International Journal of Chemical Studies

**P-ISSN: 2349–8528**
**E-ISSN: 2321–4902**
IJCS 2019; 7(1): 1484-1494

Received: 14-11-2018
Accepted: 18-12-2018

**Saurabh Singh**
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

**SS Dey**
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

**Raj Kumar**
ICAR-Indian Agricultural Research Institute, Regional Station, Katrain, Kullu, Himachal Pradesh, India

**Reeta Bhatia**
Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi, India

**Hemant Ghemeray**
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

**TK Behera**
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

**Correspondence**
**Saurabh Singh**
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

# Genetic analysis and interaction among CUPRAC, FRAP, phytochemical and phenotypic traits in cauliflower (*Brassica oleracea* var. *botrytis* L.)

**Saurabh Singh, SS Dey, Raj Kumar, Reeta Bhatia, Hemant Ghemeray and TK Behera**

**Abstract**
The lack of information regarding genetic variability and inter-relationships among antioxidant and phytochemical, phenotypic traits in snowball cauliflower evoked an experiment using 26 *Ogura* based cytoplasmic male-sterile (CMS) and doubled haploid (DH) lines to formulate breeding strategies for the development of antioxidant rich cultivars. Significant variability was observed for all the antioxidant traits suggesting the scope for improvement of these antioxidant traits in cauliflower. The cluster analysis revealed five different groups of parental lines based on phytochemical traits. The slightly higher magnitude of phenotypic coefficient of variation (PCV) than genotypic coefficient of variation (GCV) for all the antioxidant traits indicated small influence of environment on accumulation of these traits. The high heritability (>80%) accompanied with high genetic gain for the accumulation of antioxidant traits indicated the predominance of additive gene action. This study will pave the way for mapping of QTLs and breeding of bio fortified cultivars in cauliflower.

**Keywords:** antioxidant capacity, cauliflower, phytochemicals, genetic variability, heritability, correlation

## Introduction
Antioxidant compounds are widespread in the plant kingdom including fruits and vegetable crops, and they play a significant role in plants and human health. The discovery and subsequently the isolation of vitamin C evoked the interest in antioxidant activity and their extraction from plants (Kasote *et al*., 2015) [17]. The root cause for the incidence of life threatening diseases (such as cardiovascular diseases, various types of cancer and age related disease) is the oxidative stress attributed to imbalance between generation and accumulation of reactive oxygen species (ROS) like superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical, singlet oxygen ($^1O_2$) and ROS detoxification ability of a biological system (Li *et al*., 2014; Kasote *et al*., 2015) [17, 18]. Antioxidant compounds have been proved effective to significantly delay or prevent the oxidative stresses caused by these ROS products and thus, impart human health benefits (Li *et al*., 2014) [18]. Vegetable crops are potential source of important nutraceutical and antioxidant compounds including various vitamins and minerals, thus they are regarded as protective foods (Singh and Devi, 2015; Singh *et al.*, 2018) [31, 32]. *Brassica oleracea* comprises diverse group of vegetables (cauliflower, cabbage, Brussels sprout, kale, knoll khol, broccoli) commonly known as cole crops and are important part of dietary food. They are regarded as 'super-food' owing to their health promoting properties, as they are rich source of antioxidant phytochemicals, minerals and secondary metabolites such as selenium, glucosinolates, sulphoraphane, carotenoids, phenolic compounds, anthocyanins and vitamins A, C, E and K (Soengas *et al.*, 2012; Sotelo *et al.*, 2014; Dey *et al.*, 2015; Singh *et al.*, 2018; Samec *et al.*, 2018) [37, 38, 10, 32, 24]. The high intake of *Brassica* vegetables has been associated with reducing the risk of age related chronic diseases, cardiovascular diseases and various types of cancer (Soengas *et al.*, 2012; Ciska *et al*., 2015; Singh *et al.*, 2018) [37, 8, 32]. The dietary phytochemicals, such as indole-3-carbinol, sulforaphane, present in *Brasssica* vegetables have been associated with reduction of breast cancer by targeting the miRNAs involved in induction of breast cancer (Sayeed *et al.*, 2017) [25]. Anthocyanins are natural pigments, imparting red, purple, blue color to fruit and vegetable parts, and belong to the group of flavonoids having antioxidant, anti-inflammatory activity (Mizgier *et al*., 2016; Hodaei *et al.*, 2017) [19, 16].

The antioxidant activity of anthocyanins is related to their ability to impede the harmful effects of free radicals. The phenolic compounds are regarded as major group of antioxidants owing to their ubiquitous nature in plant kingdom including *Brassicaceae* crops. The antioxidant property of phenolic compounds is attributed to their redox properties and helps in neutralizing ROS, thus reduce the enhancement of many chronic diseases (Cartea *et al.,* 2010) [7]. Ascorbic acid is also an indispensable antioxidant compound prevalent in *Brassica* crops and is an essential vitamin to prevent scurvy, heart diseases, stroke and cancer in humans (Duan *et al.,* 2015; Granger and Eck, 2018; Singh *et al.,* 2018) [13, 15, 32]. In plants, vitamin C plays a vital role in pathogen defense, cell wall synthesis, regulation of growth processes and modulation in flowering time and plant morphological features (Duan *et al.,* 2015) [13]. Thus, ascorbic acid is an essential antioxidant for both plants and humans to prevent oxidative stress. As humans have lost the inbuilt mechanism to synthesize ascorbic acid, improvement in its content and bioavailability is an important breeding objective in crop breeding programmes. Carotenoids are isoprenoid group of antioxidants and acts as quenchers of ROS. They provide protection against various types of cancer, cardiovascular disease and eye related disorders (Fiedor and Burda, 2014) [14]. Among the diverse group of cole vegetables, cauliflower (*Brassica oleracea* var. *botrytis* L., 2n = 18, CC) is most important crop belonging to *Brassicaceae* family. It renders health promoting benefits including anticancer activity as it is good source of ascorbic acid, vitamin A, minerals, phytochemicals, glucosinolates and other antioxidant compounds (Soengas *et al.,* 2012; Sotelo *et al*., 2014; Singh *et al*., 2018) [37, 38, 32]. The most efficient approach for determining antioxidant capacity in crop plants has been associated with estimation of cupric ion reducing antioxidant capacity (CUPRAC), ferric reducing ability of plasma (FRAP), ascorbic acid, phenolic compounds, anthocyanins and carotenoids contents (Soengas *et al.,* 2012; Singh *et al*., 2018; Raiola *et al*., 2018) [37, 32, 22]. Due to antioxidant activity of these bioactive compounds and their presence in *Brassica* vegetables, the knowledge on genetic divergence, inheritance and interaction of these traits with morphological traits is essential for the development of antioxidant rich hybrids, breeding lines and cultivars to combat malnutrition problems. Being a major cole vegetable grown in the Indian subcontinent and across the world, the $F_1$ hybrid development in cauliflower (*B. oleracea* var. *botrytis* L.) has been given impetus owing to having better quality, uniformity and resistance to abiotic and biotic stresses (Singh *et al*., 2018) [32]. In cauliflower, the genetic phenomenon of sporophytic self-incompatibility (SSI) and cytoplasmic male-sterility (CMS) have been used commercially for the development of superior hybrid cultivars (Singh and Vidyasagar, 2012; Dey *et al*., 2015; Sehgal and Singh, 2018; Singh *et al.,* 2018) [33, 10, 28, 32]. However, the SSI system is very weak or negligible in case of snowball cauliflower owing to weak activity of S-alleles (Sehgal and Singh, 2018) [28] and thus CMS is the best alternative for hybrid development in this late group of cauliflower. For the development of hybrids rich in antioxidant concentration, the basic requirement is development and maintenance of improved inbred lines. Due to high percentage of cross pollination in *Brassica oleracea*, the development of inbred lines through traditional inbreeding is tedious and time consuming. Hence, in this regard the development of doubled haploid (DH) lines is the best alternative to produce 100% homozygous inbred lines and maintenance of CMS lines via microspore culture (Bhatia *et al*., 2017) [4]. The superior doubled haploid (DH) lines have been developed by our group previously (Bhatia *et al*., 2017) [4], which could serve as immortal mapping population for the identification of genes/QTLs related to qualitative and quantitative traits, thus accelerating the efficiency of breeding program (Sotelo *et al.,* 2014) [38].

