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Exploring the phytochemical potential of purple carrot (*Daucus carota* L. subsp. *sativus* var. *atrorubens* Alef.) developed at ICAR-IIVR, Varanasi, UP

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Abstract

The present study deals with assessment of polyphenols content (anthocyanins, phenolics and flavonoids), total carotenoids and antioxidant capacity (FRAP, ABTS and CUPRAC activity) among tropical purple carrots (*Daucus carota* L. subsp. *sativus* var. *atrorubens* Alef.) developed at ICAR-IIVR, Varanasi, UP, India. Higher amounts of monomeric anthocyanins (218.0 mg $100g^{-1}$ FW), phenolic compounds (263.9 mg GAE $100g^{-1}$ FW) and total flavonoids (122.2 mg CE $100g^{-1}$ FW) have been estimated in black carrots which are correspondingly 13.6-, 6.8- and 8.7–times more than rainbow carrots. Moreover, the polyphenols content and antioxidant capacity were very strongly associated with each other having correlation coefficients between 0.932** to 0.997**. Further, higher correlation values of total phenolics over flavonoids with anthocyanins indicating that anthocyanins represent a large fraction of the phenolics which is related with better stability of anthocyanin pigment as food colorant. Overall, this work highlights that tropical black carrot and its cultivar Kashi Krishna is one of the richest sources of plant derived anthocyanins, phenolics and flavonoids; having better anthocyanin yield potential; and greater antioxidative capacity. Therefore, considering the health and nutritional benefits, multifarious uses and industrial importance of plant derived natural anthocyanins, black carrot could be potentially used as pigment crop.

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Introduction

Cultivated carrot (*Daucus carota* subsp. *sativus*) is descended by wild carrot or Queen Anne's lace (Daucus carota subsp. carota), native to central Asia (Iorizzo et al. 2013). It was cultivated and used as a storage root similar to modern carrots in Afghanistan, Iran, Iraq and Anatolia (Asia Minor) beginning in the 10th century. Afghanistan is known as the primary center of origin because of the presence of wide diversity. Cultivated carrot has several categories varying in color, size, size and flavor of roots, and need of vernalization to complete life cycle. Hence, broadly classified into two groups on the basis of (A) presence of root pigments i.e. Carotenoid group (D. carota ssp. sativus var. sativus) and Anthocyanin or purple group (D. carota ssp. sativus var. atrorubens), and (B) need of vernalization i.e. Tropical/Eastern/Oriental carrot (does not require vernalization & annual) and Temperate/ Western/ European carrot (requires vernalization & biennial) [Singh et al. 2018]. Purple carrots together with yellow variants were found in Afghanistan in the 7-10th centuries followed by red carrots developed in Iran and northern Arabia during 10th century. Moreover, white and orange types appeared as a natural mutant, correspondingly during the 14th and 17th century in the central Europe (France, Germany and the Netherlands) and the Netherlands, and they are typical to temperate climates. Cultivated carrots extend towards Iran and northern Arabia in the 10th century; the Mediterranean area and Western Europe in the 11-14th centuries; North Africa in the 11-12th

centuries; and China, India, Japan and Korea in the 14-17th centuries. During domestication over the centuries, carrot transformed from being a small, tough and bitter root to a fleshy, sweet, colored, unbranched, smooth and edible vegetable, and good source of dietary fiber, pigments, phytochemicals, vitamins and minerals. The carrots of carotenoid group (Orange, Red and Yellow carrot) and anthocyanin/purple group (Black and Rainbow carrot) are universal and of commercial significance. Black carrot root is comprised with dark purple pigmentation of both root exterior (epidermal layer) and root interior [cortex (outer-phloem), phloem and xylem (core)]; while rainbow carrot is with purple colored root exterior and red/orange/yellow colored root interior. Black carrot is very much suitable for healthy salad; preparations of sweets, fresh & fermented juice and purple tea; and pharmaceutical & nutraceutical uses as protective food supplements, healthy food colorants & cosmetics. Black carrot (solid purple in some literatures) is one of the richest and greatest sources of anthocyanins possessing very high anti-oxidative ability. However, rainbow carrots are the good source of organic pigments containing moderate amounts of carotene, xanthophylls, lycopene and anthocyanins. The striking appearances of root section of these purple colored carrots make salad decorative, food nutritious and have many health beneficial properties.

Phytochemicals like polyphenols and carotenoids are organically active compounds produced by plants and have been identified as beneficial to living beings in various ways. However, the polyphenols are secondary phyto-metabolites, hydrophilic in nature, derivative of phenylalanine, and commonly impart to the color, astringency, bitterness, odor, flavor and oxidative ability. The major classes of polyphenols comprise phenolics, flavonoids (flavones, flavanones, anthocyanins, isoflavones & flavonols), stilbenes and lignans. Anthocyanins are the most important water-soluble pigments present in multiple organs of various plants (flowers, fruits, leaves, stems and roots), stand 2nd after chlorophyll amongst the plant pigments visible to the human eyes. Various researchers proved that the anthocyanins are protective to human health, possessing properties of anti-fungal, anti-bacterial, anti-inflammatory and anti-diabetic; lessen bad cholesterol; potentially reducing the risk of atherosclerosis, cancer and inflammation; enhanced vision and cognitive functions; and improving antioxidant status (Zhang and Hamauzu 2004; Karkute et al. 2018: Singh et al. 2018). The antioxidant properties of natural anthocyanins also help in improving the appearance of skin, which makes it a preferred ingredient in cosmetics & personal care products. Looking towards significance of natural anthocyanins and consumers' awareness, the major related industries are moving towards replacing synthetic colors with plant-derived natural pigments in their products to fulfil the demand of high-end consumers.

Black and rainbow carrots accumulate large amounts of cyanidin-based anthocyanins in their taproots. Almost all anthocyanins in purple carrots are based on cyanidin being essentially acylated; the major anthocyanins are 3-xylosyl (glucosyl)-galactosides acylated with sinapic, ferulic, hydroxybenzoic, or coumaric acids attached to the glucosyl moiety (Cammerer et al. 2004; Schwarz et al. 2004; Montilla et al. 2011). However, Kurilich et al. (2005) suggests that acylated anthocyanin derivatives are not degraded by cooking or heating and thus appear to be a key characteristic in determining bioavailability. Large quantities of mono-acylated anthocyanins of black carrot impart an excellent measure of heat-, light- and pH-stability, enhancing the color-stability of food products during food chain (Iorizzo et al. 2020). Anthocyanins concentrate extracted from the purple/black carrot is generally traded as powder, lump, mush or liquid. In pharmaceutical industries, the natural anthocyanins are being widely used as protective food supplements, and healthy food colorant (alternative to synthetic colorants like FD&C Red 40) in beverages, confectioneries, soft drinks, chips, candies and ice cream (Iorizzo et al. 2020, Nath et al. 2022). To achieve a sustainable natural colorant production system; purple/black carrot, tomato and beetroot are representing the most important industrial sources of the three major pigment classes, namely for anthocyanins, lycopene and betalain production, respectively (Baranski et al. 2016). Anthocyanin pigments extracted from purple carrot offer advantages over extraction from other fruit and vegetable sources due to higher concentration of anthocyanins as well as lower amounts of non-anthocyanin phenolics that cause hazing and precipitation (Iorizzo et al. 2020). Therefore, black carrot has attracted interest of breeders, biochemists, and health/drug industries because of superior quality and higher amounts of anthocyanins.

