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Genetic divergence and interaction among CUPRAC, FRAP and total phenolics content in cauliflower (*Brassica oleracea* var. *botrytis* L.)

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Abstract

Antioxidant capacity and total phenolics content were estimated in 46 cauliflower lines to determine genetic variability, heritability and correlation among them with the objective to formulate breeding strategies for development cultivars with higher antioxidant capacity. The genotypes comprised of six CMS lines, few advance breeding lines and other core collection of our institute. Significant variability was recorded for CUPRAC, FRAP and total phenolics content indicating the scope for improvement in these traits based on various breeding strategies. Phenotypic co-efficient of variation (PCV) was higher (43.01, 73.07 and 64.28) than genotypic co-efficient of variation (GCV) (41.34, 71.83 and 63.88) in all cases indicating the role of environment in expression of these traits. However improvement for these traits is possible through selection and heterosis breeding as broad sense heritability (92.44% to 98.75%) and genetic advance as percentage of mean (82.46% to 130.87%) was high for all the traits. Correlation coefficient analysis revealed that CUPRAC, FRAP and total phenolics had significant positive correlation among each other. However, none of them had any significant correlation with marketable yield. Fourty six genotypes were classified into 6 groups based on non-hierarchical cluster analysis and phenotypic divergence. This study will pave the way for breeding of cauliflower cultivars and hybrids with higher antioxidant capacity and phenolics content.

Key words: Cauliflower, anti-oxidant capacity, phenolics content, genetics.

Introduction

Vegetable breeding during the 21st century will continue its focus on quality traits, and capitalize on the growing demand for the unique health functionality of vegetable crops. The future of vegetable crops is in their past (Goldman 2004). In recent years, the regular intake of fruits and vegetables has been highly recommended because of numerous health beneficial nutritional properties. Cruciferous vegetables such as cabbage, cauliflower, broccoli, brussel sprouts, collard and kale have been classified as 'Super Food' (Mckersie 1996) as they possess robust oxidative defence systems, i.e. antioxidants and polyphenols. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.; 2n=2x=18) is an important cruciferae vegetable belonging to *Brassicaceae* family and grown throughout world. Breeding cauliflower with higher antioxidant capacity and polyphenols require basic understanding on genetic diversity and nature of inheritance of these traits. However, no study has been conducted till now to discover genetic diversity, inheritance and interaction of total antioxidant capacity among themselves and yield of cauliflower.

The role of antioxidant and polyphenols in human diet is well established (Deng et al. 2013). About 5% or more oxygen is converted to reactive oxygen species (ROS) such as O_2^-, H_2O_2 and OH by univalent reduction of O_2 (Singleton and Rossi 1965). Free radicals can cause oxidative damage to all bio-molecules and initiate a chain reaction which results in physiological damage. This physiological damage can be repaired but may also accumulate over a period of time and cause many degenerative diseases (Ames et al. 1993), In humans, if not neutralized the free radicals damage various body cells (cell membrane, lipids, proteins, DNA and other cell structures) causing many degenerative diseases like ageing, heart disease, cancer, arthritis, loss of memory, paralysis etc. The chemical diversity of antioxidants makes it difficult to separate and quantify individual antioxidants (i.e., parent compounds, glycosides, polymers, and

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many isomers) from the vegetable matrix. Moreover, the total antioxidant power is often more meaningful to evaluate health beneficial effects because of the cooperative action of antioxidants. The estimates of CUPRAC and FRAP have been advocated as the most effective way to determine total antioxidant capacity in plants (Apak et al. 2007; Ozyurek et al. 2008). Most information available for vegetables is about their nutritional values. There is insufficient data about antioxidant capacity of vegetables in general and cauliflower in particular.

To the best of our knowledge and available literatures no information is available on variability and heritability of antioxidant capacity in cauliflower. The knowledge of antioxidant capacity in the plant is of significance, in terms of its usefulness in human health. Moreover, variability analysis will also help in formulating breeding strategy for development of cultivars with higher concentration of antioxidant capacity. It will also pave the way for creation of mapping population and identification of QTLs responsible for higher concentration of antioxidant capacity. Thus, the present study was conceived with the objective to estimate variability in antioxidant capacity, its inheritance and correlation among cauliflower genotypes for possible exploitation to breed genotypes/cultivars having high antioxidant. The long term goal of the study is to identify genes/QTLs contributing for higher concentration of antioxidant capacity in cauliflower.