The extensive genetic variability present in any crop germplasm is the vital for efficient breeding programs, to broaden the gene pools, identification of highly homozygous parents and exploitation of heterosis. In context of antioxidant traits, the inadequate information is available on genetic divergence, inheritance, genetic gain and interaction of phytochemicals and phenotypic traits exploiting advance generation breeding lines including *Ogura* cybrid cytoplasm based cytoplasmic male sterile (CMS) lines and doubled haploid (DH) homozygous inbreds of snowball cauliflower. The availability of information regarding phytochemical characterization, antioxidant potential, biochemical systematics and their interaction with physiological and morphological traits in advance breeding lines of crop plants is of utmost importance to mitigate the problems of malnutrition and human health diseases. Moreover, the analysis of variability and heritability of antioxidant and phytochemical compounds will also help in formulating appropriate breeding strategy for the development of antioxidant rich varieties and hybrid cultivars. Further, it will also stimulate the generation of mapping population and identification of antioxidant capacity related QTLs, candidate gene analysis and understanding biosynthetic pathways of different biochemical constituents in crop plants. Thus, the present investigation was formulated with the objectives to analyze biochemical divergence, heritability, genetic gain and correlation among biochemical and morphological traits in cauliflower genotypes using CMS and DH lines and their test cross progenies for possible utilization to breed cultivars with enhanced concentration of antioxidants. The long run objective of the present investigation is to generate breeding material and mapping populations to identify heterotic QTLs related to antioxidant capacity in snowball cauliflower and to boost research on epidemiological studies, framing dietary guidelines and elucidating downstream mechanisms of biosynthesis of bioactive compounds and their mode of action.

## Materials and Methods

The plant materials included 30 cauliflower genotypes comprising 20 *Ogura* cybrid cytoplasm based CMS lines, 6 DH based testers, 4 commercial standard checks (Table 1). These were grown at Baragram Farm, ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Katrain, Kullu Valley, Himachal Pradesh, India, which is located at 32.12N latitude and 77.13E longitudes with an altitude of 1,560 m above mean sea level. All the recommended package of practices was followed to raise the crop (Sharma, 2003) [30]. The trial was conducted in 10 × 15 alpha-lattice design replicated thrice and plot size was kept 3.0 × 3.0 $m^2$ with inter-and intra-row spacing of 45 cm. Five randomly selected plants were tag-labelled for biochemical analysis in each genotype in each plot of all the three replications. During the marketable stage, the pooled sample was taken from the five curds of each genotype in replicated trial by chopping the curds and homogenized for analysis of potential antioxidant and quality traits in cauliflower genotypes.

**CUPRAC (cupric ion reducing antioxidant capacity) assay**
For the CUPRAC estimation (µ mol trolox/g) the method described by Apak *et al*. (2007) [2] was used with minor modifications. The pooled sample from 5 curds of each genotype/replication was chopped, homogenized at fresh market stage and a sample of 5g fresh weight (FW) was weighed, refrigerated and immediately stored until assay. The ethanol extract was prepared by homogenized 5g sample in 15 ml ethanol (100%) and centrifuged at 10,000 rpm for 15 min at 4ºC followed by storage of supernatant at -20°C. A sample of 100 µl was mixed with 4 ml of CUPRAC reagent (1 ml neucuproine, 1 ml ammonium acetate, 1ml $CuCl_2$ and 1 ml of distilled water; pH 7.4). Absorbance was recorded at 450 nm in double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). Analysis was performed in triplicate for each extract of each genotype of all the three replications.

**FRAP (ferric reducing ability of plasma) assay**
For the estimation of FRAP concentration (µ mol trolox/g), the method described by Benzie and Strain (1999) [3] with minor modifications was followed. For the preparation of FRAP reagent the mixing of acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and20 mM $FeCl_3$ at 10:1:1 (*v*/*v*/*v*) was done. Then 100 µL of sample was thoroughly mixed with the FRAP reagent (3.0 ml) and the absorbance was taken at 593 nm after 30 min in double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). Analysis was performed in triplicate for each extract of each genotype of all the three replications.

**Table 1:** Parental lines, testers and commercial checks analyzed in the present investigation

| Status | Line | Curd Compactness | LA | Source |
|---|---|---|---|---|
| CMS line | Ogu122-5A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu115-33A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu118-6A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu307-33A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu309-2A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu33A | Compact | E | IARI, Katrain, India |
| CMS line | OguKt-2-6A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu1A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu13-85-6A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu1-6A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu2A | Compact | E | IARI, Katrain, India |
| CMS line | OguKt-9-2A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu22-1A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu122-1A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu126-1A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu12A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu119-1A | Compact | SE | IARI, Katrain, India |
| CMS line | Ogu34-1A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu125-8A | Compact | E | IARI, Katrain, India |
| CMS line | Ogu33-1A | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-18-8-1 | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-18-8-3 | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-53-1 | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-53-6 | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-53-9 | Compact | E | IARI, Katrain, India |
| Doubled Haploid | DH-53-10 | Compact | E | IARI, Katrain, India |
| Check1 | HVCF-29 | Compact | SE | Acsen HyVeg |
| Check2 | HVCF-18 | Compact | SE | Acsen HyVeg |
| Check3 | HVCF-16 | Compact | SE | Acsen HyVeg |
| Check4 | Pahuja | Compact | SE | Pahuja Seeds |

LA:Leaf attitude; E:Erect; SE:Semi-erect; IARI, Katrain:Indian Agricultural Research Institute, Regional Station, Katrain, H.P., India-175129

**Quantification of Total phenolic content (TPC)**
The Folin–Ciocalteu method with slight modifications was used for estimation of total phenolic contents (mg of gallic acid/100 g FW) in cauliflower genotypes (Ainsworth and Gillespie, 2007) [1]. Briefly a volume of 0.50 mL of the plant extract was mixed with 2.5 mL of 1:10 diluted Folin-Ciocalteu reagent and was neutralized with 2 mL of 20% sodium carbonate ($Na_2CO_3$) solution. The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 750 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The calibration was performed with gallic acid. Each sample was analyzed in triplicate for each reading of all the three replications.

**Determination of total ascorbic acid content (TAsA)**
The ascorbic acid (mg/100g) was also determined by the direct colorimetric method as described by Ranganna (1979) [23] based on measurement of the extent to which 2, 6-dichlorophenol indo-phenol solution (dye) is decolourized by ascorbic acid available in sample extracts and standard ascorbic acid solution. The 5g sample of fresh homogenized cauliflower curds of each genotype/replication was extracted with 4% oxalic acid and volume made to 100 ml and centrifugation was done. After centrifugation the 5 ml of this supernatant was pipette out, added with 10 ml of 4% oxalic acid and was titrated against 2, 6-dichlorophenol indo-phenol dye. Analysis was performed in triplicate for each extract of each genotype of all the three replications.

**Quantification of anthocyanin and carotenoids**

For the estimation of anthocyanins content (mg/100g) in cauliflower genotypes, the homogenized sample of each genotype per replication (2g) was mixed with 15 ml of ethanol–hydrochloric acid mixture (95% $C_2H_5OH$ and 1.5 N HCl in the ratio of 85:15). Then this extraction mixture was transferred into a 50 ml volumetric flask and kept overnight at 4°C, filtered through Whatman No. 1. Eventually the optical density of filtrate was measured at 535 nm in a double beam UV-VIS spectrophotometer (UV Analyst-CT 8200) (Ranganna, 1979) [23]. For each extract the value of optical density was taken in triplicate for each sample of all the three replications. For the estimation of carotenoids content viz. β-carotene (µg/100 ml), total carotenoid content (TCC) (mg/100g), lycopene (mg/100g), the procedure described by Rangana (1979) [23] was followed. The 5g of homogenized sample of cauliflower curd was taken and mixed with acetone until the residue became colorless. The extract was decanted into a separating funnel containing 20 ml of petroleum ether (BP 60–80 ◦C) followed by addition of 5% sodium sulphate ($Na_2SO_4$) to remove excess water. Finally, uniform volume was made upto 50 ml and absorbance was taken at 503 nm and 452 nm using petroleum ether as a blank in double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). Each sample was analyzed in triplicate for each reading.

**Phenotypic traits**

All the genotypes comprising CMS and DH based parental lines were also characterized for various phenotypic traits to estimate correlation of antioxidant capacity and phytochemical traits with phenotypic traits. The data recording of phenotypic traits was done from 5 randomly selected plants of each genotype in three replications of 10 × 15 alpha lattice design. The genotypes were evaluated for the traits viz. (i) plant height: PH (cm) (ii) gross plant weight: GPW (g) (iii) marketable curd weight: MCW (g) (iv) net curd weight: NCW (g) (v) leaf length: LL (cm) (vi) leaf width: LW (cm) (vii) core length: CoL (cm) (viii) leaf size index: LSI ($cm^2$) (ix) harvest index: HI (%) (x) shape index (curd length/curd width) (Dey *et al.*, 2013; Dey *et al*., 2017) [9, 11].