Antioxidants inhibit the oxidation process and thereby ultimately reducing deleterious free radicals or reactive oxygen species (ROS). Commonly, hydrophilic antioxidants react with oxidants in the cytosol and the blood plasma; while lipophillic antioxidants guard cell membranes from lipid peroxidation. Mostly disorders/illness in living systems including plants and humans are produced through the production of harmful ROS which are constantly produced during natural metabolic activities as well as exposure to stresses. The effects of ROS can be mitigated by dietary antioxidants and antioxidant enzymes in living cells. Thus, the consumption of fruits and vegetables is associated with reduced risk of diseases as they are rich sources of natural antioxidants like anthocyanins, carotenoids, phenolics, vitamin C, flavonoids, tocopherol and organosulfides (Montilla et al. 2011; Koley et al. 2014; Karkute et al. 2018; Smeriglio et al. 2018; Cavagnaro et al. 2019; Iorizzo et al. 2020). In recent years, anthocyanins have emerged out as one the most promising ingredients for functional food industry because of their health-promoting effects, natural colorant and pharmaceutical properties; thereby anthocyanin-rich foods and black carrot become more popular and focused. The majority of works published in the literatures are dealing with anthocyanins of black/purple carrots especially of temperate origin, but there are very meagre information pertains with tropical purple carrots (Alasalvar et al. 2001; Nicolle et al. 2004; Zhang and Hamauzu 2004; Leja et al. 2013; Koley et al. 2014; Singh et al. 2018; Singh and Karmakar 2021). Therefore, the present study persuaded with the objectives to quantify phytochemical and antioxidative potential of 10 tropical cultivars/genotypes of black and rainbow carrots developed in India.

Materials and Methods

Basic experimental materials: A sum of ten varieties and genotypes of tropical purple carrot consisted of the experimental materials. Among these, six were black carrot such as VRCAR-89-1, VRCAR-124, VRCAR-125, Kashi Krishna (VRCAR-126), VRCAR-197 and Pusa Asita; and four were rainbow carrot, namely VRCAR-107-1, VRCAR-107-2, VRCAR-171-1 and VRCAR-198. All the genotypes/varieties, except Pusa Asita, are bred at ICAR-IIVR, Jakhini, Varanasi, UP, India. Two varieties of this study i.e. Pusa Asita (bred from ICAR-IARI, Pusa, New Delhi) and Kashi Krishna (bred from ICAR-IIVR, Varanasi, UP; Singh et al. 2019) were released for cultivation in 2015 and 2019, respectively.

Field trials and root sampling: Carrots crops were raised in open field during winter season of 2016-2017 and 2017-2018 at ICAR-IIVR, Varanasi, UP, India in soil with silt-loam texture, pH 7.3 and electrical conductivity of 0.28 dSm^{-1}. The experimental Farm is located at 82°52'36'' E longitude, 25°10'55'' N latitude and 85 m altitude receiving annual rainfall of 1050-1100 mm. To get better root development, all the agronomic practices were carried out unvaryingly during both years of experimentations as described by Singh and Karmakar (2015). Botanically schizocarp type carrot seeds were sown in double row of 7-8 cm apart and 25-28 cm wide ridge with the spacing of 65 cm between each pair of ridges. Each genotype comprises four ridges of 6 m and replicated thrice. The roots were uprooted at marketable maturity (105-115 days after sowing). Randomly eight roots of each selected and washed thoroughly with tap water. A representative sample of the edible root i.e. 6-7 cm long piece from middle portion were rapidly cut into thin slices, kept in transparent polyethylene bag to mix properly, and biochemical quantification on fresh weight (FW) basis was done using mixed root samples.

Estimation of phytochemical

Analysis of total monomeric anthocyanins: Composite root sample of 100 g were homogenized after addition of ascorbic acid (5%, w/w) and water (25%, w/w) using a warring blander. Commuted sample of 25 g was extracted with 100 ml of methanol/0.1% HCl (v/v) for 2 h under dark condition. After centrifugation at 13000 rpm for 15 min, the supernatant was recovered, and residue was re-extracted following same method. The combined supernatants were evaporated to dryness and re-dissolved in distilled water. The total monomeric anthocyanins content was determined on a UV–visible spectrophotometer by the pH-differential method (Wrolstad et al. 2005). Pigment content was expressed as total monomeric anthocyanins equivalents (mg $100g^{-1}$ FW).

Determination of total phenolics content and total flavonoid: The water soluble phytocompounds of carrot were extracted following Chu et al. (2002)

with minor adjustment. Five-gram sample were homogenized with 80% ethanol (1:2 w/v) through cooled warring blender for 5 min. Residue was again extracted for two times. To get a thoroughly homogenized sample, the mixed sample was further homogenized by polytron homogenizer for 3 min; homogenates were centrifuge at 13000 rpm for 15 min, and finally solvent was removed using a rotary evaporator. The supernatants were collected in 50 ml plastic centrifuge tubes and stored at -20 °C for analysis of total phenolics and flavonoids content; and activity of ferric reducing antioxidant power (FRAP), 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), and cupric reducing antioxidant capacity (CUPRAC).

Total phenolics content was analysed by spectro-photometer using Folin–Ciocalteu reagent (Singleton et al. 1999). Aliquot (100 µl) of hydrophilic extract were mixed with 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20% Na2CO3 solution. Then mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank. Results were expressed as gallic acid equivalent (mg GAE $100g^{-1}$ FW). Moreover, total flavonoid in carrot root sample was quantified using aluminium chloride method of Zhishen et al. (1999). In 10 ml of volumetric flask, an aliquot of 1 ml hydrophilic extract was mixed containing 0.3 ml of 5% NaNO2, 4 ml of distilled water and 0.3 ml of 10% AlCl3.6H2O. The mixture was allowed to stand for 6 min at room temperature, then 2 ml of 1 mol NaOH was added and the solution was diluted to 10 ml with distilled water. Instantaneously, the absorbance of the solution and blank was taken at 510 nm, and results were expressed as catechin equivalent (mg CE $100g^{-1}$ FW).

Determination of total carotenoids: Using a pestle & mortar and acetone solvent, 100 g carrot sample was repetitively extracted until the residue became colorless. The pooled acetone extracts were transferred into separatory funnel and the entire carotenoids were transferred to petroleum ether phase. The reading was measured by UV–Visible spectrophotometer at wavelength of 452 nm and calculated (mg $100g^{-1}$ FW).

