

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 273–280

DOI 10.13080/z-a.2019.106.035

Introgression of ‘Ogura’ cytoplasm in cabbage alters its nutritional quality and antioxidant activities

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Abstract

Ogura-based cytoplasmic male sterility (CMS) system is widely used for hybrid seed production in *Brassica* vegetables. The adverse effects associated with the introgression of Ogura cytoplasm on nutritional quality of cabbage are still unknown. Therefore, the effect of Ogura cytoplasm introgression on different quality traits in 17 lines of cabbage (*Brassica oleracea* var. *capitata* L.) was investigated. Experimental results revealed that introgression of Ogura cytoplasm altered different quality traits significantly. In general, the concentration of different nutritional compounds increased 3–5 times in some lines, while a 4–5-fold reduction was noticed in others. However, a drastic elevation in the concentration of cupric ion reducing antioxidant capacity (CUPRAC) and ferric reducing ability of plasma (FRAP) was observed in the CMS lines RRMA (29 times) and 5A (78 times), respectively. On the other hand, the amount of CUPRAC, anthocyanin and lycopene reduced severely in the CMS lines 9A (27 times), 1A (44 times) and CH2A (16 times), respectively. This alteration in different quality traits might be attributed to compatible / incompatible nucleo-cytoplasmic and mitochondrial gene interactions associated with the introgression of alien cytoplasm. Therefore, best performing CMS lines, viz. RRMA, RJA, PMA, 208A and 5A, with increased concentration of different nutritional compounds may be utilized in future breeding programs for quality improvement in cabbage. The positive correlations obtained between different quality traits offer the chances for selection of the genotypes with superior multiple quality traits. Further, outcomes of principal component analysis (PCA) and cluster analysis will aid in selection of suitable parental lines for quality F₁ hybrid development in cabbage.

Key words: antioxidant compounds, *Brassica oleracea* var. *capitata*, Ogura cytoplasm, plant pigments, vitamins.

Introduction

Among *Brassica* vegetables, cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important vegetable crops grown worldwide (Singh et al., 2009), and it constitutes an integral part of traditional cuisine in several countries. Because of consumer preference, common availability in local markets, reasonable market price and year-around availability, it represents a significant source of phytonutrients in the human diet (Samec et al., 2017). In general, *Brassica* vegetables are a good source of well-known antioxidant and health-promoting phytochemical compounds, viz. (videlicet) carotenoids, ascorbic acid (Kopsell et al., 2004), vitamins, minerals, glucosinolates and phenolic compounds (Jahangir et al., 2009). Cabbage is also enriched with proteins, all essential amino acids, minerals and several other antioxidant compounds with anti-carcinogenic (Singh et al., 2010) and anti-obesity properties (Williams et al., 2013). The various antioxidants, viz. phenols, vitamins, flavonoids, pigments, enzymes and minerals, protect human body from oxidative damage (Singh et al., 2009). Hence, estimation of total antioxidant power attributable to cooperative action of different antioxidant compounds found in cabbage ought to be evaluated for different health benefits. The estimation of cupric ion

reducing antioxidant capacity (CUPRAC) and ferric reducing ability of plasma (FRAP) contents is the most efficient means to determine total antioxidant capacity in plants (Ozyurek et al., 2008). Vitamins and carotenoids are essential compounds, which are responsible for accurate functioning of human metabolism and immune response (Samec et al., 2017). Ascorbic acid helps to maintain a healthy immune system and neutralizes the pollutants and production of antibodies. However, humans do not have the ability to synthesize ascorbic acid (vitamin C) and its severe deficiency can cause scurvy disease. Carotenoids are secondary plant compounds that form lipid-soluble yellow, orange and red pigments (Kopsell et al., 2004). A carotene-rich diet leads to decreased risk of chronic diseases related to vision, skin infection and reproduction. In addition to carotenoids and ascorbic acid, most of the anti-oxidative effects related to plant food intake are mainly due to the presence of phenolic compounds, which have been associated with flavour and colour characteristics of fruits and vegetables (Ahmed, Ali, 2013). Further, anthocyanins due to their multifunctional antioxidant activities and other beneficial biological properties constitute an integral part of food (Moreno et al., 2010). Though chlorophyll pigments do

Please use the following format when citing the article:

Parkash C., Kumar S., Singh R., Kumar A., Thakur N., Kumar S., Dey S. S., Bhatia R., Dhiman M. R., Kumar R. 2019. Introgression of ‘Ogura’ cytoplasm in cabbage alters its nutritional quality and antioxidant activities. *Zemdirbyste-Agriculture*, 106 (3): 273–280. DOI 10.13080/z-a.2019.106.035

not provide nutrition in human diet directly, they are correlated with concentration of other plant pigments (Dey et al., 2014). Therefore, it is imperative to develop nutritionally rich cultivars / hybrids in cabbage.

