 24 carrot specific markers in 48 genotypes of carrot

S. No	Molecular Marker	No of Alleles	PIC Value	% Polymorphism	MI	He=1-∑pi^2	Hav=He/n**
1	GSSR4	3	0.28	41.67	1.25	0.72	0.030
2	GSSR-6	3	0.38	40.97	2.46	0.78	0.032
3	GSSR-9	2	0.18	56.25	3.38	0.53	0.022
4	GSSR16	5	0.33	26.25	5.25	0.90	0.038
5	GSSR-17	2	0.43	57.29	5.73	0.64	0.027
6	GSSR44	3	0.39	38.19	6.88	0.81	0.034
7	GSSR-85	4	0.32	28.65	8.02	0.87	0.036
8	5 UTR-EXON 1*	1	0.47	62.50	0.00	0.61	0.025
9	EXON 1-EXON 2*	2	0.39	57.29	10.31	0.62	0.026
10	EXON 3-EXON 5*	2	0.46	48.96	9.79	0.74	0.031
11	EXON 4-EXON 7*	1	0.50	47.92	0.00	0.77	0.032
12	EXON 7-EXON 9*	1	0.25	85.42	0.00	0.27	0.011
13	EXON 9-EXON 11*	3	0.39	55.56	21.67	0.64	0.027
14	DCM -2	3	0.24	41.67	17.50	0.70	0.029
15	DCM -17	2	0.46	56.25	16.88	0.67	0.028
16	DCM -32	2	0.13	51.04	16.33	0.56	0.023
17	GSSR-14	3	0.42	43.06	21.96	0.78	0.032
18	GSSR-19	6	0.43	35.07	37.88	0.87	0.036
19	GSSR-63	2	0.44	58.33	22.17	0.64	0.027
20	GSSR-138	3	0.43	38.19	22.92	0.83	0.035
21	GSSR-149	4	0.27	30.21	25.38	0.84	0.035
22	GSSR-111	3	0.35	36.11	23.83	0.82	0.034
23	Y2 MARK*	1	0.35	77.08	0.00	0.41	0.017
24	DcEL1œ*	1	0.28	83.33	0.00	0.31	0.013

* Gene specific markers

**n is no of polymorphic markers (24)

MI-Marker Index, PIC-Polymorphic Information Content, He: percent Heterozygosity

Table 27: Linear regression (cumulative R2 values) analysis for Marker-trait association for 39-traits with 24 markers (62 alleles) in S-I and S-II

Traits	Season-I		Season-II	
	MARKERS_Alleles	Cumulative R2 VALUE	MARKERS_Alleles	Cumulative R2 VALUE
Root position in soil	GSSR-44_1	0.151	GSSR- 85 _2	0.2
	GSSR- 17 _1	0.257	GSSR- 111 _2	0.316
	D- 17 _2	0.37	EXON- 4 TO EXON7 _1	0.388
	GSSR- 16 _3	0.426	GSSR- 19 _4	0.443
	GSSR- 16 _1	0.469	GSSR- 19 _3	0.508
	DCEL1 ALPHA	0.524	GSSR- 6 _3	0.588
	GSSR- 44 _1	0.576	EXON- 9 TO EXON11 _2	0.647
Shoot Attachment	GSSR- 17 _1	0.067	EXON- 9 TO EXON11 _3	0.151
	GSSR- 85 _1	0.143	GSSR- 9 _2	0.242
	GSSR- 6 _2	0.213	EXON- 4 TO EXON7 _1	0.293
			EXON- 1 TO EXON2 _1	0.344
Leaf type	GSSR- 85 _4	0.241	EXON- 1 TO EXON2 _1	0.196
	GSSR- 9 _2	0.321	GSSR- 85 _4	0.316
	EXON- 1 TO EXON 2 _1	0.382	DCM 32_2	0.402
	GSSR- 14 _2	0.436	GSSR- 14 _3	0.467
	GSSR- 19 _1	0.491	GSSR- 9 _2	0.513
Root branching	GSSR- 85 _4	0.118	EXON- 9 TO EXON 11 _1	0.131
	GSSR- 149 _2	0.191	EXON- 3TO EXON 5 _2	0.221
	GSSR- 9 _2	0.247	GSSR- 4 _2	0.282
	5' UTR TO EXON-1	0.301		
	GSSR-111 _2	0.375		
Root Hairiness	GSSR- 85 _4	0.165	EXON- 1 TO EXON 2 _1	0.158
			GSSR- 85 _2	0.267
			DCM 2_2	0.339
			GSSR- 63 _1	0.392
			Y2 MARK	0.453
			GSSR- 16 _1	0.5

Root cracking	GSSR- 149 _1	0.326	GSSR- 149 _1	0.256
	GSSR- 16 _1	0.474	GSSR- 16 _1	0.368
	EXON- 1 TO EXON 2 _1	0.524	EXON- 9 TO EXON 11 _2	0.432
	GSSR- 149 _3	0.558	GSSR- 149 _3	0.47
Root tip	GSSR- 44 _3	0.151	GSSR- 17_1	0.292
	GSSR- 17 _1	0.257	GSSR- 111_1	0.379
	D-17_2	0.37	GSSR- 16 _1	0.479
	GSSR- 16 _3	0.426	GSSR- 85 _4	0.536
	GSSR- 16 _1	0.469	GSSR- 19 _5	0.597
	DCEL1 ALPHA	0.524		
	GSSR- 44 _1	0.576		
Root tapering	GSSR- 14 _2	0.215	DCM 32_2	0.172
			GSSR- 44 _3	0.3
			GSSR- 17_1	0.386
			GSSR- 17_2	0.487
			GSSR- 138 _1	0.54
Root texture	GSSR- 149 _3	0.126	DCM 2_2	0.09
	EXON- 9 TO EXON 11 _2	0.199	DCM 14_2	0.171
Root shape	GSSR- 149 _1	0.113	GSSR- 149 _1	0.197
	GSSR- 16 _3	0.186	GSSR- 16 _3	0.374
Root Shoulder shape	GSSR- 149 _3	0.127	GSSR- 19 _6	0.096
	GSSR- 138 _1	0.186	GSSR- 19 _5	0.198
	Y2 MARK	0.269	DCM 2_1	0.286
	GSSR- 85 _3	0.317		
Root colour (External)	GSSR- 16 _2	0.208	GSSR- 16 _2	0.157
	GSSR- 16 _5	0.315	GSSR- 4 _2	0.226
	EXON- 9 TO EXON11 _1	0.383	DCM 32_2	0.31
	GSSR- 138_3	0.444	GSSR- 16 _5	0.377
			GSSR- 138 _3	0.421
Shoulder colour	GSSR- 149 _2	0.089	GSSR- 149 _4	0.15
	D 32_2	0.161	GSSR- 16 _5	0.245
	GSSR- 85 _2	0.219	GSSR- 6 _3	0.352
	DCEL1 ALPHA	0.307	GSSR- 138 _3	0.443
			DCM 32_1	0.481
			GSSR- 19 _1	0.526
White lines on petioles	EXON- 3 TO EXON5 _2	0.087	GSSR- 16 _1	0.214
	D 32_2	0.164	GSSR- 4_2	0.28
	D 17_1	0.224	GSSR- 85 _2	0.33
	GSSR- 85 _2	0.277	GSSR- 9 _1	0.383
	GSSR- 16 _4	0.331		
	EXON- 9 TO EXON11 _1	0.391		
Petiole pubescence	GSSR- 85 _4	0.288	GSSR- 85 _4	0.181
	GSSR- 19_1	0.374	EXON- 1TO EXON 2 _1	0.27
	EXON- 1 TO EXON 2 _1	0.419		

Xylem colour	GSSR- 85 _4	0.257	GSSR- 16_3	0.216
	GSSR- 16 _3	0.336	GSSR- 85_4	0.288
	GSSR- 19 _1	0.387	GSSR- 111_2	0.373
	GSSR- 4 _1	0.439	GSSR- 44_3	0.438
	GSSR- 14 _2	0.482	EXON- 7 TO EXON 9	0.49
			DCM 17_1	0.576
			DCM 32_1	0.628
			GSSR- 4_4	0.656
			GSSR- 19_1	0.687
			GSSR- 19_3	0.723
Phloem colour	GSSR- 138 _2	0.164	GSSR- 138 _2	0.185
	D 32_1	0.228	GSSR- 19_3	0.259
			GSSR- 6_1	0.314
			GSSR- 149 _1	0.448
			5UTR-EXON 1	0.514
			GSSR- 85_1	0.552
Cambium colour	GSSR- 85 _1	0.074	EXON- 7 TO EXON 9	0.105
	D 17_1	0.114	DCM-2_1	0.161
	GSSR- 16 _3	0.2		
	EXON- 9 TO EXON 11 _2	0.255		
	D 2_2	0.342		
	GSSR- 149 _2	0.424		
No of petioles	EXON- 4 TO EXON 7 _1	0.098	EXON- 4TO EXON 7	0.125
	GSSR- 16 _2	0.176	GSSR- 16_2	0.264
	Y2 MARK	0.258	EXON- 9 TO EXON 11_3	0.379
	EXON- 9 TO EXON 11 _2	0.328		
	GSSR- 149 _2	0.374		
	GSSR- 17 _1	0.423		
	EXON- 7 TO EXON 9_1	0.511		
	GSSR- 19 _2	0.549		
Root width	GSSR- 63 _2	0.134	GSSR- 44_1	0.116
	GSSR- 85 _2	0.204	GSSR- 63_2	0.208
	GSSR- 111 _2	0.27	GSSR- 19_1	0.276
	EXON- 1 TO EXON 2 _1	0.345	GSSR- 19_6	0.334
	GSSR- 111 _3	0.394		
Shoulder width	GSSR- 16 _4	0.122	GSSR- 85_2	0.104
			GSSR- 63_1	0.213
			GSSR- 44_1	0.332
			GSSR- 44_3	0.402
			DCEL 1 ALPHA	0.466
			GSSR- 19_4	0.516
			GSSR- 16_5	0.557
			GSSR- 9_1	0.592

Petiole length	D 32_1	0.179	GSSR- 111_1	0.124
	GSSR- 63 _1	0.269	GSSR- 16_2	0.219
	GSSR- 16 _3	0.344	DCM-32_1	0.271
Root length	GSSR- 85 _2	0.093	GSSR- 111_1	0.136
	GSSR- 44 _2	0.182	GSSR- 111_2	0.333
	GSSR- 16 _3	0.3	GSSR- 63_2	0.424
	GSSR- 19 _2	0.351	GSSR- 16_5	0.505
	GSSR- 149 _1	0.397	EXON- 4TO EXON 7 _1	0.559
Shoot length	GSSR- 85 _4	0.255	GSSR- 85_4	0.25
	GSSR- 63 _2	0.325		
	GSSR- 9 _1	0.408		
	EXON- 1 TO EXON 2 _1	0.486		
	D 32_2	0.573		
	Y2 MARK	0.637		
Shoulder length	DCM17_2	0.112	DCM-17_1	0.149
	DCM 17_1	0.307	EXON- 9TO EXON 11_3	0.245
	GSSR- 111 _3	0.441	EXON- 9TO EXON 11_1	0.326
	GSSR- 14 _3	0.515	GSSR- 14_1	0.378
	5 UTR TO EXON-1	0.591	Y2 MARK	0.449
	GSSR- 4 _2	0.629	GSSR- 149_2	0.491
	GSSR- 16 _5	0.658		
Root yield per plant	EXON- 1 TO EXON 2 _1	0.214	DCM-2_3	0.136
	EXON- 4 TO EXON 7 _1	0.306	GSSR- 16_5	0.196
	GSSR- 16 _5	0.386	GSSR- 6_3	0.435
	GSSR- 63 _2	0.455	GSSR- 63_1	0.472
			GSSR- 44_1	0.523
			DCEL1 ALPHA	0.589
			GSSR- 111_1	0.631
Shoot weight	GSSR- 85 _4	0.257	EXON- 1TO EXON 2 _1	0.171
	GSSR- 9 _2	0.324	GSSR- 63_2	0.262
	GSSR- 6 _2	0.388	DCEL1 ALPHA	0.331
	Y2 MARK	0.457	EXON- 9TO EXON 11_2	0.38
	DCM2_1	0.497	DCM-2_2	0.457
	GSSR- 111 _2	0.535	GSSR- 44_1	0.512
			EXON- 9TO EXON 11_1	0.552
Xylem width	GSSR- 63 _2	0.074	GSSR- 44_1	0.177
	DCM2_1	0.141	GSSR- 44_2	0.261
	GSSR- 44 _1	0.203	EXON- 4TO EXON 7_1	0.354
	DCEL 1 ALPHA	0.278	GSSR- 63_1	0.419
	GSSR- 44 _2	0.325	DCEL1 ALPHA	0.459
	DCM2_1	0.375	DCM-2_2	0.593
			GSSR- 85_3	0.63

Phloem width	DCM 2_3	0.07	DCM-2_3	0.064
	DCM17_2	0.165	GSSR- 16_3	0.152
			GSSR- 63_1	0.206
Five roots weight	EXON- 1 TO EXON 2 _1	0.179	GSSR- 44_3	0.114
	GSSR- 63 _2	0.283	GSSR- 63_2	0.224
	GSSR- 16 _5	0.383	GSSR- 44_1	0.324
	EXON- 4 TO EXON 7 _1	0.45	GSSR- 16_5	0.422
			GSSR- 6_3	0.519
			EXON- 4TO EXON 7_1	0.577
			EXON- 9TO EXON 11_1	0.609
			GSSR- 138_1	0.642
			GSSR- 6_1	0.715
			GSSR- 19_1	0.726
			GSSR- 6_2	0.76
FIve shoots weight	GSSR- 85 _4	0.298	GSSR- 85_4	0.178
	GSSR- 9 _2	0.365	GSSR- 63_1	0.275
	GSSR- 6 _2	0.424	EXON- 1TO EXON 2_1	0.342
	Y2 MARK	0.476		
	DCM2_1	0.515		
	GSSR- 111 _2	0.553		
Root/shoot ratio (%)	GSSR- 85 _4	0.287	GSSR- 85_4	0.247
	GSSR- 19 _1	0.418	EXON- 1TO EXON 2_1	0.355
	GSSR- 9 _2	0.47	GSSR- 9_1	0.419
			GSSR-19_1	0.478
Harvest index (%)	GSSR- 85 _4	0.258	GSSR- 85_4	0.268
	GSSR- 19 _1	0.37	GSSR- 6 _2	0.339
	GSSR- 6 _2	0.444	EXON- 1TO EXON 2_1	0.395
	GSSR- 9 _2	0.507	GSSR-7_9	0.482
	GSSR- 6 _1	0.582	GSSR- 9_1	0.524
	EXON- 9 TO EXON 11 _2	0.632	EXON- 9TO EXON 11_2	0.572
			DCM-2_2	0.627
			GSSR-111_3	0.659
			GSSR-4_3	0.707
			GSSR-19_4	0.736
			GSSR-14_1	0.768
			GSSR-63_2	0.796
			GSSR-63_1	0.836
			GSSR-138_1	0.861
			EXON- 4TO EXON 7_1	0.818
			GSSR-138_3	0.889
			GSSR-19_1	0.903
			EXON- TO EXON 5_1	0.915