**Statistical analysis**

To analyze the antioxidant capacity parameters of extracts, the assays of each genotype in each block of each replication were performed in triplicate. The resultant data for each of the antioxidant traits were statistically subjected to analysis of variance (Singh and Chaudhary, 2014) [36] heritability in a broad sense ($H^2_b$) (Ogunniyan and Olakojo, 2014) [20], genetic advance (GA) and correlation coefficient (Ogunniyan and Olakojo, 2014) [20] and genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) (Singh and Chaudhary, 2014) [36]. The genotype means were compared using least significant difference (LSD) at $P \leq 0.05$ with Scott-Knott test grouping of genotypes for antioxidant traits (Scott and Knott, 1974) [27]. All the data for the analysis of above components were subjected to statistical analysis by software SAS (statistical analysis software) version 9.4 (SAS Institute, 2013) [26]. A dendrogram was made using neighbor joining (NJ) method with the help of DAR win v.6.0.12 software (Perrier and Jacquemoud-Collet, 2006) [21] based on Euclidian distance. Pearson correlation coefficient among antioxidant, phytochemical and phenotypic traits was calculated using R statistical software version 3.5.1. The genetic parameters are as follow: PCV (%) = $\frac{\sqrt{V_p}}{mean}$x100 and GCV (%) = $\frac{\sqrt{V_g}}{mean}$x100, where Vp is the phenotypic component of variance, Vg is genotypic component of variance, Vp = Vg + Ve, where Ve is environmental component of variance. The GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (≥ 20%) (Burton and De Vane, 1953) [6], Heritability in broad sense (%) ($H^2_b$) = Vg/Vp × 100. The extent of GA was calculated as = $H^2_b$ × P × K, where P is the phenotypic standard deviation, K is standardized selection differential constant (2.06) at 5% selection intensity. Genetic advance as percent of mean was computed as GAM% = GA/mean × 100.

**Results and Discussion**

**Quantification of phytochemicals and antioxidant traits**

The variance analysis of mean squares revealed significant differences among all the studied genotypes of cauliflower for all the antioxidant capacity and phytochemical traits (CUPRAC, FRAP, TAsA, anthocyanins, lycopene, TPC, β-carotene and TCC) at $P \leq 0.001$, whereas there was no significant difference among the three replications for all the studies traits, indicating the true presence of inherent variation among all the genotypes (data not shown here) which could be harnessed in cauliflower breeding program. The average quantification for antioxidant capacity and phytochemical traits of parental CMS and DH lines presented in Table 2 depicted significantly large variation for antioxidant capacity and quality traits in the studied genotypes of cauliflower (Fig 1). The antioxidant capacity was estimated in terms of CUPRAC and FRAP assay. CUPRAC assay detects a wider range of antioxidants and is based on the ability of sample to reduce $Cu^{2+}$ to $Cu^{1+}$ in the presence of a chelating agent such as neocuproine (Apak *et al.*, 2007) [2]. The FRAP assay is based on the ability of antioxidants presented in the sample to reduce ferric ions to ferrous ions (Benzie and Strain, 1999) [3]. The 5 CMS lines Ogu119-1A, OguKt-2-6A, Ogu13-85-6A, Ogu34-1A and Ogu122-5A performed better for CUPRAC content as compared to best check (HVCF-16) (Table 2). Similarly, CMS lines Ogu122-5A and Ogu307-33A were having more FRAP content than best check (HVCF-29). Among the six DH lines, DH-53-1 had highest mean value for CUPRAC and FRAP content. The four lines Ogu122-1A,

**Table 2:** Characterization of parental lines including commercial checks for 8 antioxidant traits

| Genotypes /Traits | CUPRAC | FRAP | Ascorbic acid | Total Phenols | Anthocyanin | Lycopene | β-carotene | Total carotene |
|---|---|---|---|---|---|---|---|---|
| Ogu122-5A | 0.83 | 0.27 | 31.39 | 284.59 | 0.09 | 0.06 | 0.31 | 0.23 |
| Ogu115-33A | 0.26 | 0.04 | 22.46 | 180.07 | 0.22 | 0.05 | 0.07 | 0.06 |
| Ogu118-6A | 0.08 | 0.13 | 20.86 | 217.83 | 0.15 | 0.03 | 0.12 | 0.10 |
| Ogu307-33A | 0.06 | 0.28 | 20.73 | 335.66 | 0.09 | 0.32 | 0.08 | 0.06 |
| Ogu309-2A | 0.15 | 0.11 | 18.58 | 365.61 | 0.14 | 0.46 | 0.42 | 0.33 |
| Ogu33A | 0.24 | 0.14 | 22.92 | 485.82 | 0.07 | 0.05 | 0.07 | 0.07 |
| OguKt-2-6A | 0.73 | 0.17 | 21.27 | 247.53 | 0.35 | 0.12 | 0.11 | 0.10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ogu1A | 0.14 | 0.20 | 21.01 | 249.18 | 0.19 | 0.04 | 0.14 | 0.12 |
| Ogu13-85-6A | 0.73 | 0.16 | 13.44 | 292.71 | 0.26 | 0.53 | 0.57 | 0.42 |
| Ogu1-6A | 0.34 | 0.07 | 28.62 | 278.87 | 0.15 | 0.14 | 0.17 | 0.13 |
| Ogu2A | 0.24 | 0.06 | 25.33 | 272.16 | 0.09 | 0.10 | 0.13 | 0.09 |
| OguKt-9-2A | 0.30 | 0.16 | 27.20 | 391.74 | 0.11 | 0.06 | 0.10 | 0.10 |
| Ogu22-1A | 0.28 | 0.09 | 24.81 | 312.59 | 0.08 | 0.05 | 0.12 | 0.09 |
| Ogu122-1A | 0.38 | 0.03 | 25.38 | 312.38 | 0.27 | 0.03 | 0.15 | 0.11 |
| Ogu126-1A | 0.20 | 0.06 | 22.79 | 298.42 | 0.19 | 0.04 | 0.18 | 0.13 |
| Ogu12A | 0.33 | 0.07 | 17.74 | 214.52 | 0.07 | 0.06 | 0.16 | 0.12 |
| Ogu119-1A | 0.72 | 0.05 | 20.27 | 324.58 | 0.12 | 0.06 | 0.22 | 0.17 |
| Ogu34-1A | 0.81 | 0.07 | 21.01 | 382.91 | 0.10 | 0.08 | 0.17 | 0.13 |
| Ogu125-8A | 0.48 | 0.06 | 11.40 | 1001.96 | 0.08 | 0.10 | 0.24 | 0.17 |
| Ogu33-1A | 0.69 | 0.05 | 21.89 | 615.44 | 0.11 | 0.11 | 0.09 | 0.08 |
| DH-18-8-1 | 0.08 | 0.07 | 24.64 | 117.75 | 0.07 | 0.03 | 0.06 | 0.05 |
| DH-18-8-3 | 0.10 | 0.08 | 19.62 | 323.63 | 0.07 | 0.04 | 0.07 | 0.07 |
| DH-53-1 | 0.45 | 0.14 | 19.23 | 379.05 | 0.14 | 0.04 | 0.04 | 0.04 |
| DH-53-6 | 0.34 | 0.04 | 17.49 | 389.05 | 0.24 | 0.04 | 0.04 | 0.04 |
| DH-53-9 | 0.34 | 0.06 | 18.29 | 455.71 | 0.12 | 0.06 | 0.05 | 0.03 |
| DH-53-10 | 0.39 | 0.05 | 18.63 | 616.41 | 0.26 | 0.07 | 0.39 | 0.31 |
| HVCF-29 | 0.56 | 0.25 | 25.34 | 536.58 | 0.25 | 0.04 | 0.12 | 0.11 |
| HVCF-18 | 0.11 | 0.04 | 20.49 | 234.48 | 0.13 | 0.04 | 0.30 | 0.22 |
| HVCF-16 | 0.71 | 0.06 | 19.21 | 314.74 | 0.11 | 0.06 | 0.14 | 0.11 |
| Pahuja | 0.34 | 0.05 | 17.02 | 178.24 | 0.13 | 0.04 | 0.11 | 0.10 |
| Standard error | 0.03 | 0.013 | 0.538 | 14.345 | 0.021 | 0.013 | 0.027 | 0.011 |
| CD (5%0 | 0.06 | 0.035 | 1.499 | 39.924 | 0.059 | 0.036 | 0.077 | 0.032 |
| CD (1%) | 0.08 | 0.046 | 1.974 | 52.592 | 0.077 | 0.047 | 0.101 | 0.042 |