Today, most of the cabbage cultivars grown in the world are hybrids as they are expected to form heads as early as possible with better quality and higher yields (Cervenski et al., 2012). The development of antioxidant-rich hybrids of cabbage has great significance relative to better nutrition and health not only for consumers, but also for growers and can fetch premium price for the produce in the market. Heterosis breeding plays a critical role in genetic improvement of any crop relative to yield and quality traits (Singh et al., 2014). Therefore, an efficient, economic, reliable and stable method of hybrid seed production is needed for successful F_1 hybrid breeding in cabbage (Parkash et al., 2018). The self-incompatibility system is effective in hybrid breeding of *Brassica* vegetables (Kitashiba, Nasrallah, 2014). Yet, this system is not always stable and may be broken down by high temperature conditions. This necessitates finding a more effective and reliable system of economic hybrid seed production in *Brassica* vegetables. Cytoplasmic male sterility (CMS) system is stable and widely used for F_1 hybrid seed production in all Brassicaceae crops (Yamagishi, Bhat, 2014). In CMS genotypes, pollen production is inhibited, whereas pistil functions normally. CMS is controlled by a gene located in the mitochondrial genome. The nuclear genome of *Brassica* vegetables which lacks fertility restorer (*Rf*) gene, and CMS inducing mitochondrial gene leads to nuclear-cytoplasmic incompatibility which results in production of CMS plants.

There are several sources of the CMS cytoplasm in *Brassica* species, but Ogura cytoplasm is the only source, which is widely used for hybrid seed production in *Brassica* vegetables. Ogura CMS was discovered in Japanese radish (*Raphanus sativus* L.) of an unknown cultivar by Ogura (1968). Later on, it was introduced into nuclear background of *Brassica* vegetables by inter-generic hybridization and repeated back-crossing (Bannerot et al., 1974). But, the introgression of Ogura cytoplasm in *Brassica* vegetables raises the question of adverse effects: poor agronomic performance, leaf chlorosis and floral deformities, such as abnormal pistils, lack of nectaries and poor female fertility, associated with an alien cytoplasm. These deformities were usually deliberated as a consequence of incompatibility between

the cytoplasmic and nuclear genes (Pelletier et al., 1983). This problem was solved by Robertson et al. (1987) through somatic hybridization.

In *Brassica* vegetables, chloroplast of Japanese radish was substituted with *Brassica oleracea* chloroplast through somatic hybridization and repeated backcrossing (Earle et al., 1992). These chloroplast substituted lines have non-significant effect on agronomic performance of *Brassica* vegetables (Dey et al., 2013).

However, the effect of introgression of chloroplast substituted Ogura cytoplasm on nutritional attributes of cabbage is still unknown. Therefore, in this paper for the first time is reporting the possible effect of chloroplast substituted Ogura cytoplasm introgression on important plant pigments, vitamins and antioxidant compounds in cabbage.

Materials and methods

Experimental location, materials and design.

The present investigation was carried out at Naggar Research Farm and Quality Analysis Laboratory of Indian Agricultural Research Institute (IARI) Regional Station in Katrain, Kullu Valley, Himachal Pradesh, India, during 2016–2017. At our station, Ogura cytoplasm was transferred from a cytoplasmic male sterility (CMS) source line EC-173419 of cabbage (*Brassica oleracea* var. *capitata* L.), received from National Bureau of Plant Genetic Resources, New Delhi, India into different nuclear backgrounds of cabbage through repeated backcrossing for more than six generations. The salient features of 17 CMS lines and their maintainers are given in Table 1. All the CMS lines (A lines) and their fertile counterparts (B lines) having similar agronomic traits were planted in a randomized complete block design (RCBD) with two replications during the winter season of 2016–2017. Standard cultural practices for raising a healthy crop stand were followed for cabbage cultivation according to IARI Regional Station's guidelines (Sharma, 2003).

Sampling and estimation of different quality traits. The concentrations of different quality traits, viz. chlorophyll *a* (mg g^{-1} fresh weight (FW)), chlorophyll *b* (mg g^{-1} FW), total chlorophyll (mg g^{-1} FW), cupric ion reducing antioxidant capacity (CUPRAC) ($\mu\text{mol trolox g}^{-1}$), ferric reducing ability of plasma (FRAP) ($\mu\text{mol trolox g}^{-1}$), lycopene ($\text{mg } 100\text{ g}^{-1}$), total carotenoids ($\text{mg } 100\text{ g}^{-1}$), β -carotene ($\text{mg } 100\text{ g}^{-1}$), ascorbic acid ($\text{mg } 100\text{ g}^{-1}$), phenolics ($\mu\text{g gallic acid g}^{-1}$ FW) and anthocyanin (mg