Estimate antioxidant capacity

FRAP assay: The ability to reduce ferric ions, FRAP activity, was quantified as per methodology of Benzie and Strain (1996). Primarily, the reagent of FRAP was made by mixing 10 mM TPTZ in 40 mM HCl, 300 mM acetate buffer (pH 3.6) and 20 mM FeCl3 in the ratio 1:10:1 (v/v/v). Subsequently, 100 µl aliquot was meticulously mixed with 3 ml FRAP reagent in a test tube and vortexed in the incubator for 30 min in a water bath maintaining 37 °C temperature. The reduction of ferric-tripyridyltriazine to the ferrous complex give rise to formation of intense blue colour solution. The absorbance was taken at wavelength of 593 nm and results were expressed in terms of trolox equivalent (µmol TE g^{-1} FW).

ABTS assay: ABTS activity (a peroxidase substrate) as Trolox equivalent antioxidant capacity (TEAC) in sample was analysed through a modified procedure using ABTS (Re et al. 1999). The ABTS*+ free radical (7 mmol) was prepared through reaction of 7 mmol ABTS and 2.45 mmol of potassium persulphate as the oxidant agent. Hydrophilic extract (10 µl) was added to 90 µl of ABTS*+ solution and absorbance readings were taken exactly 10 min after initial mixing at 734 nm. The inhibition (%) of ABTS of the test sample and known solutions of Trolox were calculated by the formula i.e. Inhibition (%)= 100×(A0-A)/A0; Where A0 was the initial absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 734 nm. Finally, radical-scavenging activity was expressed as µmol TE g^{-1} FW.

CUPRAC assay: The CUPRAC assay in terms of ability to reduce cupric ions was quantified following the method of Apak et al. (2008). As per protocol 100 µl of hydrophilic extract was mixed with 1 ml each of CuCl2 solution (1.0 × 10-2 mol/l), neocuproine alcoholic solution (7.5 × 10-3 mol/l), and NH4Ac (1 mol/l, pH 7.0) buffer solution and 1 ml of distilled water to make the final volume 4.1 ml. The mixture was incubated at 50°C in a water bath for 30 min, and absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of Trolox and results were expressed in terms of µmol TE g^{-1} FW.

Statistical analyses: Data were collected during the both years for each genotype/variety and were analyzed statistically for analysis of variance (Singh and Chaudhary 1977). Pooled data of both years were used for making histograms to compare mean differences by using standard error bars with $p<0.05$ through Microsoft Excel, and correlation analysis was done as mentioned by Singh and Chaudhary (1977).

Results and Discussion

The results of years 2016-17 and 2017-18 for the content of monomeric anthocyanins, phenolics, flavonoids and total carotenoid content, and antioxidant ability in terms of FRAP, ABTS and CUPRAC values were analyzed for analysis of variance to observe the effect of year and genotypes. Highly significant variations were observed among genotypes, especially between black and purple carrots; but considerable differences or definite pattern due to year effect among genotypes was not found. Hence, the pooled data of both years for 10 genotypes as well as mean values of black and rainbow carrot are presented (Figures 1-4). Since, the crops were raised in almost similar climatic conditions during both years, and color trait is easily distinguishable and qualitative in nature; it is obvious to get least differences over the year and lack any interactions between both years for various estimates analyzed. The significant variability among genotypes and non-significant differences between years were also reported among various colored carrots for total phenols, phenylpropanoids, flavonoids, anthocyanins and antioxidant activity (DPPH) by Leja et al. (2013); and for carotenoids, phenols, flavonoids, and antioxidant activity (FRAP, ABTS & CUPRAC) by Singh et al. (2018). The genotypic variations for carotenoids, phenols, flavonoids, anthocyanins and antioxidants have been described in colored carrots and other vegetables by various researchers (Nicolle et al. 2004; Zhang and Hamauzu 2004; Karmakar et al. 2013; Leja et al. 2013; Koley et al. 2014; Singh et al. 2018).

The content of total monomeric anthocyanins (mg $100g^{-1}$ FW, Figure 1A) in the purple carrot were significantly very high i.e. 13.63-fold in black carrot (157.5-282.3 mg) than rainbow carrots (12.9-18.1 mg), especially for cultivars/genotype Kashi Krishna (282.3 mg), VRCAR-125 (268.0 mg) and Pusa Asita (235.1 mg). It is notable that the color intensity and the extent of the root (exterior and interior portions) covered with purple pigmentation are both directly associated with the total anthocyanins accumulation in root. Thus, black carrot genotypes with dark purple color throughout the entire root section tend to have the higher amounts of anthocyanins, whereas rainbow carrots with purple pigmentation in only the epidermal layer of roots usually have low levels of anthocyanins. Globally, the food plants that are rich sources of natural anthocyanins (mg/100 g FW) are chokeberry (1480 mg), black raspberry (589 mg), raspberry (365 mg), concord grape (326 mg), red currant (80-420 mg), black currant (190-270 mg), black bean seed (213 mg), red cabbage (150 mg), cherry (122 mg), blue corn (71 mg) and black rice (60 mg) [Anonymous 2020]. Furthermore, few red/purple accessions of vegetables contain appropriate amounts of anthocyanins (mg/100 g FW) in their edible portions are red-fleshed sweet potato (25-45 mg), red/purple radish (0.46-16.6 mg), purple-podded French bean (7.1 mg), purple tomato (20-66 mg), red okra (3-5 mg) and purple cauliflower (2-7 mg) [Rodriguez-Saona et al. 1998; Teow et al. 2007; Lo-Scalzoa et al. 2008; Singh et al. 2011]. From the various reports mentioned, it is evident that the accessions of tropical black carrot (Kashi Krishna, VRCAR-125 & Pusa Asita) possesses 235-282 mg $100g^{-1}$ FW of anthocyanins, which is one of the best sources among plants and richest source among vegetables.

Worldwide, the usages of natural anthocyanins have witnessed a significant rise in the past few years owing to the increased awareness about its health benefits, biochemical significance, and multifarious use by industries related to the nutraceutical, food & beverage, pharmaceutical, and cosmetics & personal care. The global anthocyanin market is expected to grow from USD 300 mn in 2015, USD 365 mn in 2019 to USD 489 mn by the end of 2025 at a CAGR of 5.0%. Moreover, Food industry (Wild, Germany) assume that an annual global need of 10,000 ton of carrot-derived anthocyanin concentrate will meet the requirement by production of purple/black carrot in about 1,00,000 ha area (Baranski et al. 2016). In the present study, anthocyanins yield potential (kg/ha) of various promising lines and cultivars was calculated by multiplying total monomeric anthocyanins content (mg $100g^{-1}$ FW) to root yield (q/ha) and divided by 1000. Among purple carrot, total anthocyanins yield varied from 3.9-6.0 kg (5.1 kg) in rainbow carrot and 38.4-78.6 kg (56.0 kg) in black carrots (Figure 1B).