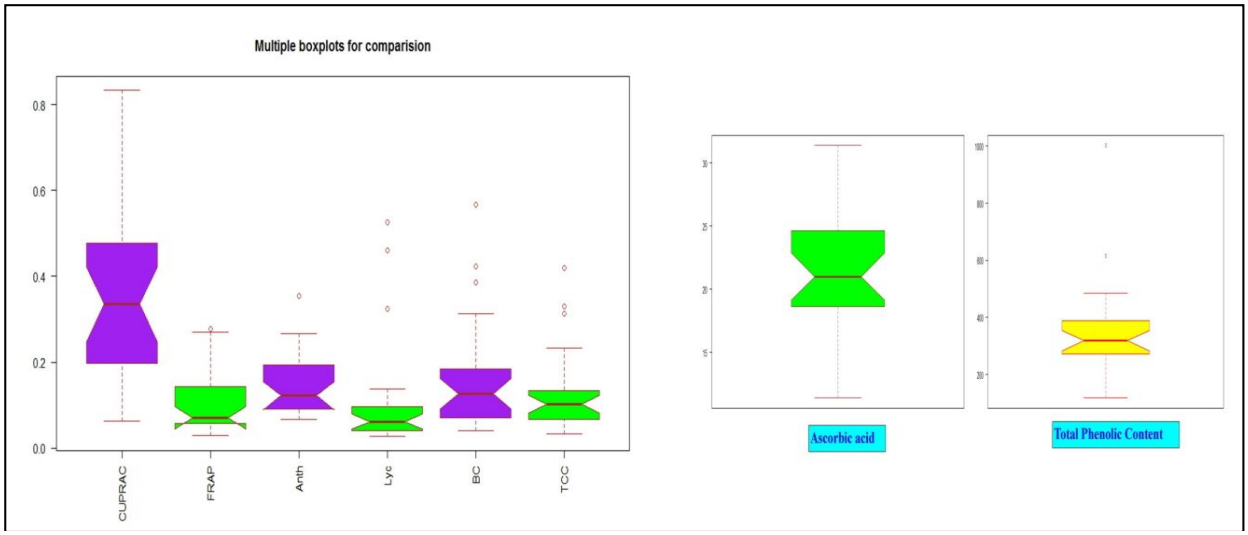



**Fig 1:** Boxplots of antioxidant traits in cauliflower genotypes

OguKt-9-2A, Ogu1-6A and Ogu122-5A had more average ascorbic acid content than best check (HVCF-29). None of the six DH based lines had more mean value of ascorbic acid content as compared to best check (HVCF-29). Among the six testers, DH-53-10 had highest mean value for both total phenolic content and anthocyanins content. The lines Ogu33-1A and Ogu125-8A gave higher mean value of total phenolic content and anthocyanins content as compared to best check (HVCF-29). Similarly, the tester DH-53-10 performed better with respect to these two compounds as compared to best check (HVCF-29). Among the CMS lines, Ogu13-85-6A had highest mean value for all the carotenoids viz. lycopene, beta-carotene and total carotenoids. The 9 CMS lines had more mean value for lycopene content as compared to best check (HVCF-16). Then, for beta-carotene content and total carotenoid, 3 lines Ogu122-5A, Ogu309-2A and Ogu13-85-6A performed better than best check (HVCF-18). Dey *et al.* (2015) [10] also observed significant variability for CUPRAC, FRAP and TPC in cauliflower genotypes and suggested the scope for development of antioxidant rich cultivars in this crop. The genetic divergence plays a vital role in identification and selection of promising genotypes which is prerequisite for efficient crop breeding programs. The highly significant variation for all the antioxidant capacity and quality parameters among the studied genotypes of cauliflower signifies their importance in cauliflower improvement for nutritional quality. Bhandari and Kwak (2015) [5] also recorded significant variability in composition of ascorbic acid, phenols, Glucosinolates and total flavonoids in different tissues of different cultivars of *Brassica* vegetables. These results clearly indicated scope for further enhancement of antioxidant capacity of cauliflower through conventional and non-conventional breeding approaches.

**Parental chemotypes and cluster analysis**

The antioxidant activity of cauliflower depends upon composition of bioactive compounds, hence it is essential to identify and classify the chemotypes with most bio actively effective phytochemical and antioxidant composition. Thus, the parental CMS and DH lines under study were investigated

to estimate the probable compositional similarities among them for CUPRAC, FRAP and phytochemical traits. For this purpose the principal component analysis (PCA) and hierarchical cluster analysis (HCA) displaying dendrogram was done utilizing data matrix comprising 26 parental CMS and DH lines based on 8 major antioxidant and phytochemical components. The dendrogram resulted from HCA based on Euclidian distance matrix (ED) revealed sufficient variability among parental CMS and DH lines, and displayed five different clusters (Fig. 2a). All the DH lines were grouped into single major cluster except DH-53-10. The CMS line Ogu307-33A was placed distantly from the rest of CMS lines based on HCA of antioxidant and phytochemical traits. Thus, the lines Ogu307-33A and DH-53-10 were quite different from rest of parental lines depending upon their quality parameters. To simplify multidimensional dataset the PCA was performed based on 8 antioxidant and phytochemical traits (Fig. 2b). The first two major axes PC1 and PC2 exhibited maximum share in the total phytochemical variance. The PCA results reaffirmed the clustering of parental CMS and DH lines based on HCA. The DH line, DH-53-10 along with CMS line Ogu125-8A was distantly placed from rest of the genotypes.

**Genetic parameters for Antioxidant capacity and phytochemical traits**

The magnitude of divergence for antioxidant and phytochemical traits (CUPRAC, FRAP, β-carotene, TCC, anthocyanins, lycopene, TAsA and TPC) present in cauliflower genotypes and genetics of these traits was estimated in terms of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV), heritability ($h^2_b$) and genetic advance as percent of mean (GAM) (Table 3). The result of GCV depicts true genetic potential of genotypes. In the present investigation, the extent of PCV was slightly higher than the corresponding GCV for all the studied traits related to antioxidant capacity and phytochemical composition. Concurrently, the magnitude of both PCV and GCV was very high as compared to corresponding ECV for all the antioxidant traits. The respective PCV and GCV were highest for Lycopene (105.96%, 101.40%), then anthocyanins (81.77%, 79.09%) followed by FRAP content (78.41%, 75.58%), TCC (72.10%, 70.20%), beta-carotene (70.41%, 63.00%), CUPRAC content (63.39%, 62.41%) and TPC (57.97%, 57.38%). The magnitude of respective PCV and GCV was lowest for ascorbic acid concentration (18.95%, 18.40%). As per Burton and DeVane (1953) [6] classification, the GCV and PCV were high for all the antioxidant compounds (which is ≥ 20%) except for ascorbic acid content, for which GCV and PCV estimated were found moderate (i.e. 10-20%). Hence, the slightly higher magnitude of PCV as compared to GCV for all the antioxidant traits and very low magnitude of ECV indicated the lesser affect of environment on accumulation of antioxidant capacity and quality traits in cauliflower curd (Table 3). Thus, the observed variations for these traits were mostly attributed to genetic factors with little environmental factors and hence selection based on phenotypic values is feasible. The results are in agreement with the findings of Singh *et al.* (2013) [35] for mineral contents in cabbage head and Dey *et al.* (2015) [10] for CUPRAC,

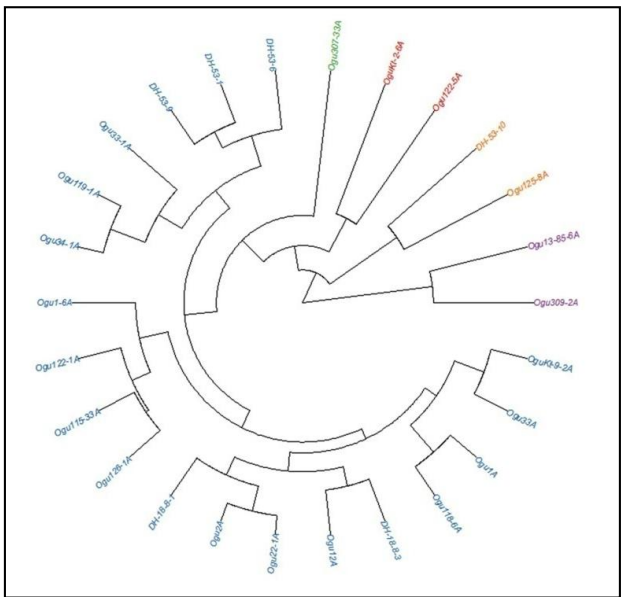



**Fig 2a:** Dendrogram of 26 parental lines (20 CMS + 6 DH) based on 8 antioxidant and phytochemical traits using hierarchical cluster analysis.