Materials and methods

Fourty-six diverse genotypes of cauliflower, including cultivars and germplasm, comprised basic experimental material. The experimental materials include CMS lines, advance breeding lines with resistance to several biotic and abiotic stresses and other core collection at our institute. The cultivar Pusa Snowball K-1 is a widely cultivated genotype throughout India and was used as control for comparison. Details of basic experimental materials used are given in Table 1.

Four weeks old seedlings were transplanted during 2011-12 at Baragram research farm, IARI Regional Station, Katrain, Kullu, HP, India. The plot size was 2.7 m x 2.7 m with inter-and intra-row spacing of 45 cm. Plots were triplicated in a randomized block design (CRBD). Five curd of each genotype in replicated trial was harvested at fresh market stage, chopped, homogenized, sample of 5 g fresh weight (FW) refrigerated immediately stored until assay. Ethanol extract was prepared by homogenized 5 g sample in 15 ml absolute ethanol. Then it was centrifuged at 10,000 rpm for 15 min at 4^{o}C. The supernatant was stored at –20^{o}C. For CUPRAC analysis, the method described by Apak et al. (2006) was followed with minor modification. A sample of 100µl 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 450 nm in spectophotometer. The results were expressed as micro mol trolox/g. The FRAP assay was performed based on the procedure described by Benzie and Strain (1996) with slight modifications. In this assay, 100 µL of the diluted sample were added to 3 mL of the FRAP reagent and the reaction was monitored after 4 min at 593 nm. The results were expressed as µmol Fe(II)/g fresh weight (FW) of vegetable. Total phenolic contents were determined with Folin-Ciocalteu method (Singleton and Rossi 1965). Briefly, 0.50 mL extract was mixed with 2.5 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 2 mL of saturated sodium carbonate solution was added. The mixture was incubated in dark for 2 h at room temperature and its absorbance was detected at 760 nm. Gallic acid was used for calibration, and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g FW of vegetable.

The data were analyzed statistically for analysis of variance (Panse and Sukhatme 1967), estimation of variability (Burton and Devane 1953) and correlation (Searle 1961). The standard error for genotypes was calculated as per Singh and Chaudhary (1977). The D^2 statistics was used for assessing the genetic divergence among the populations as suggested by Mahalanobis (1936). Based on the D^2 values thus obtained, the entire germplasm was classified into distinct clusters, grouping together the less divergent genotypes (Rao 1952).

Results and discussion

Basic statistics

The mean square of CUPRAC, FRAP and total phenolics content in cauliflower curd varied significantly among the 46 genotypes (data not presented). The mean performance, range and standard error (Table 2) also showed large variation for antioxidant activities and total phenolics content. The CUPRAC values differed by 6.8 fold among the lines and ranged from 1.35-9.24 with a general mean of 3.21

Table 1. Details of 46 cauliflower genotypes along with their commercial traits

S.No.	Lines	Curd color	Curd compactness	S.No.	Lines	Curd color	Curd compactness
1	Kt 18	Snow white	Compact	24	Kt-15	Snow white	Very compact
2	RSK-119	White	Very compact	25	HLSR 05	White	Compact
3	Supreme plaswie	White	compact	26	Sel 27	Snow white	Very compact
4	Kt-41	Cream white	compact	27	Kt-22	White	Very compact
5	DB 1305	White	Very compact	28	PHJ	White	Compact
6	RSK 1301	Snow white	compact	29	Kt-2	Snow White	Very compact
7	Suprimax Late	White	Compact	30	King king	White	Compact
8	Palam Kanchan	Green	Medium compact	31	DB 187	Cream white	Compact
9	Kt-62-6	Snow white	Very compact	32	PSB-16	Cream white	Compact
10	Lal chowk maghi	Cream white	Compact	33	Sel-21-78	Cream white	Very compact
11	Agrotech	Yellows white	Compact	34	kt-25	Snow white	Very compact
12	Lawyana-2	Yellow white	compact	35	PSB-1	Cream white	Compact
13	Sel 26	Snow white	Very compact	36	Ogu33A	Cream white	Very compact
14	Hermia	Cream white	compact	37	Ogu14A	White	Compact
15	EC-162587 New	White	compact	38	Ogu2A	Cream white	Compact
16	Sel-29	Snow white	Very compact	39	Kt-8	White	Compact
17	White fox	Snow white	Compact	40	Sel-28	White	Very compact
18	Romanesco Sel-II	Light gteen	Compact	41	Ogu12A	White	Compact
19	Kt-178	Snow white	Very compact	42	Ogu2A	Wnow white	Very compact
20	Kt-16	Cream white	compact	43	Kt-1301	Snow white	Compact
21	Mukut mani	Cream white	compact	44	Kt-71	White	Compact
22	1385	White	Very compact	45	PSB K-1	Snow white	Very compact
23	Kt-51	White	Compact	46	Ogu1A	Snow white	Very compact

