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Antioxidant potential and anthocyanin pigmentation profile of different coloured cultivars of ornamental kale (*Brassica oleracea* L. var. *acephala* DC)

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Abstract
The present investigation was carried at ICAR-IARI Regional Station, Katrain, Kullu, HP during 2016–2017. The activity of different antioxidant compounds & plant pigments in eight genotypes of ornamental kale were estimated. The experimental results on anthocyanin pigmentation profile revealed the presence of yellowish-green or bluish-purple or pink-purple colour in different genotypes of ornamental kale. Further, analysis of variance exhibited significant differences among different genotypes for all quality traits under study. The genotype, KtOK-40-1 recorded highest amount of lycopene (25.36 mg 100 g^{-1}), total carotenoids (18.30 mg 100 g^{-1}) & β-carotene (23.56 mg 100 g^{-1}), while concentration of FRAP (1.29 µ mol trolox g^{-1}) & anthocyanin (16.02 mg 100 g^{-1}) was observed maximum in the genotype KtOK-40-1. In the meanwhile, amount of CUPRAC (5.42 µ mol trolox g^{-1}), ascorbic acid (23.83 mg 100 g^{-1}) & phenolics (2582.82 µg gallic acid g^{-1} FW) was recorded highest in the genotype KtOK-45-1, KtOK-39-1 and KtOK-37-2, respectively. The Pearson's correlation coefficient analysis revealed a significant positive correlation of lycopene with total carotenoids (0.97) & β- carotene (0.97); total carotenoids with β-carotene (0.99) & ascorbic acid with anthocyanin (0.96). Based on the principal component analysis (PCA), first two components having eigen values greater than one was retained in the analysis because of the substantial amount of variation amongst them (77.68%). Further, loading of different quality traits based on two principal components indicated that CUPRAC, FRAP, anthocyanin & ascorbic acid are the main components of divergence between eight genotypes of ornamental kale, whereas contribution of rest of traits under study was found comparatively less in divergence. Hence, main emphasis should be given on these traits for quality improvement in ornamental kale. Further, neighbour-joining UPGMA dendrogram revealed that based on the trait of interest to be improved distantly placed genotypes can be selected as parental lines for the development of heterotic quality hybrids in ornamental kale.

Keywords: Ornamental kale, antioxidant activity, anthocyanin localization, PCA, & dendgrogram

1. Introduction
A traditional system of medicine such as ayurveda and Unan have provided us with the novel concepts and modalities in the healthcare area. Right from the prehistoric times, the value of traditional medicine in the treatment of different ailments is well recognized. Many physiological and biochemical processes in the human body may produce oxygen-centered free radicals and reactive oxygen species (ROS) as by-products. Overproduction of such free radicals can cause oxidative damage to bio-molecules (Lipids, proteins, carbohydrates and nucleic acids), ultimately causes mutations in genetic material, various chronic diseases and apoptosis (Kim *et al*. 2015; Bellomo, 1991; Halliwell and Gutteridge, 1986) [10, 3, 7]. Antioxidants are the substances that are broadly recognized as an important free radical scavenger and can eliminate the ROS by scavenging initiating radicals, breaking chain reaction, and binding of metal ions (Khled khoudja *et al*. 2014) [9]. The source of nutraceutical compounds such as retinol (Vitamin A), ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids, flavonoids, tannins, and other phenolic compounds in human diet is almost exclusively provided by fruits and vegetables (Duthie and Crozier, 2000; Stoclet *et al*. 2004 and Vinson *et al*. 1995) [6, 29, 31]. Recently, more attentions have been paid to natural antioxidants (Li *et al*. 2014) [17]. Because synthetic antioxidants have potential toxicological effects (Upadhyay *et al*. 2013) [30].

The potential of the antioxidant constituents of plant materials for the maintenance of health and diseases is raising awareness among scientists and food manufacturers because consumers are moving toward functional foods with specific health effects. So we need a good source of natural antioxidants which are efficient and cost effective. In order to resolve these problems, people are now interested to search plant-based antioxidants. Food rich in flavonoids and phenolic compounds acts as a good antioxidant. These phenolic compounds play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa 1994) [18].