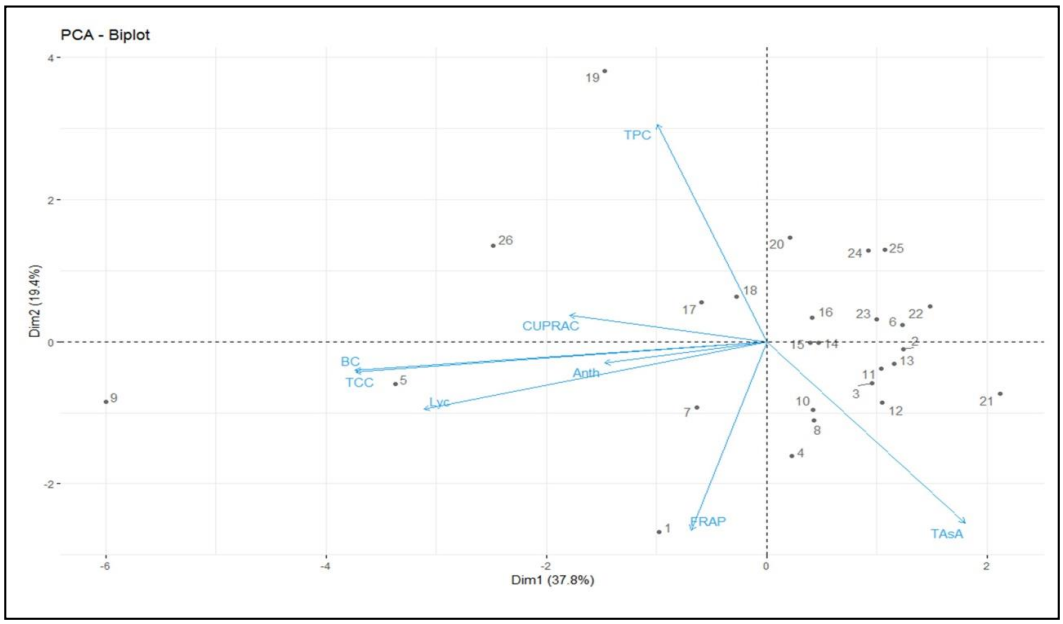



**Fig 2b:** PCA based biplot of parental genotypes and antioxidant traits

**Table 3:** Genetic parameters, heritability for antioxidant and phytochemical compounds

| Traits/Genetic Parmeters | CUPRAC | FRAP | Ascorbic Acid | TPC | Anthocyanin | Lycopene | β-carotene | TCC |
|---|---|---|---|---|---|---|---|---|
| ECV | 11.15 | 20.89 | 4.52 | 8.23 | 20.79 | 30.74 | 31.45 | 16.48 |
| GCV | 62.41 | 75.58 | 18.40 | 57.38 | 79.09 | 101.40 | 63.00 | 70.20 |
| PCV | 63.39 | 78.41 | 18.95 | 57.97 | 81.77 | 105.96 | 70.41 | 72.10 |
| h²b | 0.97 | 0.93 | 0.94 | 0.98 | 0.94 | 0.92 | 0.80 | 0.95 |
| GA 5% | 0.42 | 0.16 | 7.61 | 353.40 | 0.28 | 0.15 | 0.18 | 0.17 |
| GA 1% | 0.54 | 0.20 | 9.75 | 452.91 | 0.36 | 0.19 | 0.23 | 0.22 |
| GAM5% | 126.55 | 150.06 | 36.82 | 117.01 | 157.56 | 199.91 | 116.12 | 140.77 |
| GAM1% | 162.18 | 192.32 | 47.18 | 149.96 | 201.92 | 256.19 | 148.82 | 180.41 |

ECV= Environmental coefficient of variation; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; h2b = Broad sense heritability; GA = Genetic Advance; GAM = Genetic advance as percent of mean (Genetic gain), TPC: total phenolic content, TCC: total carotenoid content

FRAP and phenolic content in cauliflower curd. The computation of heritability and genetic advance as percent of mean (GAM) provided the explanation for heritable proportion of variation in genotypes (Table 3). The acquaintance with heritability is important as the estimates of heritability for a trait indicates the scope of improvement and the extent to which it is possible by selection (Xie *et al.*, 2018) [41]. Besides, the heritability estimates also indicate the genetic control of expression of a particular trait and phenotypic reliability in speculating its breeding value. The high broad sense heritability ($h^2_b$) (> 80%) was computed for all the studied antioxidant traits, which was 97.0%, 93.0%, 94.0%, 98.0%, 94.0%, 92.0% and 95.0% for CUPRAC, FRAP, AA, TPC, anthocyanins, lycopene and TCC respectively. The estimates of genetic advance as percent of mean were also high (> 80%) for all the antioxidant traits except ascorbic acid content for which low estimates of GAM were recorded which is 36.82%. While, the genetic advance per se was low for all the studied traits. The high heritability in the present study (Table 3) indicated that large portion of phenotypic variation is contributed by genotypic variance and less environmental influence in the observed variability and hence a reliable selection procedure can be followed for these traits. The estimates of heritability alone don't provide a clear indication of the magnitude of genetic improvement resulted from selection of individual genotypes. Therefore, knowledge of heritability accompanied with GA and coefficient of variations are very useful. The effective selection for the trait under study could be revealed by estimation of genetic gain. The results in our work indicated the role of both additive and dominant gene action in the expression of these traits in cauliflower. In the present investigation, the high heritability accompanied with high GAM for CUPRAC, FRAP, TPC, anthocyanins, lycopene and carotenoids, suggested the role of additive gene action and thus hybridization followed by selection could be practiced for high genetic gain of these antioxidant traits. However, ascorbic acid content expressed low GAM accompanied with high heritability, thus indicating the role of non-additive gene action in regulating ascorbic acid content (AA), which could be utilized for heterosis breeding through generation of synthetics and hybrids. Thus, the heterotic hybrids having high antioxidant capacity can be developed based on the information generated. Singh *et al.* (2013) [35] and Dey *et al.* (2015) [10] also reported high heritability and genetic advance for mineral content (Fe, Zn, Cu, Ca, Mn, K) in cabbage head and CUPRAC, FRAP, TPC in cauliflower curd respectively. Similarly, Xie *et al.* (2018) [41] also reported high heritability for minerals contents (Ca, Fe, Mg, Zn) in non-heading Chinese cabbage using CMS systems.

**Inter-relationships among CUPRAC, FRAP and phytochemicals**

The knowledge of correlation plays a vital role in breeding program for the prediction of correlated responses and suggesting the effective selection indices (Sehgal *et al.*, 2018) [29]. In the present investigation, no significant positive association was found among all the antioxidant traits at genotypic levels (Table 4). However, at the phenotypic levels, there was significant correlation present among the studied antioxidant traits (Table 4; Fig. 3). The genetic analysis revealed a significant positive association of CUPRAC with FRAP (r = 0.1341), total phenolic content (TPC) (r = 0.2945) and lycopene (r = 0.1118). The significant inter-relationships were also found for FRAP with ascorbic acid (r = 0.3480), TPC (r = 0.2129) and lycopene (r = 0.1395). TPC showed highly positive correlation with anthocyanins (r = 0.2395) and lycopene (0.1364), while anthocyanins and lycopene showed significant inter-relationships with β-carotene (r = 0.1320, r = 0.2280 respectively) and total carotenoid content (TCC) (r = 0.1053, r = 0.1905 respectively). Ascorbic acid (AA) showed significantly positive correlation with TPC (r = 0.1090), but significantly negative correlation was found between AA and lycopene content. Dey *et al.* (2015) [10], also reported significant positive association of CUPRAC and FRAP with total phenolic content (TPC) and among themselves in cauliflower genotypes suggesting TPC as main antioxidant component. Similarly, highly significant correlations between FRAP and TPC were also reported in chilli by Sricharoen *et al.* (2015) [39] indicating mainly the role of TPC for antioxidant activity. Deng *et al.* (2013) [9] and Li *et al.* (2014) [18] also reported positive linear correlation between FRAP assay and trolox equivalent antioxidant capacity (TEAC) assay at P < 0.001 between 56 vegetables and 51 different wild and edible flowers respectively. Likewise, highly significant correlation between vitamin C and TPC was also reported by Bhandari and Kwak (2015) [5] in *Brassica* vegetables. The strong positive inter-relationships between TPC and anthocyanins were also reported in strawberry by Singh *et al.* (2011) [34]. In general, good inter-relationships were found between TPC and other antioxidant traits and vice versa, indicating more contribution of phenolic compounds in antioxidant capacity including CUPRAC and FRAP content in cauliflower genotypes, thus phenols could be demonstrated as reliable biochemical marker, for the selection of elite genotypes having high antioxidants like phenols, CUPRAC, FRAP, anthocyanins and ascorbic acid. The significant correlations among antioxidant traits and disease resistance/tolerance in crop plants have also been reported by different workers. Verma and Singh (2018) [40] reported higher concentration of ascorbic acid (AA) and TPC in downy mildew resistant cauliflower genotypes as compared to