± 0.12. The CUPRAC value was highest in the genotype, Sel-28 followed by Pusa Himjyoti, Lal Chowk Maghi, Pusa Snowball K-1 and Ogu12A. While the genotypes with very low CUPRAC value were DB-1305, Sel-17, Kt-18, RSK-119 and Sel-29. As compared to commercially cultivated variety, Pusa Snowball K-1, CUPRAC value was higher in 3 genotypes. FRAP values ranged from 0.44-8.28 (18.8-fold difference) with a genotype mean of 2.17 ± 0.14. The maximum FRAP activity was assayed in the line, Ogu12A followed by Sel-28, RSK-1385, Ogu33A and Lal Chowk Maghi. The lines Sel-29, EC-162587, Sel-26, Pusa Snowball K-25 and RSK-1301 had lower values of FRAP. Twenty one genotypes had higher FRAP values when compared with the commercial check, Pusa Snowball K-1. Total phenolics content varied from 360.67-3576.98 (9.91-fold difference) with a general mean of 1155.16 ± 62.92. The highest values were recorded in the genotypes Sel-28 followed by Supremax late, Palam Kanchan, Pusa Himjyoti and HL-SR-05. The lines with lowest concentration of phenolics were DB-1305 followed by Sel-21-78, Pusa Snowball K-25, Kt-18 and Ogu2A. Twenty eight genotypes had higher phenolics content than Pusa Snowball K-1.

Highly significant mean squares for CUPRAC, FRAP and total phenolics activities indicate the presence of sufficient natural variation among cauliflower genotypes which could be exploited through various breeding approaches. Availability of genotypes with higher values of CUPRAC, FRAP and total phenolics than commercially cultivated check indicates the scope in developing cultivars with more anti-oxidant capacity. Similar results were reported in cabbage for different anti-oxidant enzymes by Singh et al. (2010),

Genetic variance

The extent of variability (Table 3) present among

Table 2. Mean performance, range and standard error of CUPRAC, FRAP and total phenolics in cauliflower