Table 1. Salient features of 17 cytoplasmic male sterile (CMS) lines and their respective maintainers used for estimation of different plant pigments, vitamins and antioxidant compounds in cabbage

No.	Line		Salient features						
	cms-line (a line)	fertile / maintainer line (b line)	head colour	head shape	head compactness	outer leaf waxiness	outer leaf shape of blade	leaf margin	average head weight g
1.	6A	6B	green	round head	very compact	medium	obovate	non-serrated	1165
2.	RRMA	RRMB	light purple	round head	compact	medium	obovate	serrated	960
3.	RCGA	RCGB	purple green	round head	compact	strong	obovate	non-serrated	950
4.	208A	208B	dark green	flat head	compact	strong	transverse broad elliptical	serrated	1690
5.	RCA	RCB	purple	round head	very compact	strong	obovate	non-serrated	1300
6.	MRA	MRB	bluish green	oval head	very compact	medium	obovate	non-serrated	1150
7.	5A	5B	dark green	round head	compact	weak	transverse broad elliptical	serrated	1565
8.	831A	831B	dark green	round head	compact	medium	obovate	serrated	975
9.	PMA	PMB	purple	round head	very compact	strong	obovate	non-serrated	1380
10.	ZHA	ZHB	light purple	flat head	compact	medium	elliptical	serrated	1300
11.	9A	9B	bluish green	round head	compact	strong	obovate	non-serrated	1820
12.	RJA	RJB	purple	round head	compact	medium	obovate	serrated	970
13.	1A	1B	green	flat head	compact	weak	circular	non-serrated	1670
14.	CH2A	CH2B	dark green	flat head	compact	strong	transverse broad elliptical	non-serrated	1690
15.	836A	836B	green	oval head	compact	medium	ovate	serrated	1020
16.	835A	835B	green	oval head	compact	strong	elliptical	non-serrated	1135
17.	924A	924B	green	oval head	compact	absent	transverse broad elliptical	non-serrated	750

100 g⁻¹) among CMS and fertile lines (17 each) were estimated in this study. The pooled samples of true-to-type five heads were harvested, chopped and homogenized at fresh marketable stage from both the replications of each genotype (CMS and fertile lines). Then, 5 g fresh sample for each analysis was weighed and stored instantly at -20°C temperature until needed for further analysis. For the estimation of chlorophyll *a*, chlorophyll *b* and total chlorophyll, 5 g sample was crushed in 30 ml of 80% acetone and extract was centrifuged at 10,000× rpm for 15 min at 4°C temperature. Then absorbance was recorded at 645 and 663 nm optical density (OD) via a UV-visible spectrophotometer (Thermo Fisher Scientific, USA). To estimate the CUPRAC, a procedure described by Apak et al. (2006), 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 (CuCl₂) and 1 ml of distilled water; pH 7.4). The absorbance was recorded at 450 nm OD. The FRAP concentration was estimated as per the method described by Benzie and Strain (1996) 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) 5 g 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 (Na₂SO₄) 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). 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) 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 (Na₂CO₃) 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.

Statistical analysis. In order to study the effect of genotype, cytoplasm and their interaction in determining the concentration of different quality traits, the data obtained were subjected to analysis of variance using software *OPSTAT* (Sheoran et al., 1998) as per the formulae described by Panse and Sukhatme (1967). The average data of two replications from each CMS line were also compared with the data of their respective maintainer lines through paired t-test using software *SPSS*, version 16.0 (IBM, USA). The Pearson's correlation coefficient and principal component analysis (PCA) was also done through software *SPSS 16.0*, while unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on neighbour-joining hierarchical cluster analysis was constructed using software *DARwin*, version 6.0 (Perrier, Jacquemoud-Collet, <http://darwin.cirad.fr/darwin>).