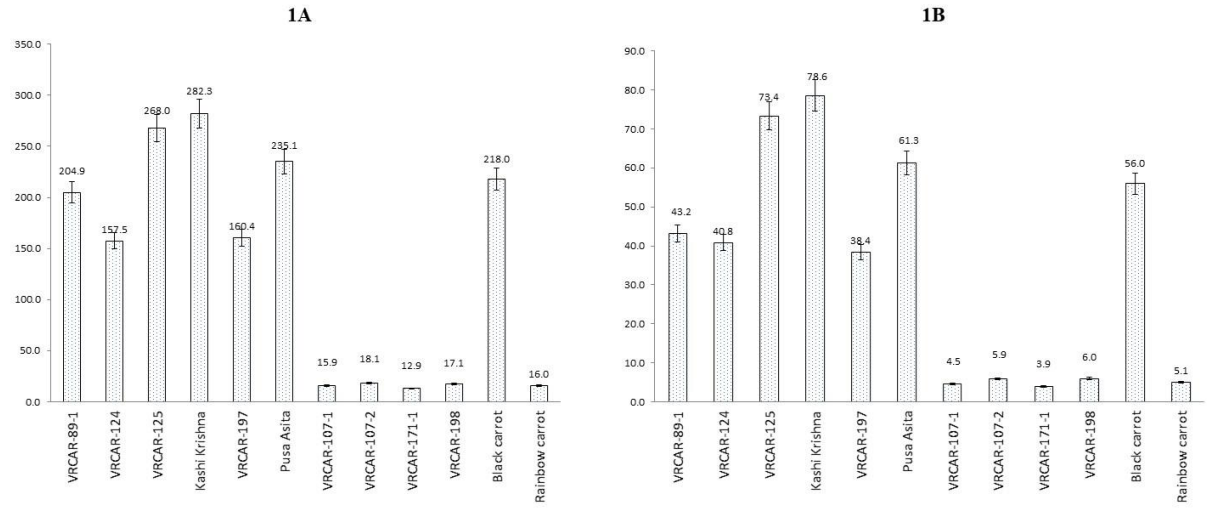

Figure 1A & 1B: Total monomeric anthocyanins content (mg100g^{-1} FW) and its yield potential (kg ha^{-1}) in black and rainbow genotypes of tropical carrot, respectively (Standard error bars with p<0.05).

Krishna (78.6 kg) followed by VRCAR-125 (73.4 kg), Pusa Asita (61.3 kg), VRCAR-89-1 (43.2 kg), VRCAR-124 (40.8 kg) and VRCAR-197 (38.4 kg). Dark purple pigmentation of root sections (exterior and interiors) in black carrot, and higher root yield potential both contribute towards higher anthocyanins yield potential per unit area. Since black carrot is an important and highest industrial source of anthocyanins of plant origin, the most potential tropical cultivars/genotype, namely Kashi Krishna, VRCAR-125 and Pusa Asita will certainly play a major role for their industrial use as well as will boost seed industry in tropical and sub-tropical regions of the world.

Moreover, total phenolics concentration (mg GAE 100g^{-1} FW) in black and rainbow carrots analyzed to be 263.9 mg (183.1-310.9 mg) and 39.0 mg (29.9-52.6 mg), respectively with significantly higher amounts in Kashi Krishna & VRCAR-125 followed by VRCAR-89-1, VRCAR-197 and VRCAR-125 (Figure 2A). Largely, black carrot roots possessed 6.8-fold more phenolics than roots of rainbow carrots. Higher concentration of phenolics in black carrot is due to presence of anthocyanins. The trend of present result is in concurrence with Algarra et al. (2014) who analysed 187.8 & 492.0 mg of phenolic compounds in two black carrot cultivars Antonina and Purple Haze, respectively. Moreover, Leja et al. (2013) estimated 9-times more phenolics in purple carrot than roots of other colors. Comparatively, greater amounts of phenolics in black carrots (solid purple) have also reported by have also reported by Alasalvar et al. (2001), Koley et al. (2014), Singh et al. (2018), and Smeriglio et al. (2018).

Another polyphenol, flavonoids content (mg CE 100g^{-1} FW, Figure 2B) was analyzed significantly higher in black carrots (122.2 mg, 8.66-fold) cultivars and promising lines such as Kashi Krishna (140.0 mg) followed by VRCAR-125 (133.1 mg), VRCAR-124 & VRCAR-197 (116.9 mg), Pusa Asita (114.6 mg) and VRCAR-89-1 (111.4 mg); and lesser concentration in the genotypes of rainbow carrot, namely VRCAR-107-2 (20.9 mg), VRCAR-107-1 (17.0 mg), VRCAR-171-1 (12.0 mg) and VRCAR-198 (6.5 mg). In various lines of black and rainbow carrots, the average phenolic content is higher (1.6-8.1 times) than the corresponding total flavonoids. Anthocyanins are responsible for the purple color of carrot roots which is being reflected in the present analysis with higher values of phenolics and flavonoids in the roots of black and rainbow carrots. Various previous researchers (Leja et al. 2013; Koley et al. 2014; Smeriglio et al. 2018) estimated higher amounts of flavonoids in the roots of black/purple coloured carrots.

The total carotenoids content (mg 100g^{-1} FW) among purple carrots ranged from 5.15-9.70 mg in

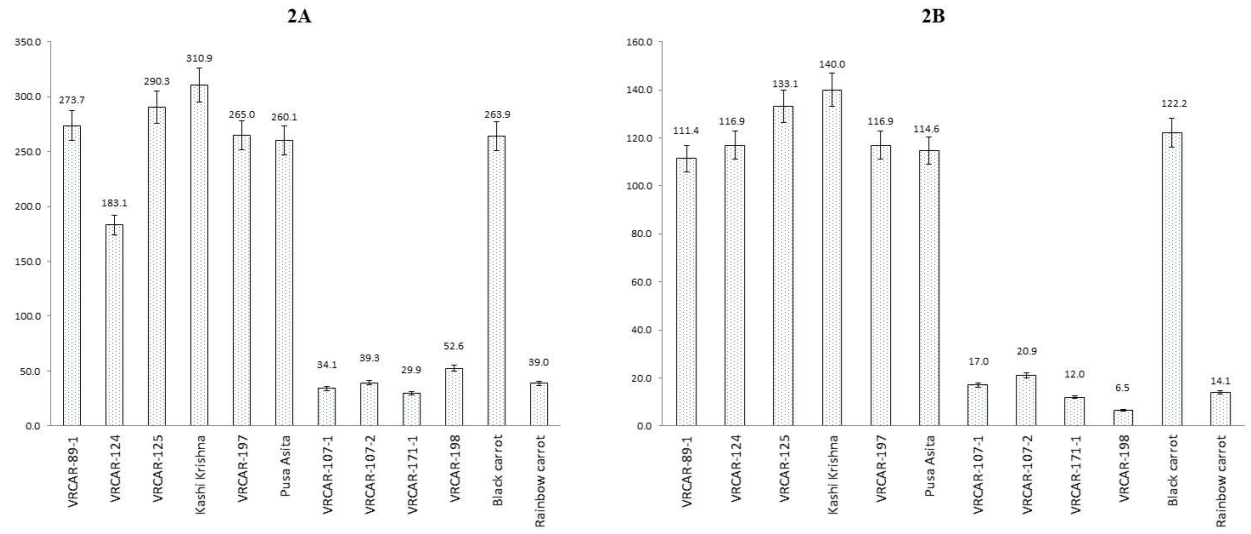

Figure 2A & 2B: Total phenolics (mg GAE 100g^{-1} FW) and total flavonoids content (mg CE 100g^{-1} FW) in the roots of black and rainbow carrots, respectively (Standard error bars with p<0.05).

rainbow genotypes and 0.46-1.10 mg in black carrots with a general mean value of 6.95 mg and 0.61 mg, respectively (Figure 3A). Rainbow carrots with orange/red/yellow root interior/core contribute towards higher amount of total carotenoids. Present result is in accordance with the previous findings of Alasalvar et al. (2001), Nicolle et al. (2004), and Singh et al. (2018) who also reported the lesser amounts of carotenoids (<2 mg 100g^{-1} FW) in black/yellow carrots. The presence of organic pigments like anthocyanins and carotenoids in rainbow carrots responsible for the brilliant colours ranging from pale yellow through bright orange to dark red make it most suitable for use as table/salad purpose which is not only attractive/decorative but also very nutritious containing reasonable amounts of carotenoids (lycopene, β-carotene, α-carotene & xanthophyll) and anthocyanins.