S.No.	Cultivar	CUPRAC (lmol Trolox/g FW)	FRAPlmol Fe(II)/g FW)	Total phenolics (mg GAE per 100 g FW)
1	Kt 18	1.73 ± 0.17	1.20 ± 0.12	455.46 ± 40.51
2	RSK- 119	1.81 ± 0.24	1.20 ± 0.21	498.26 ± 47.76
3	Supreme plaswie	3.67 ± 0.41	1.76 ± 0.35	614.75 ± 69.01
4	Kt-41	2.30 ± 0.35	0.98 ± 0.33	486.29 ± 25.10
5	DB-1305	1.58 ± 0.26	1.25 ± 0.17	387.69 ± 32.43
6	RSK- 1301	2.83 ± 0.20	2.05 ± 0.24	753.65 ± 51.80
7	Suprimax	2.23 ± 0.52	1.63 ± 0.44	3332.49 ± 263.75
8	Palam Kanchan	2.28 ± 0.77	1.83 ± 0.44	2725.87 ± 277.37
9	Kt-62-6	2.71 ± 0.35	1.25 ± 0.25	2206.41 ± 138.99
10	Lal chowk Maghi	5.93 ± 1.06	4.39 ± 0.72	1654.41 ± 138.71
11	Agrotech	3.49 ± 0.51	2.25 ± 0.57	980.14 ± 176.69
12	Kt-53-2	2.58 ± 0.51	2.41 ± 0.42	1392.60 ± 163.54
13	Sel-26	2.60 ± 0.45	0.90 ± 0.20	774.05 ± 55.91
14	Hermia	2.65 ± 0.46	1.19 ± 0.16	775.86 ± 101.18
15	EC – 162587	3.71 ± 0.55	0.81 ± 0.24	675.02 ± 120.87
16	Sel-29	2.04 ± 0.27	0.63 ± 0.23	623.47 ± 76.92
17	Whitefox	2.25 ± 0.29	1.00 ± 0.14	765.94 ± 55.14
18	Romanesco II	3.29 ± 0.35	4.25 ± 0.57	1227.00 ± 68.34
19	Kt-178	2.20 ± 0.41	1.87 ± 0.25	833.77 ± 115.26
20	Kt -16	2.16 ± 0.40	1.54 ± 0.53	839.14 ± 68.81
21	Mukut mani	4.07 ± 0.81	3.22 ± 0.61	1058.05 ± 113.20
22	1385	3.18 ± 0.29	5.24 ± 0.55	1119.60 ± 40.67
23	Kt-51	2.15 ± 0.27	1.58 ± 0.39	580.52 ± 36.20
24	Kt-15	2.59 ± 0.41	2.22 ± 0.50	884.54 ± 66.91
25	HL-SR- 05	3.35 ± 0.20	2.40 ± 0.39	2274.57 ± 138.95
26	Sel- 27	1.67 ± 0.18	1.37 ± 0.45	689.94 ± 78.39
27	Kt- 22	2.64 ± 0.30	2.27 ± 0.43	987.84 ± 97.85
28	Pusa Him Jyoti	6.47 ± 0.57	4.28 ± 0.42	2469.03 ± 84.78
29	Kt – 2	2.59 ± 0.17	1.98 ± 0.18	1926.29 ± 71.38
30	King king	2.23 ± 0.22	1.28 ± 0.18	1150.40 ± 88.71
31	DB 187	3.07± 0.47	1.12 ± 0.13	488.31 ± 37.52
32	PSB -16	2.78 ± 0.37	1.71 ± 0.45	1346.56 ± 73.75
33	Sel-21-78	2.78 ± 0.47	1.33 ± 0.08	389.80 ± 12.64
34	Kt – 25	2.46 ± 0.10	0.97 ± 0.13	441.23 ± 25.22
35	Pusa Snowball-1	2.58 ± 0.28	2.05 ± 0.29	816.33 ± 18.50
36	Ogu33A	4.28 ± 0.62	4.83 ± 0.50	1442.23 ± 78.26
37	Ogu14A	3.20 ± 0.36	1.48 ± 0.13	1226.43 ± 68.32
38	Ogu3A	4.55 ± 0.57	1.59 ± 0.48	1689.43 ± 73.19
39	Kt -8	4.06 ± 0.22	1.88 ± 0.24	831.32 ± 82.85
40	Sel-28	8.05 ± 1.10	7.29 ± 0.93	3404.40 ± 139.16
41	Ogu12A	5.49 ± 0.51	7.65 ± 0.56	1677.73 ± 66.33
42	Ogu2A	3.47 ± 0.60	1.42 ± 0.26	474.82 ± 24.49
43	1301	3.21 ± 0.24	0.98 ± 0.14	925.97 ± 92.12
44	Kt-71	4.32 ± 0.44	1.99 ± 0.14	1006.68 ± 48.28
45	Pusa Snowball K-1	5.65 ± 0.45	1.72 ± 0.38	779.99 ± 19.56
46	Ogu1A	2.71 ± 0.27	1.60 ± 0.21	1085.14 ± 889.40
Mean		3.21	2.17	1155.16
Range		1.35-9.24	0.44-8.28	360.67-3576.98
Standard error		0.12	0.14	62.92

germplasm was estimated in terms of phenotypic, genotypic and environmental variance (Vp, Vg and Ve) and phenotypic and genotypic coefficient of variation. The Vg was highest in total phenolics content followed by FRAP and CUPRAC. The magnitude of phenotypic coefficient of variation was slightly higher than the corresponding genotypic coefficient of variation for CUPAC, FRAP and total phenolics. The respective phenotypic coefficient of variation and genotypic coefficient of variation were high for FRAP (71.83 and 73.07%) and total phenolics content (63.88 and 64.28%), while it was lowest for CUPRAC (41.34 and 43.01%). Heritable portion of variation can be explained by computing the heritability and genetic advance as percentage of mean (Table 3). High heritability (>80.0%) was computed for all three antioxidant related activities, i.e. CUPRAC (92.44%), FRAP (96.94%) and total phenolics (98.75%). Genetic advance as percentage of mean were also high (>80.0%) for all the traits i.e. 82.46% (CUPRAC), 146.10% (FRAP) and 130.87% (total phenolics). In studying the genetic advance and broad sense heritability it was found that all the three assays had high genetic advance as percentage of mean and broad sense heritability. However, genetic advance per se was low for all these traits. This indicates role of both additive and dominance genetic component in expression of these traits. Thus, breeding methods based on additive genetic variance such as selection, hybridization followed by selection may be adopted in developing cultivars with higher total anti oxidant capacity. Besides, heterotic hybrids for the antioxidant capacities and total phenolics content can also be developed based on the information generated. Singh et al. (2010) also reported very high heritability and genetic advance for different anti-oxidant enzymes in cabbage.