Ornamental kale (*Brassica oleracea* var. *acephala* DC.) belonging to Brassicaceae family is a popular biennial herbaceous foliage ornamental plant cultivated worldwide for its variegated showy leaves, which are white, pink, purple, dark green, grey green or pale yellow and other complex coloured varieties (Zhu *et al*., 2016) [34]. It is widely cultivated as pot plant, cut stem (flower) as well as landscape plant particularly during late autumn through winter to early spring, when there is very few flowers available in the garden and its ability to withstand low temperature (Ren *et al*., 2015) [23]. The colourful leaves not only have ornamental value, but are also beneficial to human health due to high carotenoid and anthocyanin contents (Li *et al*. 2003) [16].

In general, *Brassica* vegetables are a good source of well-known antioxidant and health-promoting phytochemical compounds viz., carotenoids, ascorbic acid (Kopsell *et al*., 2004, 2006) [11, 12], vitamins, minerals, glucosinolates and phenolic compounds (Podsedek, 2007; Jahangir *et al*., 2009) [21, 8]. The various antioxidants, viz., phenols, vitamins, flavonoids, pigments, enzymes and minerals protect our body from oxidative damage (Singh *et al*., 2009) [27]. Hence, estimation of total antioxidant power attributable to cooperative action of different antioxidant compounds found in ornamental kale ought to be evaluated for different health benefits. The estimation of CUPRAC and FRAP contents is the most efficient means to determine total antioxidant capacity in plants (Apak *et al*., 2007; Ozyurek *et al*., 2008) [1, 19]. Vitamins and carotenoids are essential compounds which are responsible for accurate functioning of human metabolism and immune response (Samec *et al*., 2016) [25]. Ascorbic acid helps to maintain a healthy immune system, and neutralizes the pollutants and production of antibodies. Carotenoids are secondary plant compounds that form lipid-soluble yellow, orange and red pigments (Zaripheh and Erdman, 2002) [33]. Further, anthocyanins due to their multifunctional health benefits and other beneficial biological properties constitute an integral part of food (Sadilova *et al*., 2006) [24].

Today, ornamental kale is more popular in the home landscape and different cultivars are available in the market. However, there is meager information available on the biochemical composition and growth characteristics of these cultivars. Thus, the present study was conceived with the objective to estimate the antioxidant capacity and correlation among eight ornamental kale genotypes of different foliage colour and growth patterns for possible exploitation to breed genotypes/cultivars having high nutritional quality. Genotypes with higher levels of antioxidants will be used in further breeding programs and genetic studies with the aim to develop ornamental kale hybrids/cultivars of a particular colour which serve as a reference point for monitoring phytochemical and anthocyanin accumulation patterns in other plants and also to enhance the level of these compounds in edible cabbage and kale.

2. Materials and Methods

2.1 Experimental location, materials and layout plan

The present study was conducted at Sarsai Research Farm of ICAR-Indian Agricultural Research Institute Regional Station, Katrain, Kullu, HP, India, during 2016–2017. The farm is situated at 32.12°N latitude and 77.13°E longitude, at an altitude of 1650 m above mean sea level and it receives an average annual rainfall and snowfall of 110–120 and 120–150 cm, respectively. The experimental material consists of eight genotypes of ornamental kale maintained at the Regional Station, Katrain. The salient features of eight genotypes under study have been presented in the Table 1. Seeds of these lines were sown during August, 2016. The young seedlings were collected at the cotyledonary stage for anthocyanin profiling (Figure 1a and 1b) and the rest of the plants were allowed to grow until rosette stage to determine the location of the pigments, as well as to analyse anthocyanin and phytonutrient composition (Figure 1c).

2.2 Localization of pigments

For localization of the pigments**,** fresh hypocotyls were hand-sectioned as per the procedure described by Wang *et al*. (2014) [32]. The samples were placed in a petri dish containing distilled water and cut freehand with two overlapping scalpels. Transverse sections were selected for observation under a light microscope (Nikon ECLIPSE Ci-CF160, Japan).