Phloem to Xylem ratio	GSSR- 19 _6	0.107	GSSR-16_3	0.142
	GSSR- 16 _3	0.203	GSSR-9_2	0.223
	DCM2_3	0.281	GSSR-9_1	0.319
	DCM17_1	0.355	DCM-2_3	0.374
	DCM 2_1	0.406	GSSR-85_2	0.425
	EXON- 1 TO EXON 2 _1	0.484		
Beta carotenoids	GSSR- 85 _3	0.166	GSSR-4_2	0.124
	GSSR- 4 _2	0.25	GSSR-85_3	0.269
	GSSR- 16 _1	0.308	EXON- 9TO EXON 11_3	0.321
	EXON- 9 TO EXON 11 _3	0.384	GSSR-9_2	0.403
	GSSR- 17 _2	0.432	GSSR-6_2	0.445
			GSSR-16_1	0.485
Total sugars	GSSR- 149 _1	0.07		
	GSSR- 85 _2	0.143		
Reducing	GSSR-111 _2	0.088	GSSR-149_4	0.149
			GSSR-44_2	0.225
			DCM-32_2	0.313
			GSSR-6_1	0.359
			GSSR-6_2	0.487
			EXON- 1 TO EXON 2 _2	0.525
			5 UTR EXON-1	0.562
			DCM-2_2	0.596
Non reducing	GSSR- 16 _1	0.081	DCM-2_3	0.106
	GSSR- 85 _2	0.155	GSSR-4_1	0.19
			GSSR-16_1	0.255
			GSSR-138_1	0.314
			EXON- 7 TO EXON 9	0.377

4.4 Identification of superior carrot germplasm

As per the results obtained from the various statistical analyses we could find out the best superior genotypes by considering the economically important traits of carrot. Prime importance was given to the root yield. First best three genotypes were selected in each season to conclude the suitability of carrot germplasm for tropical regions. The performance of various genotypes for the 39 traits in S-I and S-II is listed in Table 1 of Appendix

In season-I, UHSBC-32 (VRCAR-20), UHSBC-44(VRCAR-62) and UHSBC-52 (VRCAR-81), showed best performance among all the forty diverse genotypes under study with the root yield of 80.75 g, 70.25g and 64.38 g respectively. UHSBC-32 had a high uniformity in root colour (score 5), xylem (score 2.5) and phloem colour (4.5) which was orange type and is most commonly preferred by consumers.

Xylem to phloem ratio was taken under consideration which was 0.41 Among the quantitative traits, the UHSBC-32(VRCAR-2), root weight of 80.75gms, root length of 16.83cm, root width of1.49 cm and harvest index- 48.71%. Other characters of this genotype includes, root colour- orange (5.35) xylem colour- light orange(2.5) phloem colour was dark orange (4.5), xylem to phloem ratio 0.41, biochemical parameters like beta carotenoid was 293.75ppm, total sugar 7.11% and reducing sugar 5.0%.

Another best genotype in S-I, was also a collection from IIVR, Varanasi, *i.e.* UHSBC-44 (VRCAR-62) where the root weight was 70.5gms with root length of 21.5cm, root width 1.77 cm and harvest index 23.13. With respect to root color external colour was yellow orange (3.5), xylem color dark yellow (2.80), phloem color near to yellowish green (5.0), xylem to phloem ratio (0.36). For biochemical parameters like beta carotenoid it was showing 384.26 ppm with a total sugar of 10.93 % and reducing sugar 4.92 %.

The third best genotype in S-I was UHSBC-52(VRCAR-81) with a root weight of 64.38gms, root length (21.5cm), root width (1.76) and harvest index (36.85). The qualitative characters of this genotype were characterized as dark orange (5.63) external root colour, light yellow xylem color, (1.63) with the phloem color was dark orange (4.0). The root width for this was 1.76; xylem to phloem ratio was 0.23. Among the

biochemical parameters, beta carotenoid was of 378ppm, total sugar 8.26% reducing sugar 5.73%.

Among the biochemical traits, such as beta carotene, the best performance was shown by UHSBC-06 (420.60ppm) followed by UHSBC-69 (394.91 ppm), where as UHSBC-36 shown highest total sugar content of 21.38 % as well as reducing sugar (11.77%) followed by UHSBC43-1 (16.51%). The lowest sugar content was 5.53 % (UHSBC-26). The best performing genotype for beta-carotene *i.e* UHSBC-06 showed a total sugar content of 10.40 % with 8.03 % of reducing sugar.

In contrast to first season, three different genotypes performed well in S-II such as UHSBC-66 (Pusa Asita), UHSBC-17 (Ghataprabha local-2) and UHSBC-22(Jatt Local).

Pusa Asita is a black coloured released carrot variety form IAR, New Delhi, showed a root yield of as high as 73.02gms, root length of 20.38cm, root width 2.27cm, harvest index-44.37 %. With respect to Root color, it was black (14.10) coloured cultivar with xylem colour- light yellow (2) phloem color- light orange (3). Root width for this cultivar was 2.27cm, xylem to phloem ratio-0.3. Among biochemical parameters, beta carotenoid was 360.19ppm, total sugar-8.22% and reducing sugar-1.29%.

Another cultivar, UHSBC-17 (Ghataprabha local-2) had a root weight of 61 gms, root length- 20.40cm root width with 1.74cm and harvest index-52.50 %. Root color of this was dark orange (5.70) xylem color was white (1.0), phloem color was pink (6.0), root width-1.74cm, xylem to phloem ratio-0.41. Biochemical parameters like beta carotenoid were 354.86 ppm, total sugar 13.93 %, reducing sugar 10.75 %.

The characters of another best performing genotype UHSBC-22 (Jutt Local) is as follows, root weight of 54.80gms, root length 23.35cm, root width of 1.89cm, harvest index 45.82%, Root color was pink (8.13) xylem color-white (1.0), phloem color was pink (6.0), root width 1.89 and xylem to phloem ratio 0.4, beta carotenoid was 338.19 ppm, total sugar 9.47 %, reducing sugar-6.9 %.

In S-II, UHSBC-66 (Pusa Asita), was superior for various characters as follows, root weight (68.36gms), root length of 20.38cm, root width (2.23cm), harvest index

(45.7), root color was black (14.55), xylem color dark orange (2.06), phloem color dark orange (4.06), root width (2.23cm), xylem to phloem ratio was 0.34, beta carotenoid was 332.18 ppm, total sugar of 8.22% and reducing sugar of 3.64 %.

Among the biochemical traits, for S-II, UHSBC-69 shown highest beta-carotene content (474.77 ppm) followed by UHSBC-68 (443.52 ppm). The total sugar content for the respective genotypes was 8.22 %.

The cultivar UHSBC-44 (VRCAR-62), which was superior in S-I, also expressed the following characters, root weight (60.93gms), root length(21.87 cm), root width of 1.87cm, harvest index of 32.15 %, root color was orange type (5.40), xylem color was light orange(~3.00), phloem color was pink(5.60), xylem to phloem ratio of 0.28, beta carotenoid content of 371.06 ppm, total sugar of 9.28% and reducing sugar was 4.71%.

Another carrot cultivar from IIVR Varanasi, UHSBC-32 (VRCAR-20) performed well across the seasons with root weight of 59.18gms, root length of 16.55cm, root width of 1.64cm, with harvest index (41.37%). The root color was of orange type (5.33), xylem color was dark orange (4.5), phloem color was orange (2.5) with the xylem to phloem ratio 0.34 and beta carotenoid of 294.79ppm, total sugar of 11.39% and reducing sugar 4.28 %.

5. DISCUSSION

Carrot, a cool season vegetable is the tenth most important food crop of the world, serve as a major source of provitamin A to the vegetarian population. Being a highly cross-pollinating and biennial crop, greater amount of genetic diversity is available especially in the root colour, nutritional quality and other morphological traits. Recently, the carrot genome has been sequenced, but the information pertaining to tropical carrots, germplasm diversity at the phenotypic or at the molecular level is meagre. Hence, in the present study, an attempt has made to study the genetic variability and diversity available in the tropical carrot along with the temperate carrots by characterizing using 35 morphological characters (including 18 qualitative and 17 quantitative), 4 biochemical traits. Genotyping was done using microsatellite markers. The detailed information of the present investigation is discussed in to phenotypic characterization, genotypic characterization, marker-trait association and selection of superior genotypes suitable to tropical region.

5.1 Phenotypic characterization

The selected forty eight diverse carrot genotypes have been extensively studied in terms of its phenotypic information. Among the 39 characters, the 21 quantitative traits were subjected to various statistical analysis, such as All the 39 characters were subjected to statistical analysis such as ANOVA (individual seasons) mean, range and genetic variability components (GCV, PCV, h^2, GA and GAM). Eighteen qualitative traits have been subjected to frequency distribution analyses as they are scored based on the descriptors and hence, did not used for other statistical analyses except the root colour characters for diversity analyses. Seventeen quantitative traits were further analyzed for principle component analyses, Pearson's correlation coefficient analyses, path coefficient analyses. Diversity analyses was carried out with the above seventeen quantitative characters along with external root colour, internal colours (Xylem and phloem) as they are the most important economic traits of carrot which directly influence the consumer acceptance and attractiveness of carrot.

Analyses of variance in both S-I and S-II the season's revealed significant variation for most of the traits studied indicating the existence of sufficient genetic variation among the carrot genotypes selected for the study.

5.1.1 Mean, Range and genetic variability estimates

Variation which is genetically controlled is the most important basic need for improvement of their respective traits. How much of the total phenotypic variation is heritable to the next generation is of great concern to a plant breeder for effective selection. This genetic variation can be studied by range and genetic variability estimates such as genotypic variance (GV) and phenotypic variance (PV). Since many characters have been studied in the present investigation and different traits are expressed in terms of different units, hence to compare these genetic variability components across the traits, they have further expressed as GCV, PCV which are unit less/expressed in percentage.

Absolute variability values of different characters do not reveal which of the characters showing high variability. This could only be assessed through standardized values of the phenotypic and genotypic variance estimates by obtaining the coefficients of variability.

The coefficient of variability indicates only the extent of variability present for a character and does not demarcate the variability into heritable and non-heritable portion. So, the extent to which variability could be transferred from parent to offspring would suggest how far variation is heritable which has close bearing on response to selection.

In the present study, wider range of variation was available for almost all the quantitative traits studied in both the seasons (S-I and S-II) indicating the presence of variation and scope for further improvement by breeding. Among the qualitative traits, lot of variation was seen in the genotypes especially for root colours (internal and external colours) indicating that depending on the choice of a breeder, or consumers requirement, multi-coloured carrots could be developed.

Also, the superior lines suitable to tropical region identified will be identified in the present study having contrasting colours such as orange, red, pink, black or purple

with yellow or white colour could be used to develop the mapping populations and further development of linkage map is also possible. Several linkage maps have been developed in carrot using various types of mapping populations (Cavagnaro *et al.*, 2011, Just *et al*., 2007, Iorizzo *et al.*, 2011).

Among the qualitative traits, the range was narrow for traits like type of shoot attachment (single/multiple), leaf type (celery, normal and fern), root cracking (absent, intermediate, sparsely), root tip (present or absent), root tapering (blunt or pointed), petiole pubescence (present or absent) and white lines on petioles (presence or absence), due to very few types observed.

Root cracking, branching and hairiness characters are the most undesirable characters leading to poor productivity of carrot. Although cracking sometimes is due to hard soil condition, but the better penetrating capacity is a genetically controlled phenomenon, which leads to least cracking or branching in the roots. Identification of carrot genotypes with least abnormalities is also one of the main objectives for tropical conditions as the soil will be usually harder than the required sandy loam type.

Other qualitative characters, especially for external root colour and internal root colour (xylem, phloem and cambium), wider range (2.0 to 15.0) of colours were found among the genotypes selected for the study ranging from white to black in both the seasons. Carotenoids are responsible for the yellow, orange, and red colours of carrots, while anthocyanins, a class of polyphenolic compounds, are responsible for the colour of purple carrots (Kurilich *et al.*, 2005; Arscott and Tanumihardjo, 2010).

The mean value for external root colour (5.65) indicates more of orange coloured carrots in the selected genotypes in S-I. The internal colour (xylem, phloem and cambium colour) ranged from white to black but the mean values (2.36, 3.9 and 2.90) for respective traits indicated more of white to yellow colours. Root position in soil ranged from shallow (3.0) to very deep (9.0), for root shape and shoulder shape, oblong to tapering (3.77 to 5.0) and flat to rounded types (1.0 to 3.0) were found respectively in both the seasons. Development of uniform root colours (internal and external) is another major objective in carrot breeding, which can fetch more prices in the market because of the attractive colour.

When these characters were analyzed for genetic variability components such as GCV and PCV, the highest root weight/root yield was obtained in S-I with an average of 80.75grams, very wide range of variation with higher PCV and GCV was observed for this trait. In both the seasons, PCV was moderate to high for most of the traits but GCV was moderate to high and also low in few cases indicating the influence of environment on these traits. All the four biochemical parameters such as beta-carotene, total sugars, reducing and non-reducing sugars showed greater genetic variation in both the seasons as depicted by their wider range, higher PCV and GCV. As high as 474.77ppm of beta-carotenoid was estimated in S-II, with as high as 21.0% of total sugars and 11.77 % of reducing sugars (S-II).