Correlation analysis

Correlation coefficient of CUPAC, FRAP and total phenolics content with net curd weight was analysed to observe the direction and magnitude of associations at genotypic and phenotypic levels (Table 4). Genotypic correlation coefficients, in general, were higher in magnitude than of the corresponding phenotypic correlation coefficients. The analysis revealed a significant positive correlation of CUPRAC with FRAP (0.711** and 0.702**), CUPRAC with total phenolics (0.470** and 0.451**) and FRAP with total phenolics (0.504** and 0.495**). Net curd weight had no correlation with any of the assayed antioxidants capacity and total phenolics content. In a study based common vegetable in Coloradfo, Zhou and Yu (2006) also found that total phenolic content and the measured antioxidant properties were correlated with each other. Deng et al. (2013) also revealed significant linear co-

Table 3. Estimates of variance, coefficient of variation, broad sense heritability and genetic advance for CUPRAC, FRAP and total phenolics content

S.No.	Traits	Vg	Vp	Ve	GCV	PCV	Heritability	Genetic advance	Genetic advance as % of means
1	CUPRAC	1.76	1.90	0.14	41.34	43.01	92.44	2.62	82.46
2	FRAP	2.43	2.51	0.08	71.83	73.07	96.64	3.15	146.10
3	Total phenolics	545253.15	552120.67	6867.51	63.88	64.28	98.75	1513.74	130.87

Table 4. Estimates of correlation coefficient for CUPRAC, FRAP, total phenolics and curd weight in cauliflower genotypes

Traits		CUPRAC	FRAP	Total phenolics	Net curd weight
CUPRAC	G	-	0.711**	0.470**	0.043
	P	-	0.702**	0.451**	0.041
FRAP	G		-	0.504**	-0.221
	P		-	0.495**	-0.231
Total phenolics	G			-	0.176
	P			-	0.164
Net curd weight	G				-
	P				-

G: genotypic level; P: phenotypic level; **Significant at 1% level; G: Genotypic; P: Phenotypic

Table 5. Grouping of 46 cauliflower genotypes based on non-hierarchical cluster analysis

Cluster	Genotypes
I	Kt-62-6, Lal Chowk Maghi, HL-SR-05, Kt – 2, Ogu3A, Ogu12A
II	Kt-53-2, Romanesco II, PSB–16, Ogu33A, Ogu14A
III	Kt-18, RSK-119, Supreme plaswei, Kt-41, DB-1305, Sel-29, Kt-51, DB-187, Sel-21-78, Kt- 25, Ogu2A
IV	Kt-59-2, Palam Kanchan, Pusa Himjyoti, Sel-28
V	RSK-1301, Sel- 26, Hermia , EC-162587, Whitefox, Kt-178, Kt-16, Kt-15, Sel- 27, Pusa Snowball- 1, Kt-8, Pusa Snowball K-1
VI	Agrotech, Mukut mani, 1385, Kt- 22, King King, RSK-1301, Kt-71, Ogu1A

relation among different antioxidant capacity (FRAP and TEAC) in a study with 56 vegetables. They have also reported very strong positive co-relation among total anti-oxidant capacities and total phenolics content. CUPRAC, FRAP and total phenolics had no significant co-relation with yield of cauliflower. In cabbage, Singh et al. (2010) also found similar results in cabbage in studying the correlation among different anti oxidant enzymes and head yield.

Grouping of the genotypes based on non-hierarchical cluster analysis

Information on nature and magnitude of variability present in a population is an important pre-requisite for starting any systematic breeding programme. Cluster analysis based on D^2 statistics is highly useful in grouping of genotypes based on phenotypic diversity. Based on D^2 analysis the 46 lines were classified into six clusters (Table 5). The cluster V had highest number of genotypes (12) to follow cluster III (11), whereas cluster IV had only four genotypes. The cluster VI and cluster I had 8 and 6 lines, respectively. There was no distinction between the genotypes with green and white curd. In cluster-II green curded Romanesco-II grouped together with four other white curded genotypes, Kt-53-2, PSB-16, Ogu14A and Ogu33A. Similarly, in cluster IV, Palam Kanchan was grouped together with Kt-59-2, Pusa Himjyoti and Sel-28. Two commercially cultivated varieties, Pusa Snowball-1 and Pusa Snowball K-1 were grouped together with 10 other genotypes in cluster V. Thus the white curded genotypes could also be as good as green curded genotypes for health beneficial properties like total anti oxidant capacity. In our previous study (Dey et al. 2014) it was observed that few white curded genotypes are as good as green curded genotypes for various important vitamins and antioxidant pigment concentration in cauliflower. Selection of genotypes based on genetic divergence will be helpful in identifying parental lines for developing heterotic hybrids and hybridization based programme.

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