2.3 Sampling and quality analysis

Antioxidant activity of different compounds viz., CUPRAC (µmol trolox/g), FRAP (µmol trolox/g), lycopene (mg/100g), ascorbic acid (mg/100 g), total carotenoids (mg/100 g), β-carotene (µg/100 g), phenolics (µg gallic acid/g f.w.) and anthocyanin (mg/100 g) among all the genotypes were estimated in this study. Samples were taken from true-to-type three plants at the rosette stage from both the replications of each genotype. Then, 5 g fresh sample for each analysis was weighed and stored instantly at -20°C temperature until needed for further analysis. To estimate the CUPRAC, a procedure described by Apak *et al*. (2006) [2], following some modifications, was used. For this, ethanol extract was prepared by mixing 5 g of each crushed sample in 15 ml of absolute ethanol. This extract was centrifuged at 10,000× rpm for 15 min at 4°C temperature. The supernatant was stored at -20°C temperature. After this, a 100 µl sample was mixed with 4 ml of CUPRAC reagent (1 ml neocuproine, 1 ml ammonium acetate, 1 ml cupric chloride ($CuCl_2$) and 1 ml of distilled water; pH 7.4). The absorbance was recorded at 450 nm OD via a UV-visible spectrophotometer (Thermo Fisher Scientific, USA). The FRAP concentration was estimated as per the method described by Benzie and Strain (1996) [4] after making some minor modifications. For this, ethanol extract was prepared by mixing 5 g of each crushed sample in 15 ml of absolute ethanol. This extract was centrifuged at 10,000× rpm for 15 min at 4°C temperature. The supernatant was stored at -20°C temperature. After this, a 100 µl sample was mixed with 3 ml of the FRAP reagent and observations were recorded after 4 min at 593 nm OD. For lycopene and carotenoids (Total carotenoids and β-carotene) 5g sample was crushed in acetone solution and centrifuged at 10,000× rpm for 15 min at 4°C temperature. Then supernatant was transferred in a separating funnel containing 20 ml of petroleum ether, mixed gently and then 5% of sodium sulphate (Na_2SO_4) and 20 ml of petroleum ether was added. Then supernatant was extracted and observations for all the pigments were recorded using a spectrophotometer at 452 and

503 nm OD. The ascorbic acid was estimated by the direct colorimetric method described by Ranganna (2008) [22]. Each sample (5 g) was homogenized in 4% oxalic acid and centrifuged at 10,000× rpm for 15 min at 4°C temperature. Then, 10 ml of supernatant was titrated against 2, 6-dichlorophenol-indophenol solution dye till the light pink colour appeared. Then the final reading was noted down as the volume of dye used for appearance of light pink colour of supernatant. Phenolic content was determined by using Folin-Ciocalteu's (Singleton, Rossi, 1965) [28] method. For this, 5 g sample was crushed in 80% ethanol and centrifuged at 10,000× rpm for 15 min at 4°C temperature. Final reaction mixture was prepared by adding 2.9 ml double distilled water in 0.1 ml supernatant. After this, 3.0 ml reaction mixture was mixed with 0.5 ml of 2 N Folin-Ciocalteu's phenol reagent. After 4 min, 2 ml of 20% sodium carbonate (Na_2CO_3) solution was added to this. Then the mixture was placed in a boiling water bath for 1 min and its absorbance was recorded at 750 nm OD. For the estimation of anthocyanin content, 5 g sample was homogenized in ethanolic hydrochloric acid solution (30 ml) and sample solution was stored overnight at 4°C temperature. The next day, solution mixture was centrifuged at 10,000× rpm for 15 min at 4°C temperature. Then supernatant was extracted and observations were recorded at 535 nm OD.

2.4 Statistical analysis

The pursual of data so obtained were subjected to analysis of variance using OPSTAT software (Sheoran *et al*., 1998) [26] as per the formulae described by Panse and Sukhatme (1967) [20]. The Pearson's correlation coefficient and principle component analysis (PCA) was done through SPSS 16.0 software, while UPGMA dendrogram based on neighbour-joining hierarchical cluster analysis was constructed using Darwin 6.0 software.

3. Results

3.1 Anthocyanin localization

Microscopic observation of inner leaf tissue of hypocotyls of different ornamental kale genotypes and their phenotypic expression are presented in Fig. 1B and 1C, respectively revealed that the genotype KtOK-2-1exhibited yellowish green pigmentation in first two layers beneath the epidermis. In the cultivar KtOK-37-1 and KtOK-37-2 bluish-purple pigmentation was observed in first three layers beneath the epidermis of the hypocotyls which provided dark purple colouration. The inner rosette leaves of these genotypes were pink in colour. In the genotype KtOK-39-1, outer layer had purple colouration which was followed by light pink ting in the inner layers. A uniform purple colouration with pink margins on all whorls of leaves were observed in the genotype KtOK-40-1. In the genotype KtOK-40-2, purple pigment was present beneath first layer of epidermis. Whereas, greenish yellow pigment was distributed as strips in the middle of rosette but inner leaves were pigmented with pink colour. The purple pigment was present in the first layer and greenish yellow pigment was distributed in all other layers beneath epidermis in the genotype KtOK-44-1. The genotype KtOK-45-1 exhibited dark purple pigmentation localized in first two layers, then slight yellow in the middle and again purple colouration in the inner layers, which is apparent from the phenotypic expression.