susceptible genotypes. Ascorbic acid also acts as co-factor for many enzymes and plays a key role in plant defense and human health by detoxifying ROS (Duan *et al.*, 2015) [13]. Phenolic compounds have been reported to exhibit toxicity against invading pathogens, therefore, the enhancement in concentration of TPC in crop plants including *Brassica oleracea* vegetables is one of the important components for rendering disease resistance (Cartea *et al.*, 2010; Verma and Singh, 2018) [7, 40]. In the present investigation, we found significant association between TPC and CUPRAC, FRAP, ascorbic acid in cauliflower genotypes, thus, based on previous findings by different researchers (Cartea *et al.*, 2010; Duan *et al.*, 2015; Verma and Singh, 2018) [7, 13, 40] the selection for genotypes having high TPC will facilitate the indirect selection for genotypes having tolerance to invading pathogens. Hence, there is strong association between antioxidant compounds and human health benefits including plant defense itself.

**Interaction of CUPRAC, FRAP and phytochemical compounds with phenotypic traits**

To investigate the interaction of antioxidant and phytochemical compounds with some phenotypic traits in cauliflower, the Pearson's correlation coefficient was calculated (Table 5). In general, no significant association was found for CUPRAC, FRAP and phytochemical traits with any of phenotypic trait (Table 5), suggesting no effect of quality traits on phenotypic performance of cauliflower genotypes. Results are in conformity with the findings of Dey *et al.* (2015) [10] who observed no significant correlation of CUPRAC, FRAP and TPC with NCW in cauliflower genotypes. However, in the present investigation, we observed significant interaction of lycopene with CoL ($r = -0.283$, $P \leq 0.01$) and total carotenoid concentration with curd shape index ($r = 0.166$, $P \leq 0.05$).

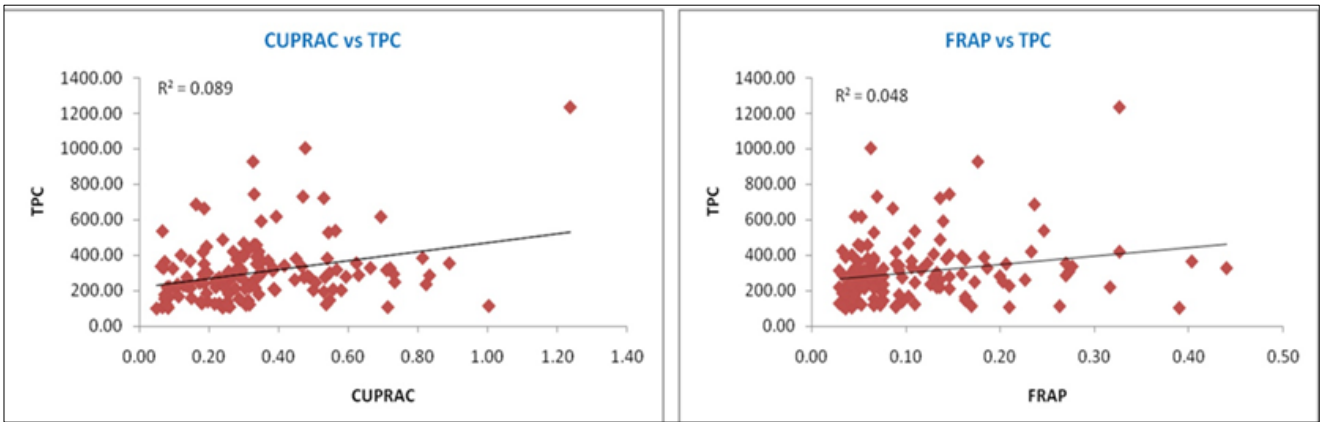
**Conclusions**

The results reported in the present investigation indicated sufficient genetic variability among the genotypes of cauliflower for antioxidant capacity and phytochemical composition (CUPRAC, FRAP, TAsA, TPC, TCC, anthocyanins and lycopene content), thus suggested ample scope for selection of promising parental lines for the development of heterotic hybrids. The information generated regarding heritability and genetic gain, helped in understanding inheritance and nature of gene action in the control of these antioxidant and phytochemical traits, thus, to formulate appropriate breeding strategy for the improvement of antioxidant capacity in cauliflower and enhance the selection efficiency.

**Table 4:** Estimates of correlation coefficient for antioxidant traits in cauliflower genotypes

| Traits | | CUPRAC | FRAP | Ascorbic Acid | TPC | Anthocyanins | Lycopene | Beta-carotene | TCC |
|---|---|---|---|---|---|---|---|---|---|
| CUPRAC | G | 1.0000 | 0.1353 | 0.0326 | 0.3027 | 0.0789 | 0.1098 | 0.0579 | 0.0471 |
| | P | 1.0000 | 0.1341** | 0.0380 | 0.2945*** | 0.0813 | 0.1118* | 0.0485 | 0.0423 |
| FRAP | G | 0.1353 | 1.0000 | 0.3686 | 0.2243 | -0.0429 | 0.1352 | -0.0255 | -0.0228 |
| | P | 0.1341** | 1.0000 | 0.3480*** | 0.2129*** | -0.0329 | 0.1395** | -0.0186 | -0.0266 |
| Ascorbic Acid | G | 0.0326 | 0.3686 | 1.0000 | 0.1097 | 0.0350 | -0.1198 | 0.0154 | 0.0301 |
| | P | 0.0380 | 0.3480*** | 1.0000 | 0.1090* | 0.0324 | -0.1070* | 0.0091 | 0.0309 |
| Phenols | G | 0.3027 | 0.2243 | 0.1097 | 1.0000 | 0.2506 | 0.1424 | 0.0517 | 0.0491 |
| | P | 0.2945*** | 0.2129 | 0.1090* | 1.0000 | 0.2395*** | 0.1364** | 0.0404 | 0.0481 |
| Anthocyanins | G | 0.0789 | -0.0429 | 0.0350 | 0.2506 | 1.0000 | 0.0203 | 0.1396 | 0.0973 |
| | P | 0.0813 | -0.0329 | 0.0324 | 0.2395*** | 1.0000 | 0.0253 | 0.1320** | 0.1053* |
| Lycopene | G | 0.1098 | 0.1352 | -0.1198 | 0.1424 | 0.0203 | 1.0000 | 0.2666 | 0.2098 |
| | P | 0.1118* | 0.1395** | -0.1070* | 0.1364** | 0.0253 | 1.0000 | 0.2280*** | 0.1905*** |
| Beta-carotene | G | 0.0579 | -0.0255 | 0.0154 | 0.0517 | 0.1396 | 0.2666 | 1.0000 | 1.0092 |
| | P | 0.0485 | -0.0186 | 0.0091 | 0.0404 | 0.1320** | 0.2280*** | 1.0000 | 0.9138*** |
| Total carotenoid | G | 0.0471 | -0.0228 | 0.0301 | 0.0491 | 0.0973 | 0.2098 | 1.0092 | 1.0000 |
| | P | 0.0423 | -0.0266 | 0.0309 | 0.0481 | 0.1053* | 0.1905*** | 0.9138*** | 1.0000 |

G: genotypic correlation coefficient, P: phenotypic correlation coefficient, *:significant at 5%, **:significant at 1%, ***:significant at 0.1% level