The antioxidants are bio-molecule substances which counteract reactive oxygen species (ROS) or free radicals, thus preventing oxidative damage. The plant materials rich in antioxidant ability like black carrot are of growing interest in the food/pharmaceutical industries because they retard oxidative degradation, and thereby improve the quality and nutritional value of foods. The dietary antioxidants from plant products are generally more effective in reducing ROS levels due to the synergistic actions of a wide range of biomolecules such as vitamin C & E, phenolic compounds, anthocyanins, carotenoids, terpenoids and other phytonutrients. In food nutrition research, the evaluation of antioxidant ability is becoming more important as it provides useful information with regard to health promoting functional quality of food items. In the present experimentation, the antioxidant activities of purple carrots were analysed by three different in-vitro methods i.e. FRAP, ABTS and CUPRAC assay to compare the potentiality of promising lines/cultivars of black and rainbow carrots.

The reducing capacity was assayed in tropical purple carrots using the FRAP method (µmol TE g^{-1} FW) that ranged from 2.5-4.9 µmol (3.3 µmol) in rainbow carrot, and 25.6-59.4 µmol (45.8 µmol) in black carrot. It is much higher in black carrot lines such as Kashi Krishna followed by VRCAR-125, VRCAR-197, VRCAR-89-1, Pusa Asita and VRCAR-124 (Figure 3B).

FRAP activity is estimated 13.63-fold higher in black carrot as compared to rainbow carrot. The higher FRAP value of black carrot is due to the presence of greater amounts of anthocyanins and phenolics. The trend of higher reducing capacity of black carrot is in concurrence with those of previous studies (Algarra et al. 2014; Koley et al. 2014; Singh et al. 2018). Further, Raman (2010) listed the most effective 12 healthy foods that are very high in antioxidant capacity (FRAP value µmol TE g^{-1} FW) which are dark chocolate (150 µmol), pecan nut (106 µmol), blueberry (92 µmol), strawberry (54

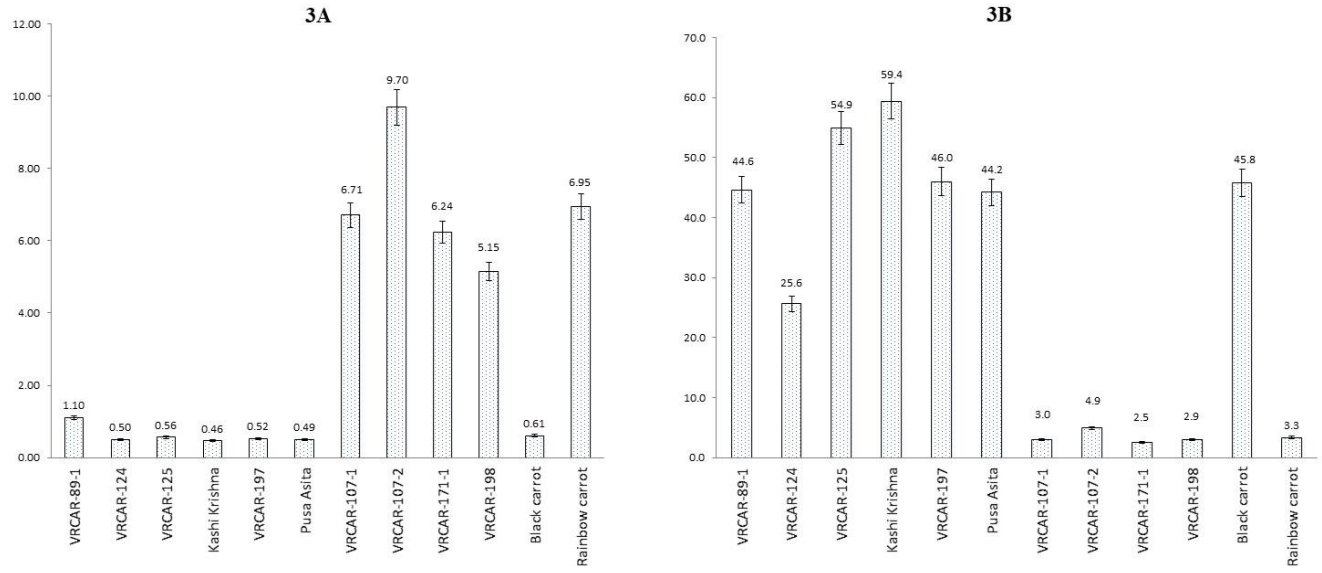

Figure 3A & 3B: Total carotenoids content (mg 100g^{-1} FW) and FRAP activity (µmol TE g^{-1} FW) in black and rainbow genotypes of tropical carrot, respectively (Standard error bars with $p<0.05$).

µmol), globe artichoke (47 µmol), goji berry (43 µmol), raspberry (40 µmol), kale and red kale (27 & 41 µmol), red cabbage (22 µmol), beans (20 µmol), garden beet (17 µmol) and spinach (9 µmol). It is revealed that FRAP antioxidant ability is higher in black carrots (25.6-59.4 µmol), particularly in cultivar Kashi Krishna (59.4 µmol) which is ranked under 5 among food items and highest among vegetables.

The free radical scavenging activity of black and rainbow carrots was analyzed following ABTS assay (µmol TE g^{-1} FW) as described in methodology. The antiradical capacities of the black carrot cultivars were 11.53-fold higher than that of the rainbow carrot that ranged from 26.4-73.1 µmol & 4.3-6.3 µmol, respectively (Figure 4A). Significantly higher ABTS radical scavenging capacities was estimated for Kashi Krishna (73.1 µmol) and VRCAR-125 (71.9 µmol) followed by VRCAR-89-1 (61.1 µmol), VRCAR-197 (60.7 µmol) and Pusa Asita (58.0 µmol). Moreover, Zhang and Hamauzu (2004) analyzed stronger radical scavenging ability (RSA) in phenolic extracts than pure chlorogenic acid, vitamin C and β-carotene in carrot. In another study, Leja et al. (2013) estimated RSA among 35 genotypes of temperate and tropical carrots, and reported 7.4-times more RSA in purple carrot than carotenoid carrots.