3.2 Quality analysis

The analysis of variance exhibited significant differences among different genotypes of ornamental kale for all quality traits under study (Table 2). The mean performance of eight genotypes of ornamental kale for different quality traits in has been presented in the table 3. The considerable variation (range) was observed among different genotypes for eight quality traits viz., CUPRAC (1.33-5.42 µ mol trolox g^{-1}), FRAP (0.09-1.29 µ mol trolox g^{-1}), lycopene (3.15-25.36 mg 100 g^{-1}), total carotenoids (3.92-18.30 mg 100 g^{-1}), β-carotene (5.04-23.56 mg 100 g^{-1}), ascorbic acid (4.53-23.83 mg 100 g^{-1}), phenolics (1080.94-2582.82 µg gallic acid g^{-1} FW) and anthocyanin (3.25-16.02 mg 100 g^{-1}). The genotype, KtOK-40-1 recorded highest amount of lycopene (25.36 mg 100 g^{-1}), total carotenoids (18.30 mg 100 g^{-1}) and β-carotene (23.56 mg 100 g^{-1}), while concentration of FRAP (1.29 µ mol trolox g^{-1}) and anthocyanin (16.02 mg 100 g^{-1}) was observed maximum in the genotype KtOK-40-1. In the meanwhile, amount of CUPRAC (5.42 µ mol trolox g^{-1}), ascorbic acid (23.83 mg 100 g^{-1}) and phenolics (2582.82 µg gallic acid g^{-1} FW) was recorded highest in the genotype KtOK-45-1, KtOK-39-1 and KtOK-37-2, respectively.

3.3 Correlation, principal component and cluster analysis

The Pearson's correlation coefficient analysis was used to explore the relationship among the different quality traits in eight genotypes of ornamental kale. The analysis revealed a significant positive correlation of lycopene with total carotenoids (0.97) and β- carotene (0.97); total carotenoids with β-carotene (0.99) and ascorbic acid with anthocyanin (0.96) (Table 4). Based on the principal component analysis (PCA), first two components having eigen values greater than one was retained in the analysis because of the substantial amount of variation amongst them. The two components had a variance of 55.50 and 22.18 per cent aggregating to total of 77.68 per cent of the total variation explained (Table 5). The first principal component (PC_1) had highest positive values for FRAP (0.68), lycopene (0.96), total carotenoids (0.88), β-carotene (0.88), ascorbic acid (0.69), phenolics (0.67) and anthocyanin (0.69), while second principal component (PC_2) exhibited highest positive value for CUPRAC (0.34) only. Further, loading of different quality traits based on two principal components indicated that CUPRAC, FRAP, anthocyanin and ascorbic acid were loaded more positively, while lycopene, total carotenoids, β-carotene and phenolics were loaded negatively on two axes (Fig. 2). The UPGMA dendrogram constructed using neighbour-joining hierarchical cluster analysis classified eight genotypes into two major groups viz. A and B ((Fig. 3). The group 'A' was further bifurcated into two sub-groups viz. A_1 & A_2, which accommodated three (KtOK-44-1, KtOK-40-2 and KtOK-45-1) and two (KtOK-37-1 and KtOK-2-1) genotypes, respectively. Similarly, group B was also divided into two subgroups viz., B_1 and B_2, which accommodated two (KtOK-37-2 and KtOK-40-1) and one (KtOK-39-1), genotypes, respectively.

4. Discussion

In ornamental kale colourful leaf is one of the most important agronomic traits. The diversity of colouration in inner leaves accelerated its usage in the garden all around the world. In the present study, we found that pigmentations were mainly distributed in the in first two layers beneath the epidermal cells in ornamental kale. Tissue distribution characteristics of pigmentations vary among different *Brassica* species. In *Brassica oleracea* var. *capitata* and *Brassica oleracea*. var. *botrytis* anthocyanins were concentrated in the outer layers of the sub-epidermal cells on both sides of the young leaf tissue.