Among the morphological traits, highest heritability was recorded for shoulder width (77.0%) followed by root tip (73.0%) in S-II, root to shoot ratio, harvest index and xylem colour (81.0%) followed by phloem colour (78.0%) in S-II.

Similarly, higher GAM was observed for shoulder length followed by root to shoot ratio in S-I, xylem colour followed by root to shoot ratio in S-II. Hence, root to shoot ratio, harvest index and xylem colour are considered to be best for effective selection for indirect improvement of root yield as revealed by the positive significant correlation of harvest index and root to shoot ratio with root yield in the present study. Jagosz (2012)

, reported a heritability close to zero for root yield indicating a very high influence of environment which is supported by their significant environmental influence on root yield and other yield components in the present study as well.

Among the biochemical traits, all the four parameters showed higher heritability with higher GAM in general in both the seasons followed by a very good amount of expected mean to the next generation indicating they are the most simply inherited characters and easy for a breeder to for effective improvement just by means of selection of superior lines in the segregating generations. The results are in accordance with the higher heritability values for carotenoids and monosaccharide sugars in the study of Jagosz 2012 and Michalik *et al*. (1988). Duan *et al* (1996) reported a significant GCA effect of carrot root yield and quality parameters such as carotenoids,

sugars, dry matter and carrot root yield and they are mainly controlled by additive as well as non-additive gene effects (Jagosz 2012).

5.1.2 Frequency distribution

Frequency distribution revealed normal distribution for external root colours in both the seasons indicating the polygenic nature of root colour. Since root colour is highly influenced by the carotenoid genes present in them and carotenoid is considered to be a quantitative traits in carrot (Simon and Peterson 2011). Qualitative traits are generally show discontinuous variation due to limited variation present in them, hence, there are very few statistical tools which are applied to estimate the variability of the qualitative traits such as chi-square test or frequency distribution. Chi-square test is generally applied in segregating population, hence, in the present study, the carrot genotypes for these quanitiave traits were further categorized based on the frequency distribution for these traits in both S-I and S-II.

Although, external root colour was showing normal distribution, but with respect to xylem and phloem colour, there was a skewed distribution towards white to yellow colour indicating the lack of uniformity in the expression of root colour especially in season II. There is a need to improve for this trait especially in tropical carrot, as the temperature has a great influence on the expression of colour. The uniformity in root colour is the most important breeding objective in carrot breeding; hence, there is a need to select uniform carrot cultivars in breeding.

In carrot although, many root shapes are available *viz.*, round, obovate, obtriangular, oblong, tapering *etc* and for shoulder shapes we can find flat, flat to rounded, rounded, rounded to conical, conical *etc* in descriptors, but we did not find any of the conical shaped shoulders, but other shapes were available in genotypes selected for the study. Majority of the root shapes were of tapering types (score 5.0) in both the seasons as shown by its skewed distribution towards the tapering side. However, the variation was present for both root shape and shoulder shape in the genotypes as shown by the distribution curve for different shapes.

When the petioles were observed carefully, we could find the white lines on them in few genotypes but not in others, hence to know the influence of this on the

economic traits, the observation was recorded as presence (2.0) or absence (1.0) of white line on the petioles in both the seasons. Majority of the genotypes showed absence of white lines although the lines were present in few genotypes in both the seasons.

With respect to root tip, maximum genotypes showed presence of root tip and pointed tapering, although both the types were present in the present study in both the seasons. Maximum genotypes showed pointed type of roots in S-II compared to S-I. The type and hardiness of the soil may have influence on the pointed or blunt type of roots with presence or absence of root tip as they will be lost during harvesting if the soil is hard leading to more number of broken carrots than complete ones though the character is controlled genetically.

Root texture is another important trait which attracts the consumers and indicating the surface of the root, is another important quality character which influences the palatability of carrot for consumption. But, coarse textured roots are not preferred for consumption. In the present study, ranging from smooth (score 1.0) and ridged (4.0) types, coarse (2.0), and dimpled (3.0) were observed in the genotypes selected for the study in both the seasons. Very few extreme types (smooth or ridged) for the root texture were found in both the seasons, but most commonly found were coarse textured (2.0) to dimpled (3.0) types as shown by the frequency distribution.

Pubescence on the leaf and petioles is another interesting character which could play an indirect role on the insect resistance. Hence, the observations were recorded for the 48 genotypes utilized for the present investigation and scoring was given as presence or absence in both the seasons. Very few genotypes were showing presence of pubescence but majority of the genotypes were not having the pubescence on leaf and petioles.

Uniformity in external and internal roots is an important breeding objective in carrot; hence, the observations of vascular tissues such as xylem, phloem and cambium colours were also recorded in both the seasons. In S-I, xylem and cambium colour were showing skewed distribution towards white colour (with score 1.0-3.0) but in phloem majority of the genotypes shown orange colour. Very few genotypes, both external and internal root colours were same like white-white, red-red, orange-orange, black-black

etc were shown. In S-II, except cambium colour, the distribution of xylem and phloem were skewed, but for cambium colour it was showing normal distribution ranging white (1.0) to black (7.0).

5.1.3 Principle component analysis

In the germplasm characterization, evaluation of number of traits which show variation in the population is prerequisite to evaluate and study the economic traits of interest. When the number of characters is large in number, it will be confusing for a breeder that, on which character he should rely on for selection. Although, economic characters like yield and quality are important, but their inheritance is complex and also highly influenced by environment and other traits. Hence, principle component analysis (PCA) is the most powerful approach to know what are the traits contribute or explains the variation among the many traits in the genotypes selected for study,

PCA is a variable-reduction technique that shares many similarities to exploratory factor analysis. Its aim is to reduce a larger set of variables into a smaller set of 'articifial' variables, called 'principal components', which account for most of the variance in the original variables. PCA) helps in identifying the most relevant characters that can be used as descriptors by explaining as much of total variation in the original set of variables as possible with as few components as possible and reducing the dimension of the problem (Ramesh 2011).

In the present study, since 48 genotypes were characterized with many (39) characters in both the seasons, hence, to know the important quantitative characters which explain the highest variation would be studied. Although, 39 traits were studied including 18 qualitative traits, the first important assumption to be fulfilled in PCA is that, the traits under study must be quantitative in nature and must show continuous variation; hence, only 21 quantitative traits were subjected to PCA analyses.

In S-I and S-II, twenty one characters were partitioned in to seven and six main components with the cumulative percent of variation 83.0%. Out of the total components extracted, only first seven and six components were retained in S-I and S-II respectively as they explained sufficient variation in both the seasons and showing the Eigen value of >1.0.

The other factors corresponding to Eigen value <1.0 were not considered as per Kaiser's (1958) recommendations. Among these components, first two components explained more than 43.66 % in S-I and as high as 54.28% in S-II with the Eigen value of >3.0. Hence, in the present study, the first two components were decided to be important based on the initial Eigen value of >3.0. The first component consisted of combination of 15 characters. Among them, individual shoot weight, harvest index, five shoot weight, no of petioles, shoot length and five root weights are the important traits present in first two principle components in S-I.

Similarly, in S-II also along with these traits, root width, xylem width, root lengths were also present in first two components with positive loadings. Ramesh *et al.*, 2011 also reported similar findings while studying the principle components involved in European germplasm characterization.

5.1.4 Correlation study among quantitative traits

Before formulating suitable strategies to breed varieties for better quality, higher productivity, understanding the relationship among and between the morphological and or biochemical characters is of paramount importance. Correlation coefficients measure the mutual relationship between various characters, which help in devising efficient strategies for indirect selection using component character and simultaneous selection of multiple traits. Genetic variability is an important tool to select desirable characters, which are heritable. For the improvement of root yield in carrot, it is necessary to have regarding the association of various quantitatively inherited characters with root yield.

In the present study, although 18 qualitative characters were used for characterization of carrot germplasm, but as per the hypothesis of correlation analysis, it is better to include only the numerical data which shows continuous variation should be included for analysis. Hence, only 21 characters were used for the correlation study and the data from individual seasons were subjected to Pearson's correlation analysis.

In general, in both the season's the relationship was common although there was a variation for the strength of the association between the characters across the seasons. There was a strong positive correlation for root yield with five roots weight, root width, shoulder width, xylem width, shoot weight, shoot length, five shoot weight in S-I.

Along with these characters, root length (S-II), phloem width, and petiole length in S-II also showed positive association with the root yield and hence considered to be important yield components which help in indirect improvement of yield. In the present study, no characters showed significant negative association with yield. Similar findings on correlation and path analysis were reported by Dod *et al.*, 2013 and indicated that root yield per plant was closely associated with number of leaves per plant at harvest, fresh weight of leaves, root length, total plant weight, chlorophyll content of leaves and days required to harvest. Hence, these characters may be given consideration while making selection for the improvement of carrot.

Other important economic traits in carrot is harvest index and root to shoot ratio, which are very important for a farmer for profitable increased productivity are having negative association with number of petioles, petiole length, shoot length as well as five shoot weight (S-I and S-II). Similarly they had the strong positive correlation with phloem to xylem ratio and shoulder length. Although shoulder length is an undesirable trait to a consumer, but the carrot with higher shoulder length can give better weight and profit to a farmer, but as a breeder, reduced shoulder length is one of the breeding objectives in carrot improvement.

Although, carrot leaves were earlier consumed as vegetable like that of coriander, but the major consumption will be the tap root, hence, the concentration must be to develop better root yield and quality that can be achieved by optimum shoot and vegetative portions. In the present study, temperate types had lower shoot to root ratio as they are more of erect type of plant habit, less number of petioles, lesser shoot length and shoot weights than the local tropical types of carrot. Harvest index, root to shoot ratio and phloem to xylem ratio are positively associated with each other and with root yield. Hence, with the increase in these components, the ultimate root yield will be improved and productivity will also be further enhanced.

Among the biochemical characters, although there is no association between beta carotenoid and sugars as they are independent traits, but there is a significant negative correlation between non-reducing and reducing sugars (S-II) and strong positive correlation with total sugars. However, there was no association between them in other season. Interestingly few morphological characters showed significant positive

correlation with biochemical traits such as, total sugar had a positive correlation with root length (S-I), reducing sugar had negative correlation with harvest index and beta carotene (S-II), and five shoot weight (S-I).

5.1.5 Path coefficient analysis

Path coefficient analysis further provides an insight into the interrelationship of various characters with seed yield. In carrot, root yield is a complex character influenced by number of interrelated component traits. The inter dependence of the component character among themselves often influence the direct relationship with seed yield, as a result, information based on correlation coefficients becomes not dependable.

Since path coefficient analysis gives a more realistic interrelationship of characters for root yield. Hence, the 21 traits have been subjected to path coefficient analysis by keeping individual plant root weight/root yield as dependent trait and other 20 traits as independent traits. All the four biochemical parameters showed highest direct effects, although the trend was same between S-I, and S-II.

Among the indirect effects of morphological traits, xylem, phloem and cambium width, five roots weight, shoot length etc had the positive indirect effects through root width on root yield indicating their role in improvement in the root yield of carrot irrespective of the seasons.

Number of petioles, shoulder width, xylem and phloem width, shoot weight *etc* also influenced positively and indirectly through five roots weight on root yield and considered as important yield components in predicting the carrot yield. Shoulder length showed negative indirect effects through shoot weight on root yield and hence, need to be careful in selecting the superior genotypes for root yield as we need to keep the number of vegetative parts as optimum as possible so that not only the photosynthesis will be optimum but also the root yield.

Among the biochemical traits, in S-I, the positive indirect effect of root length through total sugars on root yield was highest followed by indirect effect of five shoot weight through reducing sugars. The indirect effect of beta carotene through total sugars was positive and high in both the seasons.

In S-II, the negative indirect of total sugars via many characters *viz..*, phloem width, five roots weight, harvest index, phloem to xylem ration etc was negative on root yield, in contrast, root to shoot ratio and harvest index, root length, shoulder length, cambium width *etc* showed positive indirect effect through total sugars on root yield.

In general, the trend was same for both the seasons indicating their consistent results across the seasons with respect to path coefficient analysis for the traits selected in the present study.

The least residual values in the present study for S-I and S-II indicates that the 21 characters selected for the study explains the sufficient amount of variation which is also supported and explained by PCA analysis above as it shows >80.0 % cumulative phenotypic variance by the principle components with >1.0 Eigen values.

Based on the path coefficient analysis, it can be concluded that, other than biochemical parameters, number of petioles, root length, root width, shoulder length and width will either directly or indirectly influence the root yield and hence they are considered to be important yield components in carrot improvement.

5.1.6 Morphological diversity analysis

Mahalanobi's D2 analysis is one of the most powerful approach for study of genetic diversity especially when the selection of diverse parents have to be made based on the morphological characters. In this approach, irrespective of the number of traits under study, it will give the detailed information about how many characters contributing to the diversity by taking care of genetic variation of the respective traits in the population. Here, the genotypes are grouped in to number of cluster, also the number of genotypes in the respective clusters, their genetic distance (both within and between clusters) and also the cluster means for the traits under study. Hence, we have the choice of selecting diverse genotypes from the diverse clusters based on the traits of interest of a breeder.

In the present study, 21 quantitative traits and also root colour (external and internal colours) which are considered to be important in the carrot breeding were

considered to study the genetic diversity among the 48 genotypes characterized in two seasons (S-I and S-II).

In general, biochemical traits contributed maximum to the genetic diversity in both the seasons studied and among morphological traits, shoulder length, five shoot weight, root colours and root to shoot ration contributed maximum to the diversity indicating the presence of highly diverse genotypes for these yield components and we can make a selection of diverse parents for hybridization program from the present study. The study is also supported by other analysis like PCA, correlation and path coefficient analysis indicating the importance of these traits in explaining the variation.

When the genotypes were grouped in to clusters, the number of clusters in S-I and S-II were different, but interestingly, two genotypes such as UHSBC-36 and UHSBC-08 consistently were present in different clusters and the clusters having these genotypes were also diverse in both the seasons. Based on the inter cluster distance in different seasons, cluster II and cluster III in S-I, cluster II and cluster IV and cluster VI in S-I and S-II respectively. Although, the cluster means for various traits was different in different seasons, but based on the breeder's objective, the desirable traits can be concentrated and the superior clusters for that traits could be selected suitable for either summer or winter seasons. In the present study, in S-I, highest root yield was shown by cluster I, but cluster II showed superior performance for biochemical traits. Similarly, in S-I, root yield was highest for cluster III and cluster IV was superior for biochemical traits indicating their possible exploitation for respective traits if we select the superior genotypes from these clusters. The present study would provide detailed information for a breeder on selection of diverse genotypes for breeding program specially for varietal development to tropical region and improvement of desirable traits of interest may be root colour, biochemical traits or productivity related traits based on their choice.