Furthermore, the antioxidant potential in terms of CUPRAC value (µmol TE g^{-1} FW) in tropical black and purple carrots was varied from 39.7-83.2 µmol (68.6 µmol) and 3.1-7.6 µmol (5.0 µmol), respectively (Figure 4B). CUPRAC value was analysed significantly higher particularly in Kashi Krishna (83.2 µmol) and VRCAR-125 (79.8 µmol). The higher CUPRAC values for black carrot in comparison to rainbow carrot are due to the presence of anthocyanins and phenolics. CUPRAC estimates were found to be highest in black carrots in previous studies of Koley et al. (2014) and Singh et al. (2018). The ability to reduce metal ions was measured using CUPRAC and FRAP assay; and the overall values of CUPRAC assay were considerably higher than the values of FRAP assay which may be because of the presence of flavonoids such as quercetin and kaempferol.

The most common source of betalain-based food colorant is red beetroot (Beta vulgaris) which has been reported to be one of the most potent antioxidant vegetables whose antioxidative potential in terms of FRAP and ABTS values on dry weight (DW) basis quantified as 40-125 mmol TE kg^{-1} DW (i.e. ≈ 4.0-12.5 µmol TE g^{-1} FW) and 30-110 mmol TE kg-1 DW (i.e. ≈ 3.0-11.0 µmol TE g^{-1} FW), respectively (Song et al. 2010; Carrillo et al. 2017). In the present study, it is very interesting to note that the black carrot cultivars possessed 500-800% more antioxidant ability than red beetroot with respective FRAP, ABTS and CUPRAC capacity of 25.64-59.40 µmol TE g^{-1} FW, 26.39-73.14 µmol TE g^{-1} FW and 39.67-83.23 µmol TE g^{-1} FW. Therefore, black

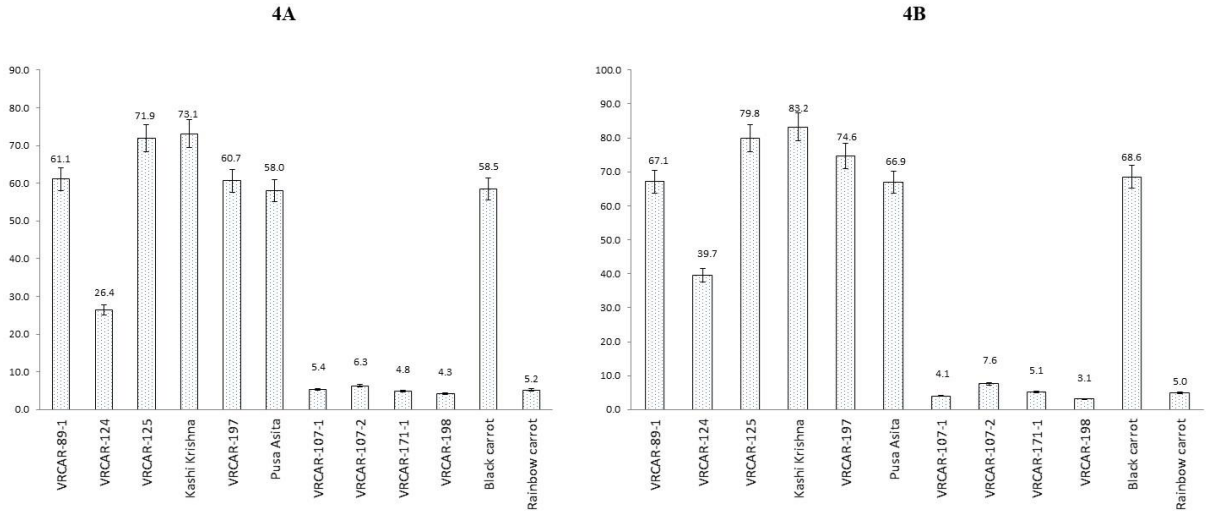

Figure 4A & 4B: ABTS and CUPRAC activity (µmol TE g^{-1} FW) in the roots of black and rainbow genotypes of tropical carrot, respectively (Standard error bars with $p<0.05$).

carrot is one of the best sources among plants and richest among vegetable for antioxidant compounds that need to be promoted in the daily diet and utilized for industrial manufacturing. Overall, the antioxidant ability is higher in anthocyanins rich black carrot followed by rainbow carrot and least in carotenoid pigmented carrot.

The correlation coefficients between polyphenols content (anthocyanins, phenolics, flavonoids), antioxidant ability (FRAP, ABTS, CUPRAC) and total carotenoids were computed to assess strength of linear association between variables (Table 1).

Table 1: Correlation coefficient between phytochemical content and antioxidant capacity in tropical purple carrot

Parameter	Monomeric anthocyanins	Total phenolics	Total flavonoids	FRAP activity	ABTS activity	CUPRAC activity	Total carotenoids
Monomeric anthocyanins	1.000	0.974**	0.963**	0.976**	0.961**	0.964**	-0.884**
Total phenolics		1.000	0.971**	0.992**	0.985**	0.993**	-0.911**
Total flavonoids			1.000	0.957**	0.932**	0.961**	-0.917**
FRAP activity				1.000	0.995**	0.997**	-0.871**
ABTS activity					1.000	0.994**	-0.881**
CUPRAC activity						1.000	-0.851**

**: significant at 1% level

Anthocyanins, phenolics and flavonoids were strongly and positively correlated to each other (0.974**, 0.963** & 0.971**), and with all three antioxidants i.e. 0.961** to 0.976**, 0.932** to 0.961** and 0.985** to 0.993**, respectively because of the presence of various polyphenols in purple carrots. The higher coefficients of total phenolics over flavonoids with anthocyanins indicate that phenolics could be used as a biochemical indicator while genetic improvement programmes; and also suggesting that anthocyanins represent a large fraction of the phenolics which is associated with better stability of anthocyanins as food colorant. Further, the activity of antioxidants FRAP, ABTS and CUPRAC were also highly associated with each other i.e. 0.995**, 0.997** & 0.994**, respectively. Positive association between polyphenols and antioxidant ability in root vegetables have also been reported in earlier studies (Leja et al. 2013; Koley et al. 2014; Carrillo et al.

2017; Singh et al. 2018). Nevertheless, there were significant negative correlation between total carotenoids and phytonutrients/antioxidative capacity (-0.851** to -0.917**) because of lower concentration of anthocyanins in the roots of carotenoid carrot.

Conclusions

The present study assayed higher amounts of monomeric anthocyanins (218.0 mg 100 g^{-1} FW), phenolic compounds (263.9 mg GAE 100 g^{-1} FW) and total flavonoids (122.2 mg CE 100 g^{-1} FW) in black carrots which are correspondingly 13.6-, 6.8- and 8.7-times more than rainbow carrots. Overall, the anthocyanins, phenolics and flavonoids have very high and direct associations with all three antioxidants i.e. FRAP, ABTS and CUPRAC. Higher content and better yield potential of anthocyanins, strong direct association between anthocyanins & phenolic compounds, health and nutritional benefits, multifarious uses & industrial importance, and greater antioxidant ability of black carrot (Figure 5) make it very suitable to be used a pigment crop.