In *Brassica rapa* ssp. *chinensis*, anthocyanins were found only in the upper epidermal cells. In agreement with the results of Zhu *et al*. (2016) [34] in different coloured cultivars of ornamental kale, we observed that red pigment was present mainly in first three layers beneath the epidermis of the hypocotyls which provide dark purple colour to the seven genotypes. The high levels of red pigment in the first and second layers beneath the epidermis provided the pink, red, and purple phenotypes. In addition, greenish yellow pigment was distributed in all the layers beneath epidermis of the inner rosette leaves of the genotype KtOK-44-1, which may be due to the accumulation of chlorophylls. In earlier study on ornamental kale, Zhu *et al*. (2016) [34] reported that the colour of hypocotyls co-segregates with the formation of anthocyanins in sub-epidermal cells of leaves and is controlled by the same semi-dominant gene, *Pi*. In the present study, we found that anthocyanins are the main factor for inner-leaf colouration of the seven genotypes. We hypothesize that the colour of the showy inner leaves of different genotypes of ornamental kale is determined by the distribution and contents of anthocyanins, in addition by the proportion of different pigment (Anthocyanin) components present.

Although ornamental kale is primarily used for their aesthetics in the home garden, the presence of high concentrations of phytochemicals (Polyphenols, flavonoids, carotenes, glucosinolates, lutein, zeaxanthin), require additional investigation into the possibility of breeding this trait into edible kale and cabbage cultivars, which have been reported to have significantly lower levels of these compounds. Carlson *et al*. (1987) [5] and Kushad *et al*. (1999) [15] in ornamental cabbage and kale found that the glucoraphanin levels were 3 to 12-fold and lucobrassicin levels were 2.5 to 4-fold higher than in the edible vegetables, respectively. In the present investigation, we have found wide variation in most of the evaluated attributes. This indicates very wide genetic base for these quality traits in ornamental kale. Ascorbic acid content varied from 4.53 mg/100g (KtOK-2-1) to 23.83 mg/100g (KtOK-39-1) indicating significantly high variability for this trait among the evaluated genotypes. The intake of natural antioxidants from food is important for healthy life. The CUPRAC and FRAP content was significantly varied among the genotypes. Therefore, genotypes with high values of CUPRAC and FRAP can be used in the development of F1 hybrids with higher antioxidant capacity in edible kale.

The concentration of lycopene, total carotenoids and β-carotene was found to be highest in the genotype KtOK-40-1 followed by KtOK-37-1, KyOK-37-2 and KtOK-40-2. Thus, these lines can be used in the development of hybrids with higher concentration of plant pigments. The phenolics content was found to be maximum in the genotype KtOK-37-2 followed by KtOK-40-1. Our results were consistent with the earlier report of higher total phenolic and flavonoid content in kale leaf extract by Kural *et al*. (2011) [13]. Anthocyanin is an important pigment present in various plant parts. Many beneficial activities have been attributed to anthocyanins and the majority of work focuses on the antioxidant characteristics of anthocyanin rich diets. When we analyzed the eight genotypes of ornamental kale it was found that significantly highest amount of this pigment was present in KtOK-40-2, which was found statistically at par with KtOK-39-1.

The correlation studies indicated that lycopene, total carotenoids, β-carotene and ascorbic acid had strong positive correlation with each other. This illustrated that these plant pigments have similar pathway of their synthesis in plant species. Hence, a positive correlation among different quality traits offers the chances for selection of genotypes with superior multiple quality traits in ornamental kale for edible purpose. Ornamental cabbage cultivars contained higher glucoraphanin, followed by sinigrin, progoitrin and glucobrassicin, while ornamental kale cultivars contained about the same concentrations of glucoraphanin, sinigrin and glucobrassicin, except 'Coral Prince' (Kushad *et al*., 2004) [14]. The outcome of PCA indicated that CUPRAC, FRAP, anthocyanin, and ascorbic acid are the main components of divergence between eight genotypes of ornamental kale, whereas contribution of rest of traits under study was found comparatively less in divergence. Hence, main emphasis should be given on these traits for quality improvement in ornamental kale and cabbage. Further, neighbour-joining UPGMA dendrogram revealed that based on the trait of interest to be improved distantly placed genotypes can be selected as parental lines for the development of heterotic quality hybrids in ornamental kale for edible purpose.