In general, from the phenotypic characterization, in the present investigation, extensive information about the root morphological characters which include, many qualitative and quantitative traits and also important quantitative traits, could be given to the researchers. This is the first report on such an extensive information of morphological and biochemical characterization of carrot genotypes involving tropical and temperate type of genotypes.

Even, with the various statistical analyses such as genetic variability estimates, principle component analyses, correlation and path coefficient analysis, diversity analysis, frequency distribution, the knowledge about the association pattern, contribution of various traits to the diversity, the frequency of various types of qualitative parameters present in the carrot germplasm could be understood. Hence, the present investigation about the root morphological traits, and biochemical traits will be definitely useful as readily available information for any students or scientists who initiate the carrot research.

5.2 Microsatellite based allelic diversity

The number of clusters obtained for 62 allelic data from 24 genotypes was mainly two with the number of sub clusters in them with 4 and 21 subclusters. The genotypes grouped in to the cluster I were all of IIVR collections obtained from Varanasi. In cluster II, 21 sub clusters were obtained of which thirteen clusters were solitary clusters with single genotypes. The genotypes belonging to these solitary clusters consisted of local types, few IIAR collection and also temperate carrots such as century super Kuroda and century super Nantes, Ghataprabha local, black wonder, other local collections from Karnataka etc. The genotypes belonging to solitary clusters are supposed to have a higher genetic distance from the VRCAR collections between and within the clusters and considered to be diverse and hence there is a possibility of utilizing these temperate and tropical carrots in hybridization program. The main problem comes for hybridization program is the flowering of temperate adopted carrots which will not flower in the tropical region. Further, this problem could be solved by either shuttle breeding or givig a vernalization treatments to the temperate carrots after harvesting by keeping them in cold storage for 6-10 weeks which is a common practice followed for temperate type of carrots.

Although, the markers used for the study were either gene specific or the markers derived from the coding regions as they were designed from the cDNA library (Cavagnaro *et al*., 2011; Budahn *et al*., 2014; Soufflet-Freslon, 2013; Maksylewicz and Baranski, 2013), the number of alleles obtained in the study (62) are considered to be significant. Hence, the carrot genotypes are supposed to be highly diverse as they are collected from different geographical regions of India, such as Karnataka, Maharashtra,

New Delhi, Varanasi etc and also contains different coloured and types (temperate and tropical) as shown by the detailed phenotypic characterization. But when compared to the available publications (Budahn *et al*., 2014; Soufflet-Freslon, 2013; Maksylewicz and Baranski, 2013; Cavagnaro *et al*., 2011) which used these markers to study the diversity of temperate genotypes using capillary electrophoresis, the number of alleles obtained for respective markers were exceptionally high in comparison to the present study. For example, Maksylewicz and Baranski (2013) reported as high as eighteen alleles for DCM 2, 14 alleles for GSSR 4, eight alleles for DcM 17 etc. the lesser number of alleles obtained in the present study is mainly due to screening of genotypes in Agarose gel, but the published papers are mainly screened them in fragment analyzer. Hence, it is necessary to screen these markers in capillary electrophoresis or at least in denaturing Polyacrylamide Gel electrophoresis.

With respect to the number of alleles, it ranged from two to six with GSSR 19 gave better resolution even in Agarose gel with as high as six alleles. Few of the gene specific markers were scored as presence/absence as per the guidelines of the publications Budahn et al., 2014; Soufflet-Freslon, 2013). Among the gene specific markers such as EXON 1-EXON 2, EXON 9-EXON 11, EXON 3-EXON 5 were showing more than one alleles and hence they were scored based on the allele sizing. The highest PIC value was shown by EXON 4-EXON 7 although it's screening was similar to dominant type of marker. The highest marker index of 37.88 for GSSR19 indicates its efficiency in screening the genotypes even in Agarose electrophoresis also. The highest heterozygosity value for GSSR16 and GSSR19 indicates their efficiency in detecting polymorphism even in the agarose gel electrophoresis.

In the present study, although allelic diversity for the selected markers was not much higher, despite using the highly diverse carrot genotype, but the study showed the utility of the gene specific as well as genic microsatellite markers in diversity analysis even in the smaller labs with no sophisticated lab equipments like fragment analyzer or denaturing PAGE.

5.3 Marker-Trait association

In the present study, although only 24 markers were polymorphic across the 48 genotypes but there were 62 alleles available in the study and all these 64 alleles were subjected to step wise linear regression analysis to identify the markers associated with all the 39 traits recorded in the study across both the seasons. The regression value (R^2) explaining the significant phenotypic variance was obtained as cumulative R^2 values for each trait by various markers.

In the first season, cumulative regression value (R^2) for root yield was 0.455 explained by 4 alleles among them Exon1-Exon2 contributed 0.214 which was highest for this trait. Exon1-Exon2 is a coding region of CRITSO gene involved in carotenoid biosynthetic pathway.

The highest phenotypic variance among all the characters was obtained for shoulder length which was together contributed by 7 alleles (6 markers) with 0.658 R^2 value, but that was an undesirable trait and commonly present in tropical type of carrots. Among the important root yield and yield components, for root yield/plant and five roots yield, the total R^2 explained was 0.455 from 4 molecular markers.

For external root colour, two markers such as GSSR16 and GSSR138, were consistently showing significant R^2 in both S-I and S-II, however the cumulative R^2 explained by 4 markers (5 alleles) in S-II is comparatively less (0.421) than S-I (0.444) with only 3 markers (4 alleles) in this season. GSSR138 also showed consistent association with phloem colour in both the seasons, however, it has no significant association with either xylem or cambium colour in both the seasons. Since, phloem colour and external root colour are the most important economic traits, hence, GSSR138 along with GSSR16 could be used for future selection of desirable root colour.

Among the quantitative traits of root morphological characters, for harvest index GSSR9, 6, 19 and GSSR85 consistently showed association in both the seasons indicating their efficiency in selection of higher harvest index genotypes of carrot grown under different environmental conditions. Root to shoot ratio is another yield

component, for which, again, GSSR9, GSSR19 and GSSR85 showed consistent significant influence on this trait in both the seasons.

With respect to phloem to xylem ratio, DCM2 and GSSR 16 were common between the seasons and other markers which showed significant influence on this trait were season dependent and hence need to be validated further.

Among the biochemical traits, for beta carotenoid content, EXON9–EXON11 was consistent across the seasons which are a derived marker from a CRTISO gene of carrot and showed significant association with beta carotene in the present study. GSSR4 and GSSR85 were other two markers consistent across the seasons for beta carotene. The cumulative effect of phenotypic variance (R^2) explained for this trait in second season was high (0.485) compared to first season (0.432) due to the contribution of more number of markers in S-II.

Among the other biochemical parameters, for total sugar content, only two markers showed marker-trait association only in first season. No significant association was found with any markers in season-II may be due to the reason that, the number of markers used in the present study is not enough to explain the phenotypic variance. Hence, it is recommended to use more number of molecular markers for marker-trait association preferably by LD mapping, which will be more meaningful.

In general, for most of the traits, the marker-trait association was consistent across the seasons for most of the traits in the present study. For traits like harvest index, as high >0.90 cumulative R^2 value was obtained in the present study. The markers identified for few economic traits such as root colour (GSSR16 and GSS32), root length (GSSR16), root yield per plant and five roots weight (GSSR16 and GSSR63), root to shoot ratio (GSSR19 and GSSR85), Harvest index (GSSR85, GSSR19) were showing consistent significant association for the respective traits. Hence, these markers could be further confirmed by either linkage disequilibrium mapping (LD) or biparental mapping for effective selection of these respective root traits of carrot in Marker assisted breeding program (MAS).

Although present study involves few genotypes and few markers, but it provided very good information about the marker trait association as well as sufficient allelic diversity which could be further useful as basic information for advanced research.

5.4 Identification of superior carrot germplasm`

The present investigation was conducted with the aim of identifying the superior carrot genotypes suitable to tropical region. Hence, elaborative characterization was done with respect to morphological and biochemical characters and the superior carrots were selected for individual seasons (summer and winter) as well as across the seasons. The main criteria followed was root colour (uniformity with external and internal), root yield, beta carotenoids total sugars *etc.*

In S-I, three best genotypes selected were UHSBC-32 (VRCAR-20), UHSBC-44 (VRCAR-62) and UHSBC-52 (VRCAR-81), with the highest root yield of 80.75 gms for UHSBC32 with uniform orange root colour which is highly preferred by consumers. However, the genotype UHSBC-44 (VRCAR-62), a collection from IIVR was superior in terms of biochemical components among the three genotypes selected with medium root yield (70.25 g).

Similarly in S-II, a black coloured carrot genotype, UHSBC-66 (Pusa Asita) performed well with the highest root yield of 73.02 g, 68.36 g respectively. Although black coloured carrots are rich in antioxidants, contain 2-3 times more carotenoid than orange coloured carrots, but generally consumer won't prefer the black coloured types. However, the health conscious people purchase or prefer to consume black coloured types from supermarkets.

The black coloured carrot can also be used as a parent in hybridization program with other contrasting coloured carrot to develop mapping population or segregating generations to study inheritance of root colour and other linked characters.

In the present study, extensive investigation has been done to characterize the local types, germplasm lines, released varieties having multiple coloured carrots with large number of phenotypic characters and few genic microsatellites and few gene specific markers such as Y2 marker, gene for CRITSO (involved in carotenoid

biosynthetic pathway). The carrot genotypes were studied in tropical region in order to identify the superior carrot lines for various biochemical and productivity traits and identified few genotypes such as UHSBC-32, UHSBC-44, UHSBC-52 for S-I and three genotypes viz., UHSBC-66 (Pusa Asita), UHSBC-17 (Ghataprabha local-2) and UHSBC-22 (Jutt Local) are considered to be superior for winter seasons in the tropical region. They are considered to be superior for various traits such as root yield and yield components like harvest index, root width and also biochemical parameters like beta carotenoids and sugars.

The present study thrown a light on the information pertaining to genetic variability and heritability, the distribution of various qualitative characters, the association pattern, the direct and indirect effects and the principle components involved in genetic variability in carrot.

Along with this, the morphological land molecular diversity analysis, identified the diverse genotypes to colours (UHSBC66 for black and UHSBC-32 for orange) and higher productivity (UHSBC-08 and UHSBC-36). Based on the molecular data analysis, the tropical and temperate carrot genotypes were grouped in to separate clusters based on the dissimilarity index and local varieties were separated from the private sector cultivars indicating the broad genetic base of carrot genotypes used in the present study.

Local types like Jatt local, Ghataprabha local *etc* were separated from temperate carrot genotypes in the diversity analysis. Hence these genotypes would serve as good parents for combining the desirable traits from temperate as well as tropical region through hybridization. A black coloured variety Pusa Asita thrives very well in the tropical region also, hence, there is a scope to promote multi-coloured carrot even to the tropical region. This type of extensive work on phenotypic characterization is the first of its kind in general in carrot and in specific to tropical carrots.

Hence, the present study would provide detailed information to plan for the following future breeding and crop improvement program of carrot.

Future line of work

- ✓ The data generated from the present study and the other seasons data will help to propose an Ideotype of carrot suitable for tropical region
- ✓ Development of mapping populations for various types such as tropical x temperate types, black x orange/white coloured genotypes
- ✓ The contrasting genotypes of coloured carrots would be subjected to metabolomic profiling as the present germplasm material consisted of various coloured carrots with different biochemical components
- ✓ The material would be useful for exploiting heterosis for productivity and development of synthetics or hybrids
- ✓ The genetics of various characters could be further studied

6. SUMMARY AND CONCLUSIONS

Carrot is among the top ten most important vegetable crops and a major source of carotenoids. Although, it is considered as a cool season crop, but the tropical genotypes adoptable to warmer climates are also available, however, systematic studies on the tropical carrot genotypes is very limited and they are less explored in the breeding program. Hence, the present study was conducted for detailed characterization of carrot genotypes of tropical region as well as temperate types for various morphological, biochemical characters. Genotypes consisted of various coloured carrot ranging from white to black with more of orange coloured types.

These thirty nine traits and twenty four markers (62 loc) were subjected to various statistical analysis such as ANOVA, genetic variability ad heritability analysis, correlation and path coefficient analysis, Principle component analysis, Mahalanobi's D^2 analysis. The 21 qualitative traits recorded based on the descriptors were also subject to frequency distribution analysis. The marker data was subjected to dissimilarity coefficient analysis to know the diversity among the genotypes used in the study. the marker and traits were combined to know the marker-trait association based on the step wise linear regression analysis.

Analysis of variance in both the seasons revealed significant variation for most of the traits among the genotypes indicating the existence of sufficient variation for the traits under study and the genotypes are diverse. Among the biochemical parameters such as beta carotenoids, total sugars, reducing sugars and non-reducing sugars, ANOVA revealed significant variation across the carrot genotypes in both the seasons.

In the present study, wider range of variation was available for almost all the quantitative traits studied in both the seasons (S-I and S-II) again indicating the presence of good amount of variation and scope for further improvement by breeding. Among the qualitative traits also, lot of variation was seen in the genotypes especially for root colours (internal and external colours) indicating further scope for selection of desirable colours as per the consumer preference. Also, the superior lines suitable to tropical region identified in the present study having contrasting colours such as orange,

red, pink black or purple with yellow or white colour to develop the mapping populations.

When these characters were analyzed for genetic variability components such as GCV and PCV, very wide range of variation with higher PCV and GCV was observed for root yield. All the four biochemical parameters such as beta-carotene, total sugars, reducing and non-reducing sugars showed greater genetic variation in both the seasons as depicted by their wider range, higher PCV and GCV. Among the morphological traits, highest heritability was recorded for shoulder width (77.0%) followed by root tip (73.0%) in S-I, root to shoot ratio, harvest index and xylem colour (81.0%) followed by phloem colour (78.0%).