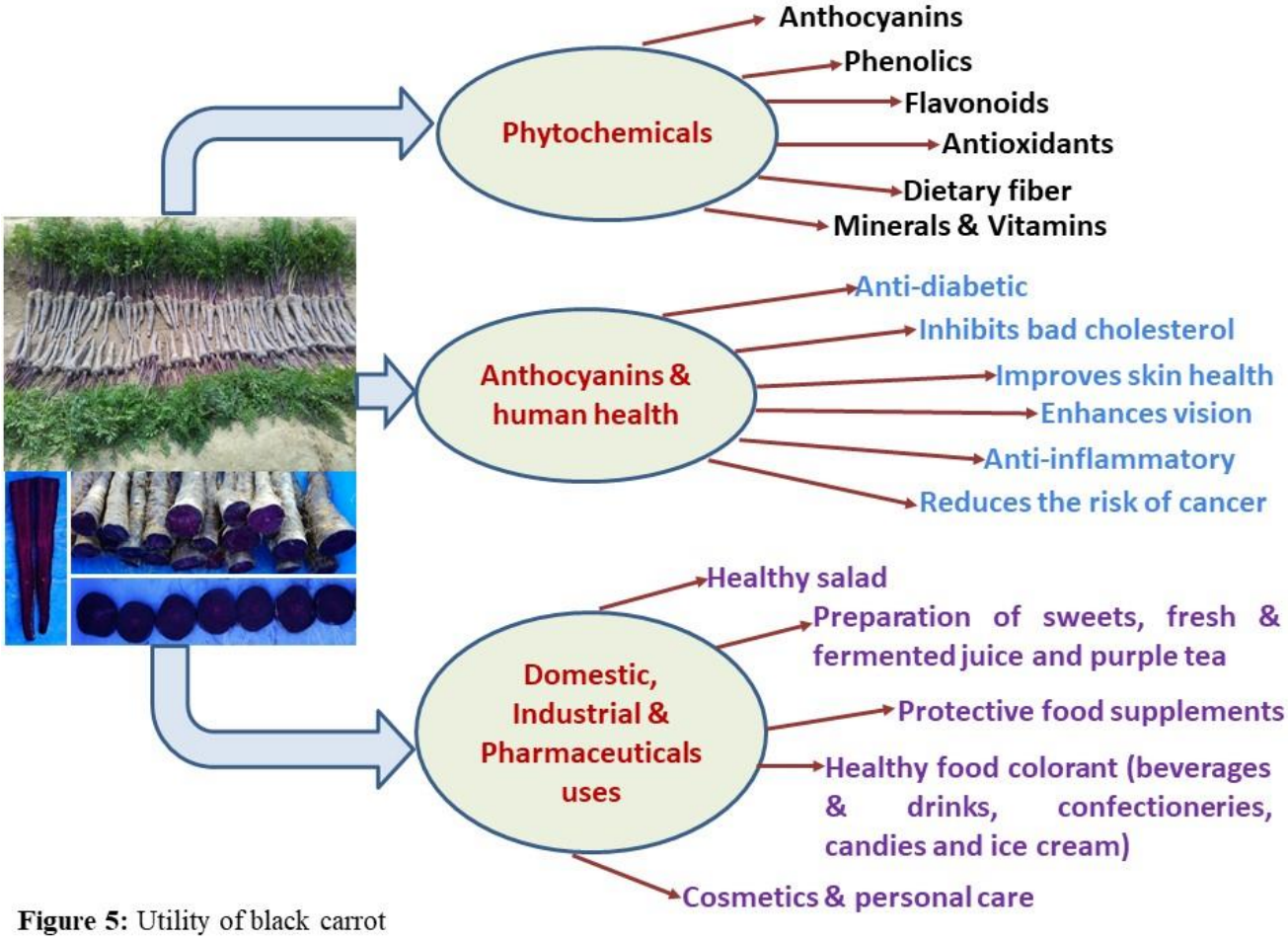

Figure 5: Utility of black carrot

सारांश

वर्तमान अध्ययन भा.कृ.अनु.प. – भारतीय सब्जी अनुसंधान संस्थान, वाराणसी, (उत्तर प्रदे”ा) द्वारा विकसित उष्ण कटिबंधीय बैंगनी गाजर (डौकस कैरोटा एल. सबस्पी”ीज सटाइवस वार. एट्रोरूबेंस एलेफ.) में उपस्थित पॉलीफेनोल्स सामग्री (एंथोसायनिन्स, फेनोलिक्स और फ्लेवोनोइड्स), कुल कैरोटीनॉयड और एंटीऑक्सीडेंट क्षमता (एफ.आर.ए.पी., ए.बी.टी.एस. एवं सी.यू.पी.आर.ए.सी. गतिविधि) का मूल्यांकन किया गया। उच्च मात्रा में मोनोमेरिक एंथोसायनिन (218.0 मिग्रा. प्रति ग्राम 100 फल भार), फेनोलिक यौगिकों (263.9 मिग्रा. जी.ए.ई. प्रति 100 ग्राम फल भार) और कुल फ्लेवोनोइड्स (122.2 मिग्रा. सी.ई. प्रति 100 ग्राम फल भार) काले गाजर में पाया गया जो इंद्रधनुषी गाजर की तुलना में 13.6, 6.8 एवं 8.7 गुना अधिक था जबकि फेनोलिक्स यौगिक एवं एंटीऑक्सीडेंट क्षमता में सहसंबंध गुणांक 0.93 से 0.997 दृढ़ रूप से पाया। इसके अलावा, एंथोसायनिन के साथ फ्लेवोनोइड्स पर कुल फेनोलिक्स के उच्च सहसंबंध मूल्य यह दर्शाता है कि

एंथोसायनिन फेनोलिक्स के एक बड़े अंश का प्रतिनिधित्व करते हैं, जो एंथोसायनिन वर्णक की बेहतर स्थिरता के साथ खाद्य रंग के रूप में संबंधित है। कुल मिलाकर, यह शोध इस बात पर प्रकाश डालता है कि उष्णकटिबंधीय काले गाजर और इसकी किस्म ''काशी कृष्णा'' पौध व्युत्पन्न एंथोसायनिन, फेनोलिक्स और फ्लेवोनोइड्स के सबसे समृद्ध स्रोतों में से एक है जो बेहतर एंथोसायनिन उपज और अधिक एंटीऑक्सीडेंट क्षमतायुक्त है। इसलिए, पौध व्युत्पन्न प्राकृतिक एंथोसायनिन के स्वास्थ्य और पोषण संबंधी लाभों, विविध उपयोगों और औद्योगिक महत्व को देखते हुए, काले गाजर को वर्णक फसल के रूप में संभावित रूप से उपयोग किया जा सकता है।

References

Alasalvar C, Gregor JM, Zhang D, Quantick PC and Shahidi F (2001) Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot cultivars. J Agric Food Chem 49:1410–1416.

Algarra M, Fernandes A, Mateus N, Freitas VD, da Silva JCGE and Casado J (2014) Anthocyanin profile and antioxidant capacity of black carrots (Daucus carota L. ssp. sativus var. atrorubens Alef.) from Cuevas Bajas, Spain. J Food Compos Analysis 33:71–76.