Table 1: Salient features of different genotypes of ornamental kale used in present study

Genotype	Morphological characteristics
KtOK-2-1	White colour with green margins; highly fringed leaves; average head size, 36.8 cm; coloured head portion, 25.9 cm; average plant height, 16.34 cm.
KtOK-37-1	Variegated head with greenish to pinkish centre; round to moderately curved leaves; average head size, 28.4 cm; coloured head portion,17.2 cm; average plant height, 30.9 cm.
KtOK-37-2	Dominantly pink coloured with pinkish whitish centre; curved wavy leaves; average head size, 32.1 cm; coloured head portion, 19.1 cm; average plant height, 32.1 cm.
KtOK-39-1	Pinkish white heads with pink centre; curved and wavy leaves; average head size, 19.0 cm; coloured head portion, 11.2 cm; average plant height, 28.4 cm.
KtOK-40-1	Pinkish head with pink centre; average head size, 24.9 cm; coloured head portion, 15.7 cm; average plant height, 35.4 cm.
KtOK-40-2	Pink coloured heads with pinkish white centre; average head size, 34.4 cm; coloured head portion, 20.8 cm; average plant height, 33.0 cm.
KtOK-44-1	Greenish coloured with white centre; highly fringed leaves; average head size, 33.2; coloured head portion, 18.1 cm; average plant height, 16.9 cm.
KtOK-45-1	Greenish pink heads with dark pink centre; highly fringed leaves; average head size, 46.2 cm; coloured head portion, 26.6 cm; average plant height, 34.8 cm.

Table 2: Analysis of variance (ANOVA) for different quality traits in ornamental kale

Source of variation	*df	Mean sum of squares							
		†CUPRAC	‡FRAP	Lycopene	Total carotenoids	β-carotene	Ascorbic acid	Phenolics	Anthocyanin
Replication	1	0.07	0.00	9.73	8.81	14.65	1.98	14965.84	0.74
Genotype	7	4.13**	0.26**	164.22**	61.08**	101.22**	97.75**	632393.72**	46.27**
Error	7	0.11	0.01	3.734	4.88	8.08	0.27	12303.14	1.35

†CUPRAC, cupric ion reducing antioxidant activity; ‡FRAP, ferric reducing ability of plasma.
*degree of freedom; **Significant at P ≤0.01.

Table 3: Mean performance of eight genotypes of ornamental kale for different plant pigments, vitamins and antioxidant compounds

Genotype	†CUPRAC (μ mol trolox g^{-1})	‡FRAP (μ mol trolox g^{-1})	Lycopene (mg 100 g^{-1})	Total carotenoids (mg 100 g^{-1})	β-carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	Phenolics (μg gallic acid g^{-1} FW)	Anthocyanin (mg 100 g^{-1})
KtOK-2-1	1.39±0.03	0.53±0.01	3.35±0.06	4.14±0.05	5.32±0.06	4.53±0.38	1718.44±139.69	3.25±0.25
KtOK-37-1	3.07±0.20	0.56±0.06	23.10±1.64	17.17±2.14	22.10±2.75	12.50±0.25	1974.38±91.88	10.77±0.90
KtOK-37-2	3.04±0.20	0.60±0.07	22.09±2.59	15.22±2.41	19.59±3.11	14.84±0.34	2582.82±122.82	7.30±0.26
KtOK-39-1	3.00±0.25	0.53±0.02	13.19±1.83	9.14±2.24	11.77±2.88	23.83±0.50	2261.25±30.00	15.94±1.76
KtOK-40-1	4.66±0.15	0.65±0.06	25.36±1.67	18.30±2.33	23.56±3.01	11.50±0.25	2522.82±62.82	8.38±0.19
KtOK-40-2	3.93±0.09	1.29±0.14	21.04±1.44	12.30±0.51	15.83±0.66	22.96±0.38	1447.50±18.75	16.02±0.99
KtOK-44-1	1.33±0.07	0.09±0.04	9.04±0.61	9.40±0.64	12.09±0.82	7.35±0.94	1330.32±14.07	4.69±0.28
KtOK-45-1	5.42±0.49	0.18±0.01	3.15±0.08	3.92±0.11	5.04±0.14	8.91±0.55	1080.94±45.94	6.44±0.13
Mean	3.23	0.55	15.04	11.20	14.41	13.30	1864.81	9.10
±SE(m)	0.23	0.07	1.37	1.56	2.01	0.37	78.43	0.82
$C.D._{(0.05)}$	0.79	0.22	4.65	5.32	6.84	1.25	266.81	2.80

†CUPRAC, cupric ion reducing antioxidant activity; ‡FRAP, ferric reducing ability of plasma.