Based on the frequency distribution analysis, most of the qualitative traits showed skewed distribution for the genotypes used in the study. Uniformity in external and internal roots is an important breeding objective in carrot; hence, the observations of vascular tissues such as xylem, phloem and cambium colours were also recorded in both the seasons. In S-I, xylem and cambium colour were showing skewed distribution towards white colour (with score 1.0-3.0) but in phloem majority of the genotypes shown orange colour. Very few genotypes, both external and internal root colours were same like white-white, red-red, orange-orange, black-black *etc* were shown. In S-II, except cambium colour, the distribution of xylem and phloem were skewed, but for cambium colour it was showing normal distribution ranging white (1.0) to black (7.0).

In the present study, since 48 genotypes were characterized with many (39) characters in both the seasons, hence, to know the important quantitative characters which explain the highest variation would be studied. Although, 39 traits were studied including 18 qualitative traits, the first important assumption to be fulfilled in PCA is that, the traits under study must be quantitative in nature and must show continuous variation; hence, only 21 quantitative traits were subjected to PCA analyses. Twenty one characters were partitioned in to seven and six main components with the cumulative percent of variation 83.0% in individual seasons. Individual shoot weight, harvest index, five shoots weight, no of petioles, shoot length and five roots weight are the important traits present in first two principle components

Twenty one quantitative traits were subjected to correlation analysis to know the association pattern among them in both the seasons. In general, the relationship was common and there was variation for strength of the association between the characters across the seasons. There was a strong positive correlation for root yield with five roots weight, root width, shoulder width, xylem width, shoot weight, shoot length, five shoot weight in S-I.

Along with these characters, root length, phloem width, and petiole length in S-II hence considered to be important yield components which help in indirect improvement of yield. In the present study, no characters showed significant negative association with yield. Among the biochemical characters, although there is no association between beta carotenoid and sugars as they are independent traits, but there is a significant negative correlation between non-reducing and reducing sugars (S-II) and strong positive correlation with total sugars. Interestingly, few morphological characters showed significant positive correlation with biochemical traits such as, total sugar had a positive correlation with root length (S-I), reducing sugar had negative correlation with harvest index and beta carotene (S-II) and five shoot weight (S-I).

Path coefficient analysis partitioned the total of twenty one quantitative traits in to direct and indirect effects on a dependent variable root yield per plant. Among the morphological traits, root width, shoot weight, five roots weight and harvest index have direct positive influence on root yield and xylem width, five shoot weight had negative direct influence on root yield. Among the indirect effects of morphological traits, xylem, phloem and cambium width, five roots weight, shoot length etc had the positive indirect effects through root width on root yield indicating their role in improvement in the root yield of carrot irrespective of the seasons.

Mahalanobi's D^2 analysis partitioned twenty one traits in to different clusters (3 in S-I and 4 in S-II) in both the seasons. Based on the inter cluster distance in different seasons, cluster II and cluster III in S-I and cluster II and cluster IV in S-II were identified as diverse clusters.

In the present study, in S-I, highest root yield was shown by cluster I, but cluster II showed superior performance for biochemical traits. Similarly, in S-I, root yield was

highest for cluster III and cluster IV was superior for biochemical traits indicating their possible exploitation for respective traits if we select the superior genotypes from these clusters.

A total of 62 loci were subjected to molecular marker diversity analyses across the 48 genotypes based on dissimilarity index. The number of clusters obtained for 62 allelic data from 24 genotypes was mainly two with the number of sub clusters in them with 4 and 21 sub clusters. The genotypes grouped in to the cluster I were all of IIVR collections obtained from Varanasi. In cluster II, 21 sub clusters were obtained of which thirteen clusters were solitary clusters with single genotypes. The genotypes belonging to these solitary clusters consisted of local types, few IIAR collection and also temperate carrots such as century super Kuroda and century super Nantes, Ghataprabha local, black wonder, other local collections from Karnataka .

Although, the markers used for the study were either gene specific or the markers derived from the coding regions as they were designed from the cDNA, the number of alleles obtained in the study (62) is considered to be significant. Hence, the carrot genotypes are supposed to be highly diverse as they are collected from different geographical regions of India, such as Karnataka, Maharashtra, New Delhi, Varanasi etc and also contains different coloured and types. With respect to the number of alleles, it ranged from two to six with GSSR 19 gave better resolution even in Agarose gel with as high as six alleles. The highest heterozygosity value for GSSR16 and GssR19 indicates their efficiency in detecting polymorphism even for segregating population. The highest PIC value was shown by EXON 4-EXON 7 although it's screening was similar to dominant type of marker and the highest marker index of 37.88 for GSSR19.

In the marker-trait association, many markers showed consistent association across the seasons for most of the traits in the present study. For traits like harvest index, as high >0.90 cumulative R^2 value was obtained in the present study. The markers identified for few economic traits such as root colour (GSSR16 and GSS32), root length (GSSR16), root yield per plant and five roots weight (GSSR16 and GSSR63), root to

shoot ratio (GSSR19 and GSSR85), Harvest index (GSSR85, GSSR19) were showing consistent significant association for the respective traits.

In the present study, three best genotypes for respective seasons were selected based on the economic traits such as root yield root colour and biochemical parameters as selection criteria. Few genotypes such as UHSBC-32, UHSBC-44, and UHSBC-52 for summer season and three genotypes *viz*., UHSBC-66 (Pusa Asita), UHSBC-17 (Ghataprabha local-2) and UHSBC-22 (Jutt Local) are considered to be superior for winter seasons in the tropical region.

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Appendix I: Mean data for 39 characters for the 48 genotypes used in the study in Season-I

S. No	GEN NO.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22
1	UHSBC02	7.00	1.00	2.00	1.20	1.90	1.00	2.00	2.00	3.10	5.00	1.00	1.60	1.00	7.00	2.00	2.60	5.80	6.00	6.60	1.81	2.95	1.00
2	UHSBC06	7.00	1.13	3.00	1.25	1.00	1.63	2.00	2.00	1.63	4.75	2.25	1.13	2.00	7.00	3.75	7.00	7.00	1.00	13.00	1.68	2.07	0.98
3	UHSBC07	6.00	1.00	3.00	1.00	1.00	1.00	1.00	1.50	1.00	5.00	1.00	1.00	1.75	6.00	1.50	3.00	3.00	3.00	7.50	1.51	1.98	0.91
4	UHSBC08	7.00	1.00	3.00	1.00	1.00	1.40	2.00	2.00	2.70	4.30	1.50	1.10	2.00	7.00	1.20	7.00	6.00	1.00	9.60	1.56	1.94	0.84
5	UHSBC11	5.40	1.00	2.10	1.00	1.10	1.10	2.00	2.00	1.80	4.90	2.70	1.00	1.60	7.00	1.00	6.00	7.00	1.00	10.60	1.47	1.19	1.09
6	UHSBC13	6.00	1.00	2.00	1.00	1.00	1.17	1.00	1.50	1.50	4.25	1.67	1.00	1.25	6.00	1.50	3.00	3.00	3.00	7.58	2.07	1.98	1.05
7	UHSBC14	9.00	1.00	1.80	1.00	1.60	1.00	2.00	2.00	3.00	5.00	1.20	1.80	1.00	5.60	2.00	3.70	6.00	3.90	8.30	1.98	3.32	1.12
8	UHSBC17	6.60	1.00	1.00	2.00	1.80	1.00	1.80	1.00	2.10	5.00	1.40	1.90	1.00	5.70	6.60	1.00	6.00	1.00	7.80	1.74	3.28	1.18
9	UHSBC19	7.00	1.00	1.90	1.45	1.90	1.00	2.00	2.00	2.70	5.00	1.70	1.40	1.00	3.00	1.70	1.00	2.00	1.00	9.80	1.65	2.37	0.89
10	UHSBC21	7.00	1.00	2.00	2.00	2.40	1.10	2.00	2.00	2.00	4.60	1.20	1.80	1.10	8.40	2.00	3.20	7.20	5.40	9.50	1.56	3.29	0.98
11	UHSBC22	7.00	1.00	1.90	1.20	1.20	1.00	2.00	2.00	1.80	4.60	1.50	1.50	1.00	8.13	2.00	1.00	6.00	6.00	8.10	1.89	3.22	1.24
12	UHSBC23	9.00	1.00	1.70	1.00	1.20	1.00	2.00	2.00	2.30	5.00	1.60	1.00	1.00	9.60	2.00	2.10	8.10	3.60	8.00	1.75	2.56	0.91
13	UHSBC24	7.00	1.10	1.90	1.00	1.90	1.00	1.90	1.90	2.70	4.90	1.90	1.00	1.00	8.70	2.00	1.50	6.80	3.80	12.95	1.27	1.41	0.55
14	UHSBC25	9.00	1.00	1.90	1.00	1.60	1.00	2.00	2.00	1.60	5.00	1.50	1.10	1.00	10.30	2.00	1.00	9.00	6.00	8.40	1.74	2.54	1.08
15	UHSBC26	7.00	1.00	2.00	1.00	1.70	1.00	2.00	2.00	2.60	5.00	1.60	1.20	1.00	4.90	2.70	1.50	5.60	5.50	9.90	1.14	1.69	0.73
16	UHSBC27	7.00	1.00	1.90	1.80	2.10	1.10	2.00	2.00	2.20	5.00	1.60	1.00	1.00	6.80	1.70	1.00	6.10	6.00	9.00	1.39	2.11	0.91
17	UHSBC28	7.00	1.30	2.00	1.00	2.90	1.00	2.00	2.00	2.90	5.00	1.00	1.00	1.00	9.10	2.00	3.60	7.50	2.70	9.20	1.49	1.93	0.86
18	UHSBC29	6.60	1.06	1.90	1.40	2.34	1.00	2.00	2.00	1.76	5.00	1.60	1.30	1.10	5.24	1.30	4.90	6.44	4.92	10.25	1.34	1.64	0.78
19	UHSBC30	7.00	1.00	2.00	1.20	2.10	1.00	2.00	2.00	2.00	5.00	1.30	1.80	1.00	8.00	1.90	1.00	6.90	6.00	9.90	1.55	2.66	0.86
20	UHSBC31	5.90	1.20	2.00	1.20	2.60	1.00	2.00	2.00	1.70	5.00	1.95	1.00	1.00	6.35	1.80	1.50	4.00	4.75	10.10	1.55	2.21	0.98
21	UHSBC32	3.20	1.10	2.00	1.20	1.90	1.10	2.00	2.00	2.20	4.80	1.30	1.60	1.00	5.30	2.90	2.50	4.50	1.80	10.30	1.80	2.70	1.27
22	UHSBC33	3.00	1.00	1.90	2.60	1.60	1.00	2.00	2.00	1.90	5.00	1.50	1.30	1.00	3.30	1.90	2.60	5.70	4.80	10.50	1.80	2.26	0.99
23	UHSBC34	7.00	1.25	2.00	1.50	2.00	1.25	2.00	2.00	1.50	5.00	2.00	1.00	1.00	2.00	1.50	1.00	6.00	6.00	10.75	1.89	2.17	1.03
24	UHSBC34-1	7.00	1.08	1.43	1.15	1.30	1.08	2.00	2.00	1.77	5.00	1.53	1.00	1.57	2.80	1.95	1.00	7.40	6.00	10.18	1.59	2.13	1.03
25	UHSBC35	8.00	1.00	2.00	1.00	2.10	1.10	2.00	2.00	3.10	5.00	2.40	1.00	1.00	7.60	4.20	1.00	6.70	6.70	9.70	1.54	2.02	0.77

26	UHSBC36	7.60	1.00	2.00	1.00	2.80	1.10	2.00	2.00	2.10	5.00	2.10	1.00	1.20	9.00	4.30	1.90	8.30	3.20	12.40	1.73	2.22	0.80
27	UHSBC37	8.20	1.10	1.90	1.00	2.40	1.20	2.00	2.00	2.80	5.00	1.80	1.00	1.20	8.20	2.00	2.30	8.10	2.00	10.30	1.62	2.30	1.03
28	UHSBC38	9.00	1.00	1.90	1.80	2.40	1.00	2.00	2.00	2.00	5.00	2.10	1.50	1.40	5.80	3.20	1.00	6.30	2.00	9.10	1.76	2.61	0.96
29	UHSBC39	7.20	1.10	2.00	1.00	2.20	1.10	2.00	2.00	3.60	4.80	2.00	1.00	1.60	6.40	2.50	1.50	5.90	1.90	13.90	1.97	2.68	1.01
30	UHSBC40	9.00	1.10	1.70	1.00	2.20	1.00	2.00	2.00	3.00	5.00	1.60	1.00	1.20	7.40	5.60	1.30	7.20	1.30	10.70	3.07	2.04	1.15
31	UHSBC41	9.00	1.00	2.00	1.00	2.50	1.50	2.00	2.00	3.33	4.50	1.50	1.00	1.25	3.00	1.83	1.00	5.00	2.00	8.42	1.97	2.84	1.20
32	UHSBC41-1	9.00	1.00	2.00	1.00	1.50	2.50	2.00	2.00	2.50	4.00	2.50	1.00	1.25	2.00	2.00	1.00	2.00	2.00	10.25	1.87	2.49	1.13
33	UHSBC42	9.00	1.30	2.00	1.00	1.80	1.00	2.00	2.00	2.70	5.00	2.30	1.10	1.10	9.50	6.00	1.00	7.50	2.00	13.40	1.65	1.98	0.83
34	UHSBC43	7.00	1.00	2.10	1.60	3.00	1.00	2.00	2.00	1.90	5.00	2.00	1.90	1.00	2.25	1.00	2.00	8.30	4.20	10.50	2.03	2.88	1.22
35	UHSBC43-1	9.00	1.00	2.00	1.00	2.56	1.00	2.00	2.00	2.64	5.00	3.08	1.00	1.00	3.83	1.00	2.00	6.75	5.83	10.11	1.74	2.44	0.88
36	UHSBC44	9.00	1.20	1.90	1.60	3.00	1.10	2.00	2.00	3.10	5.00	1.90	1.10	1.30	7.30	2.40	3.50	6.20	4.80	18.70	1.98	3.03	0.99
37	UHSBC45	7.00	1.30	2.00	1.00	2.98	1.00	2.00	2.00	2.68	5.00	2.04	1.42	1.66	7.04	3.66	1.00	6.00	2.00	13.70	1.40	2.26	0.87
38	UHSBC46	7.00	1.00	2.00	1.00	1.30	1.00	2.00	2.00	3.10	5.00	1.90	1.10	1.30	5.10	1.00	3.30	6.90	3.80	8.90	1.91	2.58	1.07
39	UHSBC47	8.00	1.00	2.00	1.60	2.00	1.00	2.00	2.00	2.40	5.00	1.90	1.40	1.10	7.60	3.80	1.60	9.00	4.00	10.40	1.73	2.56	1.05
40	UHSBC48	7.00	1.30	2.10	1.00	2.30	1.00	2.00	2.00	3.30	5.00	2.00	1.40	1.00	8.40	3.80	2.00	8.40	4.00	16.10	1.66	2.28	0.93
41	UHSBC49	7.20	1.00	1.70	1.00	2.40	1.00	2.00	2.00	2.40	5.00	2.20	1.50	1.10	8.30	3.80	2.40	7.50	3.80	12.40	1.86	2.28	1.10
42	UHSBC52	7.80	1.00	2.00	1.40	3.10	1.10	2.00	2.00	2.30	4.80	2.53	1.00	1.10	9.90	6.00	2.40	9.00	4.00	12.20	1.45	1.75	0.75
43	UHSBC53	7.20	1.20	2.00	1.80	2.90	1.00	1.00	2.00	2.80	4.60	1.30	1.10	1.00	8.60	3.60	1.00	8.30	2.00	12.60	1.47	2.31	0.97
44	UHSBC59	8.60	1.40	1.90	1.00	2.30	1.40	1.90	2.00	3.20	5.00	2.40	1.50	1.60	7.60	1.50	2.40	8.70	3.80	10.80	2.20	2.42	1.32
45	UHSBC64	8.00	1.30	2.10	1.00	2.40	1.10	2.00	2.00	2.60	4.40	1.60	1.10	1.20	6.80	1.30	2.50	9.00	2.00	14.40	1.51	2.39	0.89
46	UHSBC66	7.80	1.00	1.60	1.00	5.15	1.00	2.00	2.00	2.30	5.00	1.90	1.00	1.20	14.10	7.70	2.00	3.00	2.10	11.90	2.27	3.35	1.43
47	UHSBC68	5.90	1.00	2.75	1.50	1.00	1.50	2.00	2.00	2.45	4.45	2.55	1.50	1.90	6.00	2.65	6.50	1.50	6.50	11.15	1.33	1.57	0.73
48	UHSBC69	6.80	1.10	2.90	1.00	1.00	1.20	2.00	2.00	2.10	4.40	1.30	1.10	1.50	7.00	2.10	5.80	7.00	6.00	11.50	1.61	1.84	0.76