Anonymous (2020) Anthocyanin. https://en.wikipedia.org/wiki/Anthocyanin. Accessed on 15.08.2020.

Apak R, Guclu K, Ozyurek M and Celik SE (2008) Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. Microchimica Acta 160:413–419.

Baranski R, Goldman I, Nothnagel T and Scott JW (2016) Improving color sources by plant breeding and cultivation. In: Carle R, Schweiggert R (eds) Handbook on natural pigments in food and beverages. Woodhead Publishing, Cambridge, UK, p 429–472.

Benzie IFF and Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analyt Biochem 239:70–76.

Carrillo C, Rey R, Hendrickx M, Cavia MDM and Alonso-Torre S (2017) Antioxidant capacity of beetroot: traditional vs novel approaches. Plant Foods Human Nutri 72:266–273.

Cavagnaro P, Bannoud F, Iorizzo M, Senalik D, Ellison S and Simon PW (2019) Carrot anthocyanins: Nutrition, diversity and genetics. Acta Hortic 1264:101–106.

Chu YF, Sun J, Wu X and Liu RH (2002) Antioxidant and anti-proliferative activities of common vegetables. J Agric Food Chem 50:6910–6916.

Iorizzo M, Curaba J, Pottorff M, Ferruzzi MG, Simon PW and Cavagnaro PF (2020) Carrot anthocyanins genetics and genomics: Status and perspectives to improve its application for the food colorant industry. Genes 11: 906 doi:10.3390/genes11080906.

Iorizzo M, Senalik DA, Ellison S, Grzebelus D, Cavagnaro P, Allender C, Brunet J, Spooner DM, Van Deynze A and Simon PW (2013) Genetic structure and domestication of carrot (Daucus carota subsp. sativus) (Apiaceae). Am J Bot 100(5):930–938.

Kammerer D, Carle R and Schieber A (2004) Quantification of anthocyanins in black carrots (Daucus carota ssp. sativus var. atrorubens Alef.) and evaluation of their colour properties. Europ Food Res Technol 219:479–486.

Karkute SG, Koley TK, Yengkhom BK, Tripathi A, Srivastava S, Maurya A and Singh B (2019) Anti-diabetic phenolic compounds of black carrot (Daucus carota sps sativus var. atrorubens Alef.) inhibit enzymes of glucose metabolism: An in silico and in vitro validation. Medic Chem 14(6):641–649.

Karmakar P, Munshi AD, Behera TK, Kumar R, Sureja AK, Kaur C and Singh BK (2013) Quantification and inheritance of antioxidant properties and mineral content in ridge gourd (Luffa acutangula). Agri Res 2(3):222–228.

Koley TK, Singh S, Khemariya P, Sarkar A, Kaur C, Chaurasia SNS and Naik PS (2014) Evaluation of bioactive properties of Indian carrot (Daucus carota L.): A chemometric approach. Food Res Int 60:76–85.

Kurilich AC, Clevidence BA, Britz SJ, Simon PW and Novotny J (2005) Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. J Agric Food Chem 53:6537–6542.

Leja M, Kaminska I, Kramer M, Maksylewicz-Kaul A, Kammerer D, Carle R and Baranski R (2013) The content of phenolic compounds and radical scavenging activity varies with carrot origin and root color. Plant Foods Hum Nutri 68:163–170.

Lo-Scalzoa R, Gennaa A, Brancab F, Chedinc M and Chassaignec H (2008) Anthocyanin composition of cauliflower and cabbage and its

stability in relation to thermal treatments. Food Chem 107(1):136–144.

Montilla EC, Arzaba MR, Hillebrand S and Winterhalter P (2011) Anthocyanin composition of black carrot (Daucus carota ssp. sativus var. atrorubens Alef.) cultivars Antonina, Beta Sweet, Deep Purple and Purple Haze. J Agric Food Chem 59:3385–3390.

Nath P, Dukare A, Kumar S, Kale S, Kannaujia P (2022) Black carrot (Daucus carota subsp. sativus) anthocyanin-infused potato chips: Effect on bioactive composition, color attributes, cooking quality, and microbial stability. J Food Process Preser 46(3): https://doi.org/10.1111/jfpp.16180.

Nicolle C, Simon G, Rock E, Amouroux P and Remesy C (2004) Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. J Am Soc Hortic Sci 129:523–529.

Raman R (2018) Twelve healthy foods high in antioxidants. https://www.healthline.com/nutrition/foods-high-in-antioxidants. Accessed on 22.08.2020.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26:1231–1237.

Rodriguez-Saona LE, Giusti MM and Wrolstad RE (1998) Anthocyanin pigment composition of red-fleshed potatoes. Food Sci 63(3):458–465.

Schwarz M, Wray V and Winterhalter P (2004) Isolation and identification of novel pyranoanthocyanins from black carrot (Daucus carota L.) juice. J Agric Food Chem 52:5095–5101.

Singh BK and Karmakar P (2015) Improved production technology for root crops. In: Singh N, Roy S, Karmakar P, Chaurasia SN, Gupta S, Singh B (eds) Improved production technologies in vegetable crops. IIVR Training Manual No. 59, ICAR-IIVR, Varanasi, UP, India, p 120–133.

Singh BK and Karmakar P (2021) Introgression of cytoplasmic male sterility (CMS) in tropical carrots (Daucus carota subsp. sativus Schubl. & Martens). Vegetable Science 48(2): 203-208.

Singh BK, Koley TK, Maurya A, Singh PM and Singh B (2018) Phytochemical and antioxidative potential of orange, red, yellow, rainbow and black coloured tropical carrots (Daucus carota subsp. sativus Schubl. & Martens). Physiol Mol Biol Plants 24(5):899–907.

Singh BK, Pathak KA, Ramakrishna Y, Verma VK and Deka BC (2011) Purple-podded French bean with high antioxidant content. ICAR News: A Sci Technol Newsl 17(3):9.

Singh BK, Singh B and Singh PM (2019) Kashi Krishna: Black carrot variety. ICAR News: A Science and Technology Newsletter 25(2):18-19.

Singh RK and Chaudhary BD (1977) Biometrical methods in quantitative genetic analysis. Kalyani Publishers, Ludhiana, India.

Singleton VL, Orthofer R and Lamuela-Ranventos RM (1999) Analysis of total phenols other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol 299:152–178.

Smeriglio A, Denaro M, Barreca D, DAngelo V, Germano MP and Trombetta D (2018) Polyphenolic profile and biological activities of black carrot crude extract (Daucus carota L. ssp. sativus var. atrorubens Alef.). Fitoterapia 124:49–57.

Song W, Derito CM and Liu MK (2010) Cellular antioxidant activity of common vegetables. J Agric Food Chem 58:6621–6629.

Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV and Yencho GC (2007) Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem 103:829–838.

Wrolstad RE, Durst RW and Lee J (2005) Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 16(9):423–428.

Zhang D and Hamauzu Y (2004) Phenolic compounds and their antioxidant properties in different tissues of carrots (Daucus carota L.). J Food Agri Environ 2(1):95–100.

Zhishen J, Mengcheng T and Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555–55.