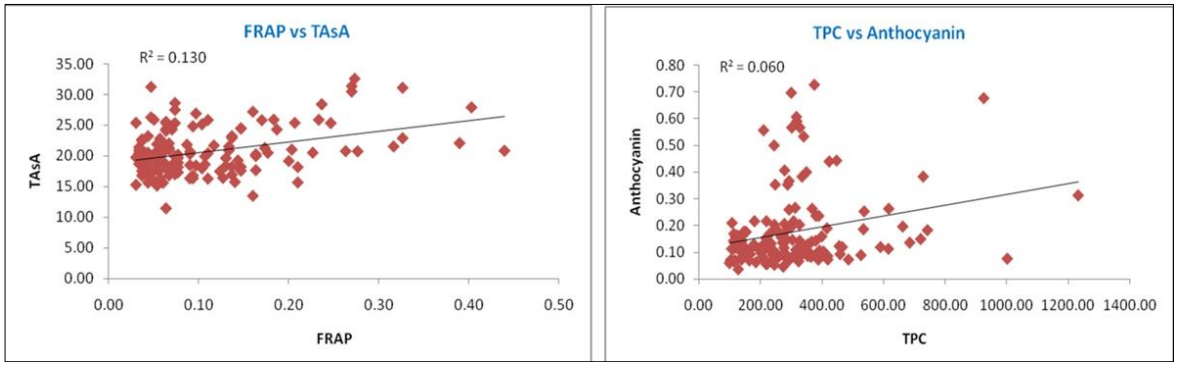



**Fig 3:** Scatterplots depicting pearson correlation. (a) CUPRAC and TPC (b) FRAP and TPC (c) FRAP and Ascorbic Acid (d) TPC and Anthocyanin

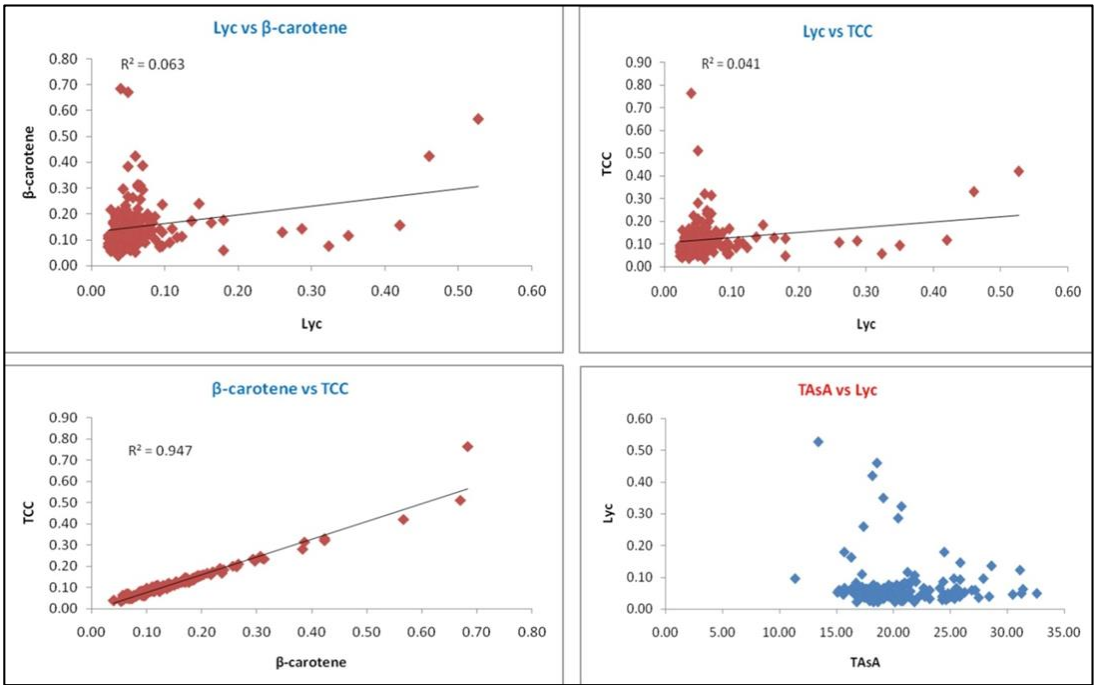



**Fig 3:** Continue. Scatterplots depicting pearson correlation and no correlation. (e) Lycopene and beta-carotene (f) Lycopene and TCC (g) beta-carotene and TCC (h) No correlation was found between Ascorbic acid and lycopene

**Table 5:** Correlation of antioxidant and phytochemical compounds with some phenotypic traits in cauliflower

| Traits | PH | GPW | MCW | NCW | LL | LW | LSI | CoL | HI | ShI |
|---|---|---|---|---|---|---|---|---|---|---|
| CUPRAC | -0.073$^{NS}$ | -0.021$^{NS}$ | -0.038$^{NS}$ | -0.051$^{NS}$ | 0.012$^{NS}$ | 0.035$^{NS}$ | 0.006$^{NS}$ | 0.045$^{NS}$ | -0.008$^{NS}$ | -0.146$^{NS}$ |
| FRAP | 0.079$^{NS}$ | 0.067$^{NS}$ | 0.089$^{NS}$ | 0.001$^{NS}$ | 0.076$^{NS}$ | 0.075$^{NS}$ | 0.092$^{NS}$ | 0.066$^{NS}$ | 0.041$^{NS}$ | -0.010$^{NS}$ |
| TAsA | -0.002$^{NS}$ | -0.014$^{NS}$ | -0.017$^{NS}$ | -0.068$^{NS}$ | 0.028$^{NS}$ | 0.026$^{NS}$ | 0.026$^{NS}$ | 0.049$^{NS}$ | -0.013$^{NS}$ | -0.057$^{NS}$ |
| TPC | -0.047$^{NS}$ | -0.054$^{NS}$ | -0.115$^{NS}$ | -0.139$^{NS}$ | 0.001$^{NS}$ | 0.007$^{NS}$ | 0.001$^{NS}$ | -0.037$^{NS}$ | -0.068$^{NS}$ | -0.123$^{NS}$ |
| Antho | -0.156$^{NS}$ | -0.087$^{NS}$ | -0.120$^{NS}$ | -0.085$^{NS}$ | -0.034$^{NS}$ | -0.115$^{NS}$ | -0.086$^{NS}$ | 0.026$^{NS}$ | -0.057$^{NS}$ | -0.046$^{NS}$ |
| Lyc | -0.109$^{NS}$ | -0.083$^{NS}$ | -0.133$^{NS}$ | -0.148$^{NS}$ | -0.084$^{NS}$ | 0.035$^{NS}$ | -0.015$^{NS}$ | -0.284$^{**}$ | -0.028$^{NS}$ | -0.116$^{NS}$ |
| BC | -0.128$^{NS}$ | -0.111$^{NS}$ | -0.096$^{NS}$ | -0.129$^{NS}$ | -0.029$^{NS}$ | 0.063$^{NS}$ | 0.020$^{NS}$ | -0.074$^{NS}$ | 0.023$^{NS}$ | 0.163$^{*}$ |
| TCC | -0.132$^{NS}$ | -0.104$^{NS}$ | -0.090$^{NS}$ | -0.125$^{NS}$ | -0.040$^{NS}$ | 0.059$^{NS}$ | 0.011$^{NS}$ | -0.082$^{NS}$ | 0.019$^{NS}$ | 0.166$^{*}$ |

*:significant at 5% probability, **:significant at 1%, ***:significant at 0.1% level, PH:Plant height, GPW:gross plant weight, MCW:marketable curd weight, NCW:net curd weight, LL:leaf length, LW:leaf width, LSI:leaf size index, CoL:core length, HI:harvest index, ShI:shape index, TCC:total carotenoid content, BC:beta-carotene, Lyc:lycopene, Antho:anthocyanins, TPC:total phenolic content, TAsA:total ascorbic acid content, NS:non significant

Similarly, the knowledge of inter-relationships among the studied antioxidant traits indicated the scope for selection of genotypes for the simultaneous improvement of different antioxidant compounds in cauliflower crop. Thus, there is ample scope to develop functional food products from cauliflower for the prevention and treatment of diseases resulted from oxidative stress. The enrichment and profiling of studied genotypes of cauliflower for antioxidant traits, indicates capability of *Brassica* vegetables for scavenging the free radicals as generated in the form of ROS and thus having health promoting properties through reduction of risk of cardiovascular diseases and various types of cancer. Further study also provide scope for generation of pre-breeding material for understanding the mechanism and mapping of QTLs/genes controlling the expression of antioxidant capacity and phytochemical compounds in Brassica vegetables. The results of this investigation will also provide support for for epidemiological research, suggesting dietary guidelines and generation of antioxidant rich cultivars.

**Acknowledgements**
The first author is sincerely thankful to IARI, New Delhi for providing Senior Research Fellowship during the Ph.D. research programme. We also acknowledge the Head, IARI, RS Katrain, H.P. and Head, Division of Vegetable Science, IARI, New Delhi for providing the necessary facilities during the research period.