Appendix II: Mean data for 39 characters for the 48 genotypes used in the study in Season-I (X23 to X39)

S. No	GEN NO.	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39
1	UHSBC02	0.53	0.28	24.87	19.38	50.12	0.82	42.00	39.00	192.00	209.00	0.87	54.07	0.53	327.08	7.17	2.65	4.52
2	UHSBC06	0.39	0.32	15.45	17.93	28.55	1.10	30.25	15.25	122.00	60.00	2.06	66.55	0.40	420.60	10.40	8.03	2.37
3	UHSBC07	0.31	0.29	15.08	17.13	28.75	1.00	24.60	12.10	122.00	46.00	2.65	66.81	0.41	304.40	9.07	3.95	5.13
4	UHSBC08	0.40	0.32	16.23	18.49	33.80	0.87	30.60	12.20	151.00	59.00	2.47	70.11	0.51	379.86	7.29	4.43	2.86
5	UHSBC11	0.19	0.19	15.61	19.20	17.24	1.64	21.60	10.00	107.00	32.00	3.41	68.67	0.17	367.59	9.07	7.44	1.63
6	UHSBC13	0.44	0.58	14.75	14.88	28.33	1.40	48.60	57.00	244.00	277.00	0.89	45.91	0.42	336.34	8.88	6.63	2.25
7	UHSBC14	0.39	0.48	27.66	22.43	54.33	1.75	49.20	63.20	244.00	313.00	0.78	43.64	0.35	317.13	11.07	8.43	2.64
8	UHSBC17	0.48	0.08	26.80	20.40	56.50	1.27	61.00	54.80	273.00	297.00	0.93	52.50	0.41	354.86	13.93	10.75	3.17
9	UHSBC19	0.25	0.51	22.98	25.20	58.00	1.10	19.75	52.72	126.00	284.00	0.44	27.21	0.29	295.14	11.27	10.40	0.87
10	UHSBC21	0.33	0.25	25.01	20.38	60.70	0.89	51.80	89.00	258.00	429.00	0.62	36.99	0.34	340.05	5.84	3.95	1.90
11	UHSBC22	0.49	0.16	23.36	23.35	54.57	1.46	54.80	64.80	269.00	320.00	0.84	45.82	0.40	338.19	9.47	6.90	2.57
12	UHSBC23	0.33	0.52	20.94	23.10	44.62	1.42	42.60	66.70	202.00	329.00	0.64	39.50	0.36	337.96	12.81	9.24	3.57
13	UHSBC24	0.30	0.42	10.97	12.30	32.03	0.70	15.80	26.60	73.00	152.00	0.48	37.48	0.55	293.98	10.24	7.95	2.29
14	UHSBC25	0.45	0.21	18.70	23.43	39.36	0.93	32.60	39.40	157.00	194.00	0.81	45.43	0.42	339.12	10.71	7.90	2.81
15	UHSBC26	0.22	0.19	20.07	18.61	44.86	0.83	19.80	48.20	99.00	242.00	0.40	28.64	0.30	306.94	10.24	8.54	1.70
16	UHSBC27	0.25	0.23	14.97	16.47	37.89	0.48	23.40	48.40	116.00	243.00	0.49	33.09	0.27	330.09	10.24	8.54	1.70
17	UHSBC28	0.36	0.27	16.31	15.85	39.42	0.59	28.20	45.90	140.00	221.00	0.66	38.21	0.41	315.74	16.30	10.68	5.62
18	UHSBC29	0.27	0.29	15.63	13.48	39.64	0.90	14.60	34.50	61.00	127.00	0.40	28.29	0.34	317.36	13.87	10.21	3.66
19	UHSBC30	0.35	0.35	20.65	23.56	47.15	1.32	34.00	58.60	170.00	283.00	0.58	35.98	0.41	305.56	14.38	10.24	4.14
20	UHSBC31	0.37	0.21	16.86	15.50	42.29	0.65	28.00	44.60	98.00	157.00	0.66	38.53	0.41	326.16	12.22	7.12	5.10
21	UHSBC32	0.35	0.18	23.67	16.26	45.74	0.88	37.60	70.50	182.00	347.00	0.52	34.03	0.27	295.83	15.68	3.55	12.12
22	UHSBC33	0.30	0.51	22.67	18.94	51.70	0.54	34.60	77.60	173.00	380.50	0.45	30.82	0.30	317.59	7.11	5.14	1.97
23	UHSBC34	0.33	0.54	19.90	22.15	50.05	0.89	40.00	49.00	202.00	319.00	0.65	44.71	0.32	304.17	9.23	7.29	1.93
24	UHSBC34-1	0.42	0.13	19.08	19.39	49.92	0.82	41.90	63.57	202.00	319.00	0.65	39.73	0.41	314.35	6.70	6.66	0.04
25	UHSBC35	0.27	0.50	21.49	20.48	43.09	1.39	24.60	34.80	123.00	171.00	0.72	41.37	0.35	370.60	9.42	7.24	2.18

26	UHSBC36	0.21	0.72	19.38	19.50	48.26	1.15	39.80	46.40	127.00	154.00	0.76	46.25	0.27	376.39	21.38	11.77	9.61
27	UHSBC37	0.20	0.39	16.46	24.73	41.36	1.16	32.00	29.80	150.00	151.00	1.10	52.60	0.19	371.53	9.19	5.10	4.09
28	UHSBC38	0.39	0.41	22.10	21.65	43.49	2.02	37.80	33.20	188.00	163.00	1.15	53.20	0.41	359.26	9.42	6.62	2.80
29	UHSBC39	0.33	0.63	18.37	18.41	47.14	1.08	37.40	53.40	188.00	254.00	0.76	41.26	0.33	337.50	21.38	6.34	15.04
30	UHSBC40	0.27	1.65	21.87	24.88	49.31	1.25	49.80	64.00	246.00	318.00	0.77	43.76	0.24	368.98	12.10	7.65	4.45
31	UHSBC41	0.35	0.42	31.70	22.16	44.90	1.75	41.50	42.00	180.00	241.00	0.75	50.20	0.29	310.19	12.17	8.69	3.48
32	UHSBC41-1	0.28	0.47	19.08	22.98	44.40	1.33	39.50	57.50	180.00	241.00	0.75	39.93	0.25	309.72	15.02	11.29	3.74
33	UHSBC42	0.31	0.51	15.76	21.46	43.41	1.33	30.40	36.80	186.00	148.00	1.29	45.07	0.37	381.94	11.22	9.14	2.08
34	UHSBC43	0.38	0.43	22.80	18.08	54.00	1.64	44.00	58.20	174.00	224.00	0.68	43.05	0.31	368.06	14.49	6.73	7.76
35	UHSBC43-1	0.34	0.53	20.05	23.98	47.56	0.69	32.67	34.33	163.33	171.67	0.79	48.76	0.38	339.81	16.51	8.50	8.01
36	UHSBC44	0.20	0.79	20.21	22.23	51.01	1.10	51.60	74.80	256.00	373.00	0.70	41.16	0.20	357.87	7.63	4.51	3.13
37	UHSBC45	0.25	0.28	16.92	13.91	41.89	0.85	19.80	34.93	99.00	176.00	0.55	35.01	0.29	337.27	11.41	6.95	4.45
38	UHSBC46	0.63	0.21	23.74	23.50	56.05	0.74	40.20	55.40	200.00	279.00	0.74	42.61	0.61	298.61	7.63	3.62	4.02
39	UHSBC47	0.25	0.43	24.61	19.99	56.89	0.98	38.60	49.00	192.00	243.00	0.79	44.08	0.24	366.90	9.19	4.61	4.59
40	UHSBC48	0.27	0.46	19.08	24.00	45.12	1.16	48.56	44.40	233.00	291.00	0.75	53.03	0.29	386.11	8.04	5.06	2.97
41	UHSBC49	0.19	0.57	20.40	21.35	52.13	0.73	35.40	54.40	179.00	269.00	0.67	39.47	0.18	340.74	8.22	1.29	6.92
42	UHSBC52	0.25	0.45	17.17	15.71	42.84	1.17	23.60	29.40	120.00	149.00	0.81	44.70	0.33	378.24	15.46	5.06	10.40
43	UHSBC53	0.32	0.18	16.31	16.78	38.94	0.87	29.00	40.20	143.00	203.00	0.81	42.79	0.33	396.30	12.82	3.72	9.10
44	UHSBC59	0.21	0.67	19.65	21.77	46.99	1.02	38.60	38.80	147.00	164.50	0.96	50.93	0.17	374.07	12.82	2.53	10.29
45	UHSBC64	0.38	0.24	17.00	17.84	37.56	1.27	33.80	34.60	169.00	173.00	0.98	49.42	0.43	392.59	9.32	1.75	7.57
46	UHSBC66	0.50	0.34	20.21	20.38	51.50	1.45	73.02	93.60	387.00	469.00	0.84	44.37	0.35	360.19	8.22	1.29	6.92
47	UHSBC68	0.38	0.23	12.51	13.74	27.62	1.25	19.20	10.20	67.00	32.00	1.15	65.28	0.52	443.52	8.22	1.29	6.92
48	UHSBC69	0.50	0.35	13.64	16.13	28.85	1.05	26.40	12.20	132.00	60.00	2.21	68.40	0.66	474.77	8.22	1.29	6.92

Appendix III: Mean data for 39 characters for the 48 genotypes used in the study in Season-II