Table 4: Pearson's correlations coefficient among different quality traits studied in ornamental kale

Trait(s)	†CUPRAC	‡FRAP	Lycopene	Total carotenoids	β-carotene	Ascorbic acid	Phenolics	Anthocyanin
CUPRAC	1.00	0.21	0.26	0.18	0.18	0.28	0.00	0.32
FRAP		1.00	0.58	0.39	0.39	0.64	0.22	0.65
Lycopene			1.00	0.97**	0.97**	0.48	0.66	0.49
Total carotenoids				1.00	0.99**	0.30	0.67	0.32
β-carotene					1.00	0.31	0.67	0.33
Ascorbic acid						1.00	0.29	0.96**
Phenolics							1.00	0.19
Anthocyanin								1.00

†CUPRAC, cupric ion reducing antioxidant activity; ‡FRAP, ferric reducing ability of plasma; **Significant at p≤0.01.

Table 5: Eigen vectors for first two principal components of different quality traits in ornamental kale

Trait(s)	*Principal Component	
	#PC_1	PC_2
CUPRAC	0.33	0.34**
FRAP	0.68	0.43
Lycopene	0.96	-0.24
Total carotenoids	0.88	-0.44
β-carotene	0.88	-0.44
Ascorbic acid	0.69	0.63
Phenolics	0.67	-0.45
Anthocyanin	0.69	0.66
Eigen Value	4.44	1.77
Percentage of variance	55.50	22.18
Cumulative % of variance	55.50	77.68

#PC: Principal component
*Extracted through principal component analysis
**Bold value indicates the highest Eigen vector for the corresponding trait amongst the two principal components

Fig 1: Phenotypes and magnified sections of different genotypes of ornamental kale; Frame A: Photographs taken at cotyledonary stage; Frame B: Images shown are transverse sections of hypocotyls observed under light microscope at 10X magnification; Frame C: Photographs taken at rosette stage