**Competing Interest**
All the authors declare that they have no competing interest.

**References**
1. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin- Ciocalteu reagent. Nat. Protoc. 2007; 2:875–877. https://doi.org/10.1038/nprot.2007.102.
2. Apak R, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektaşoğlu B, Berker KI, Ozyurt D. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules. 2007; 12:1496-1547. https://doi.org/10.3390/12071496.
3. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay:direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999:299:15–23. https://doi.org/10.1016/S0076-6879 (99)99005-5.
4. Bhatia R, Dey SS, Sood S, Sharma K, Parkash C, Kumar R. Efficient microspore embryogenesis in cauliflower (*Brassica oleracea* var. *botrytis* L.) for development of plants with different ploidy level and their use in breeding programme. Sci. Hortic. 2017; 216, 83-92. https://doi.org/10.1016/j.scienta.2016.12.020.
5. Bhandari SR, Kwak J-H. Chemical composition and antioxidant activity in different tissues of *Brassica* vegetables. Molecules. 2015; 20:1228-1243.
6. Burton GW, DeVane EH. Estimating heritability in tall fescue *(Festuca arundinacea*) from replicated clonal material. Agron. J. 1953; 45, 478-481.
7. Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. Molecules. 2010; 16, 251-280. https://doi.org/10.3390/molecules16010251.
8. Ciska E, Drabinska N, Honke J, Narwojsz A. Boiled Brussels sprouts:a rich source of Glucosinolates and the corresponding nitriles. J. Funct. Foods. 2015; 19:91-99.
9. Dey SS, Bhatia R, Sharma SR, Parkash C, Sureja AK. Effects of chloroplast substituted *Ogura* male sterile cytoplasm on the performance of cauliflower (*Brassica oleracea* var. *botrytis* L.) $F_1$ hybrids. Sci. Hortic. 2013; 157:45-51.
10. Dey SS, Bhatia R, Sharma S, Sharma K, Kumar R, Sharma VK, Parkash C. Genetic divergence and interaction among CUPRAC, FRAP and total phenolics content in cauliflower *(Brassica oleracea* var. *botrytis* L.). Indian. J. Genet. Pl. Br. 2015; 75:79-85.
11. Dey SS, Bhatia R, Bhardwaj I, Mishra V, Sharma K, Parkash C, Kumar S, Sharma VK, Kumar R. Molecular-agronomic characterization and genetic study reveals usefulness of refined *Ogura* cytoplasm based CMS lines in hybrid breeding of cauliflower (*Brassica oleracea* var. *botrytis* L.). Sci. Hortic. 2017; 224:27-36.
12. Deng G-F, Lin X, Xu X-R, Gao L-L, Xie J-F, Li H-B. Antioxidant capacities and total phenolic contents of 56 vegetables. J. Funct. Foods. 2013; 5:260-266. https://doi.org/10.1016/j.jff.2012.10.015.
13. Duan W, Songh X, Liu T, Huang Z, Ren J, Hou X, Du J, Li Y. Patterns of evolutionary conservation of ascorbic acid-related genes following whole-genome triplication in *Brassica rapa*. Genom. Biol. Evol. 2015; 7:299-313. https://doi.org/10.1093/gbe/evu293.
14. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014; 6:466-488. https://doi.org/10.3390/nu6020466.
15. Granger M, Eck P. Dietary vitamin C in human health. Adv. Food. Nutr. Res. 2018; 83:281-310. https://doi.org/10.1016/bs.afnr.2017.11.006.
16. Hodaei M, Rahimmalek M, Arzani A. Variation in morphological characters, chemical composition, and anthocyanin content of different *Chrysanthemum morifolium* cultivars from Iran. Biochem. Syst. Ecol. 2017; 74, 1-10.
17. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int. J. Biol. Sci. 2015; 11:982-991. https://doi.org/10.7150/ijbs.12096.
18. Li, A-N., Li, S., Li, H-B., Xu, D-P., Xu, X-R., Chen, F., 2014. Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. J. Funct. Foods. 6, 319-330. https://doi.org/10.1016/j.jff.2013.10.022.
19. Mizgier P, Kucharska AZ, Sokol-Letowska A, Kolniak-Ostek J, Kidon M, Fecka I. Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts. J. Funct. Foods. 2016; 21:133-146.
20. Ogunniyan DJ, Olakojo SA. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L.). Niger. J. Genet. 2014; 28:24-28.
21. Perrier X, Jacquemoud-Collet JP. 2006; DA Rwin software. http://darwin.cirad.fr/
22. Raiola A, Errico A, Petruk G, Monti DM, Barone A, Rigano MM. Bioactive compounds in *Brassicaceae* vegetables with a role in the prevention of chronic diseases. Molecules. 2018; 23:15. https://doi.org/ 10.3390/molecules23010015
23. Ranganna S. Manual of analysis of fruits and vegetable products. Tata McGraw Hill Book Co., New Delhi, 1979.
24. Samec D, Urlic B, Salopek-Sondi B. Kale (*Brassica oleracea* var. *acephala*) as a superfood:Review of the scientific evidence behind the statement. Crit. Rev. Food. Sci. Nutr. 2018; 20:1-12. https://doi.org/ 0.1080/10408398.2018.
25. Sayeed MA, Bracci M, Lazzarini R, Tomasetti M, Amati M, Lucarini G, Primio RD, Santarelli L. Use of potential dietary phytochemicals to target miRNA:promising option for breast cancer prevention and treatment. J. Funct. Foods. 2017; 28, 177-193. https://doi. org/10.1016/j.jff.2016.11.008.
26. SAS Institute Inc. SAS Online Doc, Version 9.4. Cary, NC. 2013; https://www.sas.com./en_in/home.html
27. Scott AJ, Knott MA. Cluster analysis method for grouping means in the analysis of variance. Biometrics. 1974; 30:507-512.
28. Sehgal N, Singh S. Progress on deciphering the molecular aspects of cell-to-cell communication in *Brassica* self-incompatibility response. 3Biotech. 2018; 8:347. https://doi.org/10.1007/s13205-018-1372-2.

29. Sehgal N, Chadha S, Kumar N, Kaur M, Kanwar S. Correlation and path coefficient analysis for fruit yield and its component traits among bacterial wilt resistant $F_4$ progenies of tomato (*Solanum lycopersicum* L.). Int. J. Curr. Microbiol. App. Sci. 2018; 7:1052-1059.
30. Sharma SR. Cabbage. In: Sirohi PS (ed) Vegetable crop production. Division of Vegetable Sciencce, IARI, New Delhi, 2003.
31. Singh S, Devi MB. Vegetables as a potential source of nutraceuticals and phytochemicals: A review. Int. J. Med. Pharmaceut. Sci. 2015; 5, 1-14.
32. Singh S, Bhatia R, Kumar R, Sharma K, Dash S, Dey SS. Cytoplasmic male sterile and doubled haploid lines with desirable combining ability enhances the concentration of important antioxidant attributes in *Brassica oleracea*. Euphytica. 2018; 214:207. https://doi.org/10.1007/s10681-018-2291-3.
33. Singh S, Vidyasagar. Effect of common salt (NaCl) sprays to overcome the self-incompatibility in the S-allele lines of *Brassica oleracea* var. *capitata* L. SABRAO J. Breed. Genet. 2012; 44:339-348.
34. Singh A, Singh BK, Deka BC, Sanwal SK, Patel RK, Verma MR. The genetic variability, inheritance and inter-relationships of ascorbic acid, β-carotene, phenol and anthocyanin content in strawberry (*Fragaria* × *ananassa* Duch.). Sci. Hortic. 2011; 129, 86-90.
35. Singh BK, Sharma SR, Singh B. Genetic variability, inheritance and correlation for mineral contents in cabbage (*Brassica oleracea* var. *capitata* L.). J. Hort. Res. 2013; 21:91-97.
36. Singh RK, Chaudhary BD. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, 2014.
37. Soengas P, Cartea ME, Francisco M, Sotelo T, Velasco P. New insights into antioxidant activity of Brassica crops. Food Chem. 2012; 134:725-733.
38. Sotelo T, Cartea ME, Velasco P, Soengas P. Identification of antioxidant-capacity related QTLs in *Brassica oleracea*. PLoS ONE. 2014; 9:e107290.
39. Sricharoen P, Techawongstein S, Chanthai S. A high correlation indicating for an evaluation of antioxidant activity and total phenolics content of various chilli varieties. J. Food Sci. Technol. 2015; 52:8077-8085.
40. Verma A, Singh Y. Inheritance of downymildew resistance and its relationships with biochemical traits in cauliflower (*Brassica oleracea* L. var. *botrytis*). Crop Prot. 2018; 106:132-138.
41. Xie F, Zha J, Tang H, Xu Y, Liu X, Wan Z. Combining ability and heterosis analysis for mineral elements by using cytoplasmic male-sterile systems in non-heading Chinese cababage (*Brassica rapa*). Crop Pasture Sci. 2018; 69:296-302.