Sl. No.	GEN NO.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22
1	UHSBC02	5.69	1.27	1.51	2.20	2.99	1.18	1.00	1.62	1.64	3.77	2.37	1.51	1.17	5.31	1.34	2.60	5.80	6.00	11.14	1.89	3.52	0.96
2	UHSBC06	7.00	1.13	3.00	1.25	1.00	1.63	2.00	2.00	1.63	4.75	2.25	1.13	2.00	7.00	3.75	7.00	7.00	1.00	13.00	1.68	0.21	0.98
3	UHSBC07	6.00	1.00	3.00	1.00	1.00	1.00	1.00	1.50	1.00	5.00	1.00	1.00	1.75	6.00	1.50	3.00	3.00	3.00	7.50	1.44	1.98	0.73
4	UHSBC08	6.67	1.00	1.67	1.00	1.67	1.00	1.00	2.00	1.00	5.00	1.00	1.00	1.67	6.00	1.00	3.00	3.00	3.00	9.17	1.52	2.38	0.81
5	UHSBC11	5.00	1.00	2.10	1.00	1.10	1.00	1.50	1.50	1.20	5.00	2.70	1.00	1.90	6.50	1.50	4.00	5.00	1.50	9.30	0.99	0.43	0.69
6	UHSBC13	6.00	1.00	2.00	1.00	1.00	1.17	1.00	1.50	1.50	4.25	1.67	1.00	1.25	6.00	1.50	3.00	3.00	3.00	7.58	2.11	1.98	1.05
7	UHSBC14	4.42	1.08	1.21	3.00	2.75	1.00	1.00	2.00	1.63	4.00	1.75	1.42	1.00	4.67	1.78	1.88	3.00	1.63	10.13	1.93	3.18	1.12
8	UHSBC17	6.89	1.00	1.94	3.00	2.56	1.06	1.00	2.00	1.67	4.56	1.75	1.66	1.25	4.67	1.40	2.58	2.89	2.78	7.69	1.79	3.00	1.18
9	UHSBC19	6.80	1.58	1.10	3.67	4.23	1.00	1.00	2.00	2.00	5.00	1.60	1.00	1.25	11.80	3.63	2.00	2.38	2.13	16.88	2.19	3.51	1.53
10	UHSBC21	5.80	1.00	1.37	3.00	3.10	1.20	1.00	2.00	1.60	4.00	1.00	2.00	1.00	5.10	1.57	2.87	3.00	2.97	8.07	1.90	3.71	1.00
11	UHSBC22	6.44	1.06	1.14	3.40	2.56	1.35	1.17	1.47	1.17	4.39	1.56	1.67	1.00	4.86	1.40	2.25	3.00	3.00	10.33	2.01	3.95	1.38
12	UHSBC23	6.67	1.00	1.67	1.67	1.83	1.00	1.00	1.50	1.33	5.00	2.33	1.67	1.17	3.33	1.00	1.50	3.67	2.67	9.83	1.58	1.86	0.92
13	UHSBC24	5.47	1.00	1.47	3.60	4.30	1.10	1.83	2.00	1.77	4.60	2.40	1.50	1.00	4.93	1.50	1.63	4.38	2.38	13.07	1.81	3.48	1.06
14	UHSBC25	5.45	1.13	2.00	2.50	2.38	1.00	1.88	1.63	1.38	4.80	2.15	1.00	1.13	5.63	1.23	2.50	4.20	2.70	10.58	1.64	2.59	0.89
15	UHSBC26	7.00	1.13	1.50	1.92	2.58	1.00	2.00	2.00	1.42	5.00	2.58	1.75	1.13	3.63	2.54	2.00	2.50	1.88	16.29	1.35	1.98	0.83
16	UHSBC27	6.17	1.47	1.47	3.27	3.70	1.00	1.75	1.18	1.52	4.83	1.73	1.73	1.00	4.20	1.38	1.50	2.50	1.88	18.37	1.83	3.29	1.01
17	UHSBC28	5.63	1.25	1.50	1.50	3.19	1.00	2.00	1.56	1.88	5.00	2.50	1.06	1.13	8.25	1.38	1.50	2.31	1.81	13.25	1.57	2.46	1.11
18	UHSBC29	5.88	1.07	1.44	1.45	2.65	1.07	1.54	1.79	1.61	4.38	1.93	1.00	1.00	4.52	1.14	2.63	2.88	4.38	9.04	1.46	2.15	0.63
19	UHSBC30	6.67	1.01	1.81	2.16	3.60	1.01	1.87	1.90	1.57	4.62	1.63	1.40	1.40	4.32	1.12	1.99	2.53	2.71	12.50	1.65	2.56	0.83
20	UHSBC31	4.37	1.00	1.72	2.27	3.53	1.00	2.00	1.45	1.97	4.40	2.08	1.17	1.25	7.18	3.18	2.22	4.82	2.00	21.65	1.89	2.85	1.01
21	UHSBC32	5.83	1.17	1.83	1.83	1.67	1.00	1.58	1.58	2.33	5.00	2.50	1.08	1.00	5.35	1.17	2.50	4.50	1.80	10.58	1.49	2.36	0.73
22	UHSBC33	6.83	1.00	1.45	3.67	1.75	1.00	2.00	2.00	1.75	4.83	2.50	1.18	1.18	2.57	1.25	2.60	5.70	4.80	19.52	1.75	2.02	1.22
23	UHSBC34	6.00	1.33	1.67	2.00	1.83	1.25	1.83	1.67	2.08	5.00	2.33	1.08	1.17	3.83	1.50	1.42	4.17	6.00	14.08	1.76	0.84	0.91
24	UHSBC34-1	7.00	1.08	1.43	1.15	1.30	1.08	2.00	2.00	1.77	5.00	1.53	1.00	1.57	2.80	1.95	1.00	7.40	6.00	10.18	1.59	0.21	1.03
25	UHSBC35	5.17	1.00	2.13	1.33	1.50	1.00	2.00	1.75	1.58	5.00	2.08	1.00	1.00	4.88	1.88	2.67	2.96	3.04	9.46	1.45	1.91	0.53

26	UHSBC36	7.60	1.00	2.00	1.00	2.80	1.10	2.00	2.00	2.10	5.00	2.10	1.00	1.20	9.00	4.30	1.90	8.30	3.20	12.40	1.73	0.22	0.80
27	UHSBC37	7.00	1.25	1.75	3.00	2.25	1.00	2.00	1.75	1.75	5.00	2.00	1.25	1.00	3.00	1.00	1.50	3.00	2.50	11.25	1.45	1.82	0.80
28	UHSBC38	5.00	1.00	1.50	3.00	5.00	1.00	2.00	1.50	1.50	5.00	2.00	1.50	1.00	5.50	1.00	2.50	5.50	2.50	11.50	1.59	2.34	1.00
29	UHSBC39	6.50	1.00	1.25	3.50	2.50	1.00	2.00	2.00	2.00	5.00	2.00	1.25	1.25	3.50	2.50	2.00	2.75	2.75	16.00	1.92	1.77	1.10
30	UHSBC40	5.67	1.00	1.38	2.75	2.58	1.00	1.75	1.50	2.08	5.00	2.00	1.00	1.29	5.92	1.25	2.08	3.50	3.25	12.71	1.88	2.02	1.15
31	UHSBC41	7.00	1.00	2.00	1.90	3.15	1.00	1.67	1.58	1.67	5.00	2.64	1.00	1.00	3.83	1.32	2.25	1.83	2.25	12.48	2.01	2.72	1.20
32	UHSBC41-1	9.00	1.00	2.00	1.00	1.50	2.50	2.00	2.00	2.50	4.00	2.50	1.00	1.25	2.00	2.00	1.00	2.00	2.00	10.25	1.87	0.25	1.13
33	UHSBC42	6.02	1.06	1.81	2.02	2.27	1.00	2.00	2.00	2.00	5.00	2.07	1.00	1.06	8.71	1.21	2.38	3.18	2.00	13.05	3.03	2.74	1.20
34	UHSBC43	5.75	1.13	1.75	2.00	2.75	1.00	1.88	1.50	2.75	5.00	2.25	1.25	1.13	7.25	1.13	2.13	4.13	1.75	21.25	2.07	3.24	1.22
35	UHSBC431	9.00	1.00	2.00	1.00	2.56	1.00	2.00	2.00	2.64	5.00	3.08	1.00	1.00	3.83	1.00	2.00	6.75	5.83	10.11	1.74	0.24	0.88
36	UHSBC44	7.00	2.00	2.00	4.00	2.50	1.00	2.00	1.50	2.00	5.00	2.50	1.50	1.00	3.50	3.50	2.80	5.00	3.20	42.00	1.77	3.63	0.97
37	UHSBC45	6.75	1.13	1.88	2.50	3.75	1.00	2.00	1.88	1.00	5.00	1.88	1.00	1.00	5.50	1.63	2.63	2.75	2.75	22.25	1.62	2.36	0.81
38	UHSBC46	6.17	1.13	2.00	2.33	3.58	1.00	2.00	1.83	1.42	4.75	2.58	1.25	1.00	4.75	1.29	3.79	3.50	3.75	18.29	1.85	2.54	1.18
39	UHSBC47	3.00	1.00	1.50	2.67	2.67	1.00	2.00	1.83	1.33	5.00	2.17	1.00	1.17	5.00	1.33	1.25	2.67	2.83	11.83	1.48	2.05	0.87
40	UHSBC48	7.00	1.30	2.10	1.00	2.30	1.00	2.00	2.00	3.30	5.00	2.00	1.40	1.00	8.40	3.80	2.00	8.40	4.00	16.10	1.66	0.23	0.93
41	UHSBC49	8.80	1.00	1.90	1.40	2.80	1.00	1.80	1.80	1.50	5.00	2.30	1.00	1.10	5.60	1.00	1.10	2.60	2.70	17.50	1.07	1.75	0.59
42	UHSBC52	6.50	1.00	1.88	2.00	3.00	1.00	1.88	1.75	1.25	4.63	2.25	1.13	1.00	5.63	1.25	1.63	4.00	4.50	14.88	1.76	2.32	0.71
43	UHSBC53	6.78	1.11	1.29	2.02	1.40	1.00	1.36	1.28	1.83	5.00	3.00	1.18	1.31	6.46	1.65	1.88	3.39	3.46	16.51	1.65	2.56	1.11
44	UHSBC59	5.33	1.00	2.33	2.50	1.25	1.00	1.75	1.75	1.33	4.50	3.00	1.00	1.42	5.33	1.50	2.83	2.83	3.00	12.25	1.83	2.18	1.22
45	UHSBC64	6.54	1.06	1.92	1.25	1.98	1.00	1.83	1.00	1.92	4.71	1.71	1.00	1.40	7.17	1.19	4.25	4.25	2.50	12.58	1.51	0.24	0.76
46	UHSBC66	6.01	1.17	1.94	1.69	3.80	1.00	1.67	2.00	1.92	5.00	3.32	1.19	1.12	15.00	1.00	2.11	5.11	2.00	15.00	2.18	3.05	1.16
47	UHSBC68	6.88	1.09	2.34	1.00	1.00	1.00	1.91	1.89	2.31	4.94	2.56	1.00	1.41	6.00	1.09	2.78	3.00	2.53	7.13	1.75	2.24	0.96
48	UHSBC69	6.17	1.00	3.00	1.00	1.25	1.00	1.00	2.00	1.50	4.50	2.75	1.13	1.00	6.88	1.63	2.42	3.00	3.33	7.08	1.82	2.04	0.71

Appendix IV: Mean data for 39 characters for the 48 genotypes used in the study in Season-II

S NO	GEN NO.	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33	X34	X35	X36	X37	X38	X39
1	UHSBC02	0.53	0.41	26.04	14.65	57.27	1.18	49.00	63.05	245.00	315.25	0.69	39.29	0.56	327.08	7.17	2.65	4.52
2	UHSBC06	0.39	0.32	15.45	17.93	28.55	1.10	30.25	15.25	122.00	60.00	2.02	66.55	0.40	420.60	10.40	8.03	2.37
3	UHSBC07	0.45	0.26	15.08	17.13	28.75	1.00	22.25	8.63	111.25	43.13	2.68	72.72	0.64	314.81	9.07	3.95	5.13
4	UHSBC08	0.45	0.26	13.90	17.03	36.25	0.90	27.00	34.25	135.00	171.25	1.18	51.37	0.57	275.00	7.29	4.43	2.86
5	UHSBC11	0.13	0.17	16.13	14.19	22.80	1.42	10.10	7.65	49.50	32.75	2.15	55.95	0.21	308.33	8.63	7.24	1.39
6	UHSBC13	0.44	0.62	14.75	14.88	28.33	1.40	16.33	34.83	81.67	174.17	0.48	32.25	0.42	336.34	8.44	5.72	2.72
7	UHSBC14	0.39	0.43	24.85	16.73	59.25	1.58	54.60	61.00	273.00	305.00	0.90	47.37	0.35	308.33	7.05	6.53	0.52
8	UHSBC17	0.48	0.13	24.79	18.37	51.35	1.53	50.88	36.75	254.38	183.75	1.33	56.31	0.41	354.86	13.93	10.75	3.17
9	UHSBC19	0.33	0.33	24.70	21.73	62.75	1.10	40.80	118.92	204.01	594.60	0.37	26.76	0.22	278.24	7.75	3.36	4.40
10	UHSBC21	0.51	0.39	19.33	16.77	54.90	1.28	59.38	47.63	296.88	238.13	1.24	55.28	0.51	310.65	7.15	4.51	2.64
11	UHSBC22	0.48	0.16	23.26	18.70	58.24	1.04	61.00	84.00	305.00	420.00	0.76	42.79	0.35	302.55	6.93	3.90	3.03
12	UHSBC23	0.33	0.33	18.30	18.43	48.00	1.42	28.29	73.33	141.44	366.67	0.34	24.68	0.36	302.78	7.23	3.47	3.76
13	UHSBC24	0.35	0.40	20.18	17.92	54.00	0.95	55.10	112.55	275.50	562.75	0.43	29.47	0.32	310.42	7.45	4.00	3.46
14	UHSBC25	0.31	0.44	14.41	15.11	41.38	0.90	29.69	54.50	148.44	272.50	0.55	35.28	0.36	339.12	6.65	3.79	2.86
15	UHSBC26	0.19	0.34	22.48	18.29	50.83	0.83	18.88	113.38	94.38	566.88	0.25	19.29	0.23	286.81	5.53	2.81	2.72
16	UHSBC27	0.44	0.38	15.63	20.08	48.78	1.45	57.20	106.00	286.00	530.00	0.54	35.04	0.43	330.09	11.01	3.99	7.02
17	UHSBC28	0.39	0.07	17.90	17.25	45.81	0.85	47.16	57.00	235.78	285.00	0.81	44.14	0.35	315.74	5.70	2.35	3.35
18	UHSBC29	0.29	0.55	18.41	18.85	41.96	1.40	29.31	66.25	146.56	331.25	0.44	30.77	0.46	290.05	8.44	5.44	3.00
19	UHSBC30	0.33	0.49	20.01	18.67	50.51	0.48	29.57	66.34	147.84	331.70	0.44	30.37	0.40	279.17	7.28	5.07	2.22
20	UHSBC31	0.39	0.50	17.49	30.34	47.05	0.19	28.30	115.80	141.50	579.00	0.26	20.67	0.38	326.16	7.15	4.34	2.82
21	UHSBC32	0.30	0.46	17.58	16.83	43.63	0.13	80.75	67.50	403.75	337.50	1.33	48.71	0.41	293.75	7.11	5.00	2.10
22	UHSBC33	0.28	0.26	24.79	15.33	53.77	0.14	53.28	81.67	266.38	408.33	0.58	35.40	0.23	312.27	6.26	3.61	2.65
23	UHSBC34	0.33	0.52	16.98	17.11	44.63	0.56	29.50	66.83	182.50	411.17	0.45	31.74	0.36	300.93	6.84	4.99	1.85
24	UHSBC34-1	0.42	0.13	19.08	19.39	49.92	0.82	41.90	63.57	202.00	319.00	0.65	39.73	0.41	314.35	6.70	6.66	0.04
25	UHSBC35	0.25	0.67	17.74	16.07	38.48	0.18	17.63	34.25	88.13	171.25	0.52	34.06	0.46	297.69	8.55	6.47	2.08
26	UHSBC36	0.21	0.72	19.38	19.50	48.26	1.15	39.80	46.40	127.00	154.00	0.76	46.25	0.27	376.39	21.38	11.77	9.61