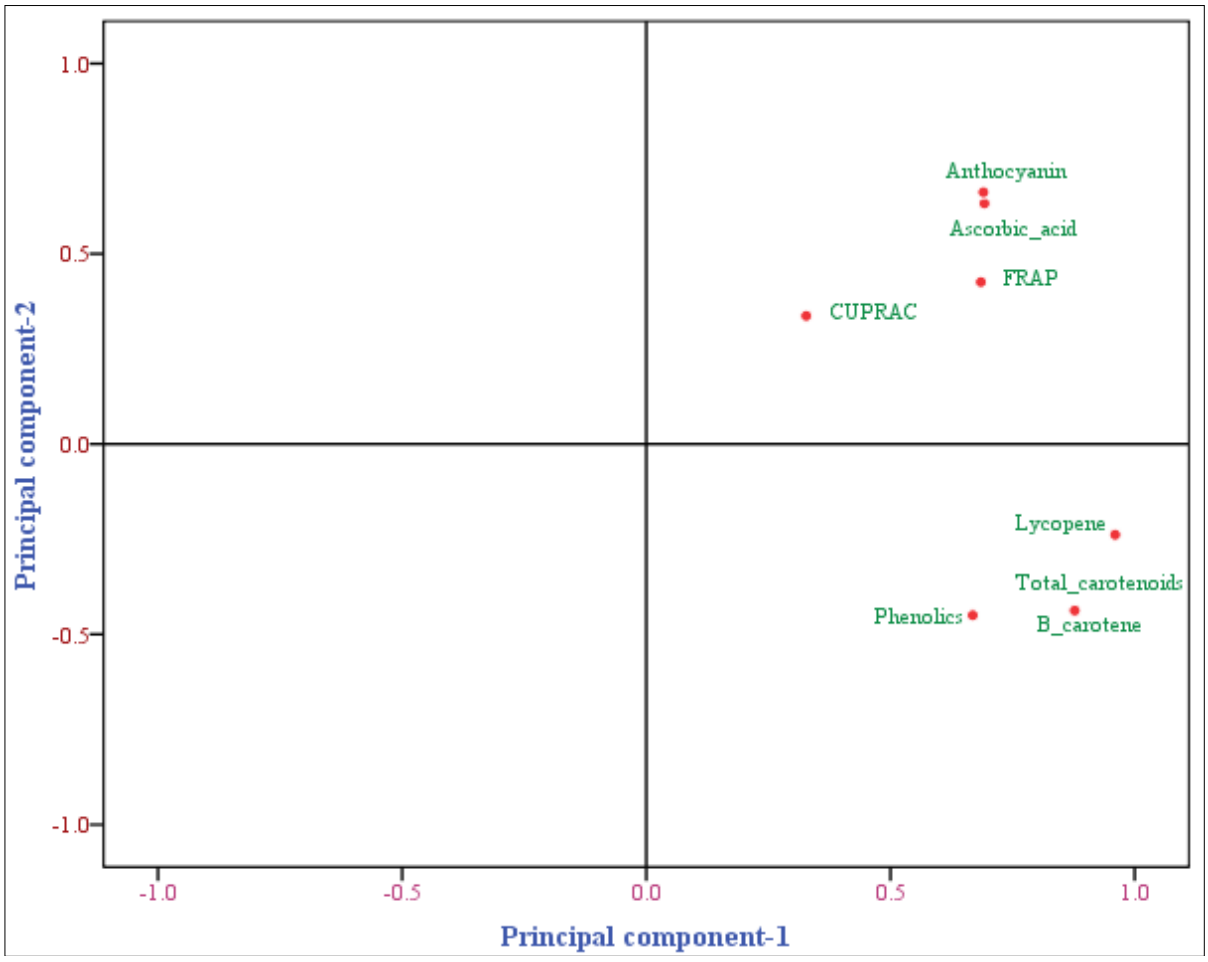

Fig 2: Loading of different quality traits based on first two principal components

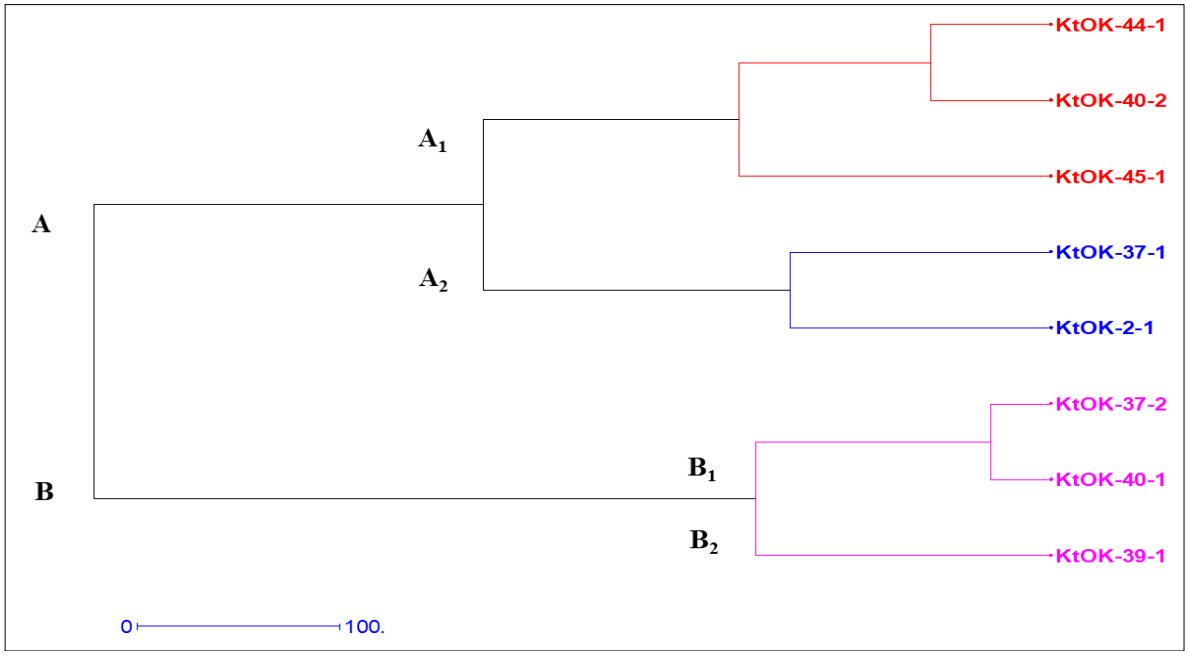

Fig 3: UPGMA dendrogram showing clustering pattern of eight genotypes of ornamental kale constructed using neighbour-joining hierarchical cluster analysis

5. References

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