27	UHSBC37	0.20	0.45	20.08	26.38	34.38	0.00	29.75	78.13	148.75	390.63	0.38	27.38	0.23	371.53	7.47	5.24	2.23
28	UHSBC38	0.28	0.31	14.50	14.25	43.00	0.00	28.00	78.75	140.00	393.75	0.37	26.76	0.29	326.39	8.09	3.87	4.21
29	UHSBC39	0.23	0.60	17.63	19.40	46.85	0.60	35.75	89.25	178.75	446.25	0.48	31.77	0.20	298.61	7.91	4.17	3.74
30	UHSBC40	0.27	0.46	16.23	16.78	47.29	1.21	41.25	84.25	206.27	421.25	0.48	32.29	0.24	303.24	7.15	3.78	3.37
31	UHSBC41	0.35	0.46	15.59	19.85	44.13	0.19	53.66	76.92	268.30	384.61	0.69	40.89	0.29	310.19	8.16	5.25	2.91
32	UHSBC41-1	0.28	0.47	19.08	22.98	44.40	1.33	39.50	57.50	180.00	241.00	0.75	39.93	0.25	309.72	15.02	11.29	3.74
33	UHSBC42	0.39	1.44	18.25	18.38	47.36	0.31	53.21	88.88	266.05	444.42	0.60	37.53	0.32	312.04	9.95	8.04	1.90
34	UHSBC43	0.38	0.47	22.14	16.50	51.75	0.06	57.75	130.38	288.75	651.88	0.44	30.49	0.31	368.06	7.17	4.59	2.58
35	UHSBC43-1	0.34	0.53	20.05	23.98	47.56	0.69	32.67	34.33	163.33	171.67	0.79	48.76	0.38	339.81	16.51	8.50	8.01
36	UHSBC44	0.35	0.45	23.50	21.50	40.00	0.40	70.25	204.75	351.25	1023.75	0.32	23.13	0.36	384.26	10.93	4.92	6.02
37	UHSBC45	0.35	0.45	30.03	17.44	49.31	0.19	31.75	127.38	158.75	636.88	0.25	19.76	0.42	281.25	9.37	4.48	4.89
38	UHSBC46	0.33	0.34	21.66	16.43	48.73	0.18	39.27	116.19	196.35	580.94	0.34	25.26	0.28	278.94	9.07	4.34	4.74
39	UHSBC47	0.27	0.34	24.43	16.62	46.70	0.13	21.58	89.75	107.92	448.75	0.24	19.25	0.29	307.41	9.45	6.06	3.39
40	UHSBC48	0.27	0.46	19.08	24.00	45.12	1.16	48.56	44.40	233.00	291.00	0.75	53.03	0.29	386.11	8.04	5.06	2.97
41	UHSBC49	0.23	0.25	17.48	22.10	38.40	0.00	30.65	111.20	153.25	556.00	0.28	21.62	0.39	386.81	7.08	5.03	2.05
42	UHSBC52	0.16	0.89	15.60	17.43	38.94	0.29	64.38	90.75	321.88	453.75	0.62	36.85	0.23	378.24	8.26	5.73	2.53
43	UHSBC53	0.36	0.17	19.93	31.77	49.50	0.90	49.21	137.67	246.07	688.33	0.36	26.24	0.32	300.69	12.82	5.32	7.50
44	UHSBC59	0.41	0.21	17.12	41.54	33.51	0.75	52.21	52.13	261.04	260.63	0.95	48.62	0.33	323.61	12.82	4.36	8.45
45	UHSBC64	0.33	0.42	17.00	17.84	37.56	1.27	54.13	58.13	270.63	290.63	1.03	50.04	0.44	301.85	9.32	4.67	4.65
46	UHSBC66	0.37	0.65	20.21	20.38	51.50	1.45	63.71	72.56	318.55	362.80	0.94	47.03	0.32	304.17	8.22	5.99	2.23
47	UHSBC68	0.55	0.25	15.68	19.08	30.69	0.00	38.56	31.78	192.81	158.91	1.30	56.13	0.57	336.57	8.22	3.60	4.62
48	UHSBC69	0.40	0.70	16.48	15.79	31.35	1.17	22.69	11.47	113.44	57.35	1.98	66.00	0.59	394.91	8.22	4.72	3.50

Appendix V: Meteorological data recorded during experimental period at Bagalkot 2015-16

Month	Temperature. (°C)				Relative Humidity (%)				Avg. Rainy days*	Actual No. of Rainy days of 2015	Avg Rainfall (mm)*	Actual Rainfall -2015 (mm)
	Max.Avg Temp*	Actual Max Temp.-2015	Mini.Avg Temp*	Actual Min Temp-2015	Avg Mrng. RH*	Actual Mrng. RH-2015 (at 7:30AM)	Avg Afternoon. RH*	Actual Afternoon RH-2015 (at 2:30PM)				
Season-I, Haveli Farm, Bagalkot												
Apr-15	35.75	35.99	23.00	21.77	43.00	50.96	27.00	50.34	1	4	21.00	48.50
May-15	31.9	35.70	23.00	24.74	55.00	53.37	31.00	53.75	1	5	51.00	44.00
Jun-15	29.0	32.17	22.00	23.40	84.00	68.91	65.00	69.10	4	7	69.00	51.50
Season-II, Main Campus, UHS Bagalkot												
Oct-15	30.9	31.00	18.00	21.00	84.00	NA	69.00	NA	4	5	93.00	43.00
Nov-15	28	30.00	17.00	20.00	70.00	NA	42.00	NA	4	1	29.00	4.00
Dec-15	28.4	31.00	15.20	18.00	62.00	NA	33.00	NA	2	0	7.00	0.00

Avg. Rainfall of Bagalkot = 552 mm
But, Total Rain Fall from Jan-2015 to Dec 2015= 424.5mm
Deficit of rainfall for the year 2015= 24 %
And Total Rainy days from Jan-2015 to Dec 2015 = 34 days

MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CARROT (*Daucus carota* L.) GENOTYPES UNDER TROPICAL REGION

CHAITRA A. POLESHI **2016** **SARVAMANGALA CHOLIN**
Major advisor

ABSTRACT

In the present investigation, forty eight carrot genotypes representing temperate and tropical types were extensively characterized with 35 morphological (qualitative and quantitative) and 4 biochemical characters in two seasons at Haveli farm (S-I) and main campus of UHS Bagalkot during 2015-16 using RCBD with 2 replications. These genotypes were also characterized using 24 microsatellite markers.

Wider range, higher PCV, GCV and heritability and GAM was observed for all the biochemical and almost all the quantitative traits studied in both the seasons indicating the existence of sufficient amount of variation among the genotypes studied. Among the morphological traits, high heritability was recorded for shoulder width, root to shoot ratio, harvest index, internal root colour *etc*.

For many of the qualitative traits, skewed distribution was observed especially for root shape, internal colour of roots *etc*, but for most of the quantitative traits the distribution was normal indicating their polygenic inheritance. PC analysis involving 21 traits partitioned the variation into 6 principle components with approximately 83.0% of variation in both seasons. Correlation and path analysis revealed significant contribution of root width, shoulder width, xylem width, shoot length *etc* on root yield in both the seasons.

D^2 analysis partitioned the 48 genotypes in to 3 and 4 clusters in S-I and S-II respectively from 21 traits contributed more to the diversity indicating the scope for improvement of nutritional quality from the present genotypes.

The genotypes were further partitioned in to 2 main clusters with 25 sub clusters based on the 24 microsatellite markers. The number of alleles per locus ranged from two to six with the highest PIC value of 0.50. Based on the marker-trait association, few markers such as GSSR16, GSSR63 showed significant R^2 value for root yield, five roots weight *etc*.

Superior carrot genotypes such as UHSBC-32, UHSBC-44, UHSBC-52 for S-I and UHSBC-66, UHSBC-17 and UHSBC-22 for S-II were performed well in the tropical region. They were superior for root yield, harvest index, root width and biochemical traits. The identified superior genotypes from the present study would be useful for future carrot breeding program.

GµÀÀ ¥ÀæÃ±ÀÀ UÀÄjAiÀÄDPÀÄªÀÄ «eÁ£À fÃªÀgÁ¸ÁAiÀÄ¤PÀªÀÄvÀÄ fÃªÀªÀÄ«zsÀÄÉÀ UÀuÁvÀÀÄCzÀAiÀÄ£À

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¸ÁgÁA±

¥ÀæÀv CzÁAiÀÄ£zº MIÄ 48 UdÌj v½U¼£ÀÄß ºªÃº ¥ÁªÄð, vÆÃIUÁjP «±«zÁ®AiÄ, ¨ÁU®PÆÃI, vÆÃIUÁjP «eÁ£U¼ ªÄºÁ«zÁ®AiÄ, ¨ÁU®PÆÃI. JgqÀ IÄvÄU¼º 2015 ¨¼AiÄ¯ÁVzÄ. MIÄ 35 DPÌw, 4 fÃª gÁ¸ÁAiÄ¤P ªÄvÄ ªÄÌPÆÃæ¸I¯Ìmï UÄgÄvÄU¼£ÀÄß §¼¹ CzÀAiÄ£ ªiÁq¯ÁVz.

ºa£ ªÁ¦ ªÌ±Á®Ìv, ºa£ ªÄIÌz (¦¹«, f¹« ºÁUÆ C£ÄªA²Ã¬ÄvAiÄ ¸ÁªÄxÌð ªÄvÄ fÃªgÁ¸ÁAiÄ¤P UÄtU¼£ÀÄß ¤jÃQ¸¯ÁVz C®z JgqÀ IÄvÄU¼º ¥juÁªiÁvPªÁV v½ªÌ«zÌv C¹vª£ÀÄß vÆÃg¥r¸Äªz£ÀÄß F CzÀAiÄ£ªÀ WÆÃ¶¹z. F CzÀAiÄ£zº DPÌw «eÁ£z ®PtU¼Ä, C¢P C£ÄªA²Ã¬ÄvAiÄ C£Ä¥Áv, ¸ÄVÌAiÄ ¸ÄZÌAP, ¤ÃUÆð®ªAiÄ §t (81.0%) awÀætð ªiÁq®Äæ¨Ädz CU® (77.0%) ºÁUÆ DºÁg PÆÃ¼ªAiÄ §t (78.0%) ªÄÆ®vÄ¢ (73.0%) F CA±U¼Ä zÁR¯ÁzªÀ.

UÄuÁvÌP ®PtU¼º «±ÃµªÁV ¨Ãj£ DPÁgzº wgÄaz «vgu ºÁUÆ ¨Ãj£ DAvjP §t ªÄÄAvÁzªÀ PAqÄ§Azª Dzg ¥juÁªiÁvP ®PtU¼º «vguAiÄÄ ¸ÁªiÁ£ÌªÁVvÄ. EzgÆ¼U ¨ÃjU¼ ªÌ«zÌ v½U¼£ÀÄß ºÆA¢gÄªÌzÄ PAqÄ§A¢vÄ. ¦¹J a±ÃµuAiÄº 21 ¥jªiÁtvÁP UÄtU¼Ä 6 ªÄÄRÌ ¨ÁU¼º (CAUU¼Ä) «¨f¹ ºÆÃVzÄ PAqÄ §A¢vÄ. CAzÁdÄ 83.0% ª«zÌvAiÄÄ JgqÀ IÄvÄU¼º PAqÄ§A¢vÄ. ¥g¸g ¸A§Az ªÄvÄ ªiÁUð «±ÃµuÀ¬ÄAz ªÌPªÁVzÌ£Azg ¨Ãj£ CU®, 5 ¨ÃgÄ vÆP, ¨Ædz CU®, ¤UÆÃð¼£AiÄ CU®, aUÄj£ Gz aUÄj£ vÆP ªÄvÄ JgqÀ IÄvÄU¼º ¨Ãj£ E¼ÄªjAiÄ£ÀÄß F «±ÃµuAiÄÄ vÆÃg¥r¹vÄ.

r^2 «±ÃµuAiÄÄ 48 v½ ªÌ«zÌvU¼Ä «¨f¸¥ngÄªz£ÀÄß J¸ï-1 ªÄvÄ J¸ï-2 3 ªÄvÄ 4 ¸ªÄÆºU¼º ¸Æa¸ÄvÄ.

fÃªgÁ¸ÁAiÄ¤P UÄtU¼Ä UdÌjAiÄ ¥Ë¶PÁA± ¸ÄzÁguU FU EgÄª v½ªÌ«zÌvU½VAv GµªÄ®AiÄz ¨¼U¼º ºa£ PÆqÄU CªPÁ± EgÄªz£ÀÄß ¸Äa¹vÄ.

fÃª v½U¼£ÀÄß ªÄv 25 G¥¸ªÄÆºU¼£ÀÄß ªÄÌPÆÃæ¸m¯ÌI UÄt ºÆA¢gÄª JgqÀ ªÄÄRÌ ¸ªÄÆºU¼º «¨f¸¯Á¬ÄvÄ. Cº® ¸ASÌAiÄÄ ¥Àæ¯ÆÃP¸ïU JgqjAz DggµÄ ¥ÀæiÁtzº Cw ºZÄ ¦L¹ ªiË®Ì¢Az PÆrzÀ ªiÁPðgï mÉæmï ¸º ¸A§Azz Dzgz ªÄÃ¯ fJ¸ïJ¸ïDgï 16, fJ¸ïJ¸ïDgï 63 EªÀ ¨Ãj£ E¼ÄªjAiÄº «²µªÁz R^2 ªÄ®ÌªÁ£ÀÄß ºÆA¢gÄªÌzÄ ªÌPªÁ¬ÄvÄ.

GµªÄ®AiÄz ¥ÀæÃ±zº Gz ªÄI UdÌj AiÄÄJZïJ¸ï©¹-32 AiÄÄJZïJ¸ï©¹-44, AiÄÄJZïJ¸ï©¹-52 J¸ï-I ªÄvÄ AiÄÄJZïJ¸ï©¹-66, AiÄÄJZïJ¸ï©¹-17 AiÄÄJZïJ¸ï©¹-22 EªÀ Z£ÁV ¨¼ §A¢z£ÀÄß vÆÃg¥r¹zªÀ. CªÀ ¨Ãj£ GvÌµ E¼ÄªjU, ¸ÄVÌ ¸ÄZÌAP, ¨Ãj£ CU® ªÄvÄ fÃªgÁ¸Á¤P ªiÁ£zAqU¼Áz ©mÁ PÁÌgÆÃn£ï ªÄvÄ ¸Pg CA±U¼£ÀÄß ºÆA¢gÄªz£ÀÄß ¸Æa¹zªÀ.