Results

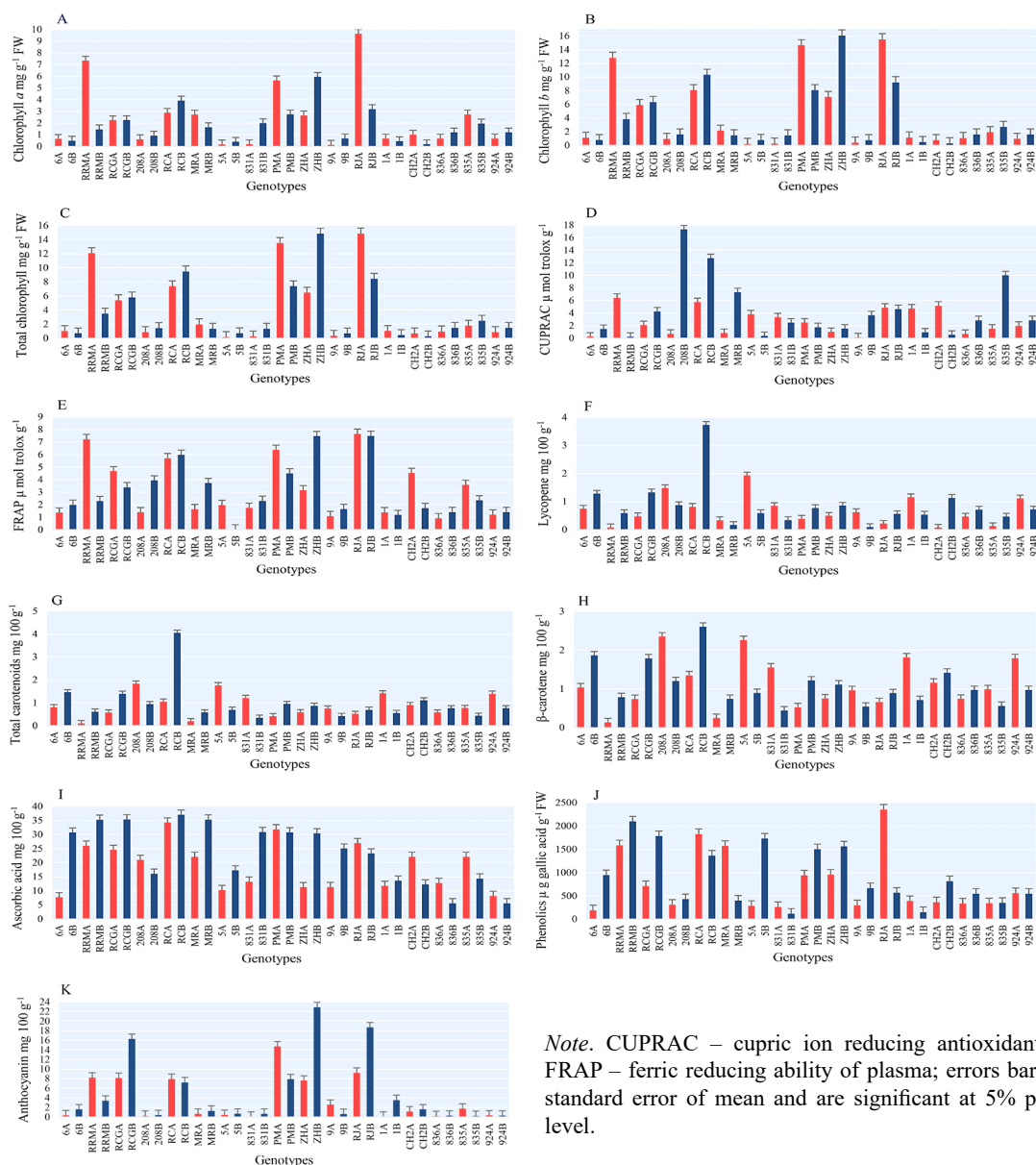
Analysis of variance (ANOVA) revealed highly significant effect of genotype and cytoplasm ($P = 0.01$) on all the 11 quality traits (Table 2). Similarly, the interaction between the genotype × cytoplasm was also found significant ($P = 0.01$) for all the traits under study. It means that all the genotypes (CMS and maintainer lines) are genetically different from each other, which offers the scope for selection of most appropriate parental lines for quality breeding in cabbage.

Effect of Ogura cytoplasm introgression on different quality traits. Chlorophyll *a* content (mg g⁻¹ FW). Among 17 Ogura based CMS lines (A lines) of cabbage, 15 lines differed significantly ($P = 0.01$) for chlorophyll *a* content from their respective fertile counterparts (B lines) with normal cytoplasm (Fig. 1A). The chlorophyll *a* content in 7 CMS lines increased, while in 8 CMS lines it was lower than in their respective maintainer lines. In the meanwhile, 2 CMS lines, viz. 6A and RCGA recorded no alteration in the concentration of chlorophyll *a* content as compared with its respective fertile analogues. The magnitude of increase or decrease in chlorophyll *a* content varied among different CMS lines. The concentration of chlorophyll *a* content increased up to 5-fold in the lines RRMA (7.34) and CH2A (1.01), while it reduced drastically to 1/10th in line 831A (0.19). Overall, A lines RJA (9.65), RRMA (7.34) and PMA (5.64), and B lines

Table 2. Analysis of variance (ANOVA) for the effect of genotype, cytoplasm and their interaction on different quality traits in cabbage

Source of variation	Mean sum of squares			
	genotype	cytoplasm	genotype × cytoplasm	error
Trait / degree of freedom	16	1	16	33
Chlorophyll <i>a</i>	13.36*	5.92*	6.10*	0.01
Chlorophyll <i>b</i>	81.66*	3.35*	15.65*	0.02
Total chlorophyll	70.33*	3.68*	14.22*	0.02
CUPRAC	23.55*	50.41*	31.34*	0.00
FRAP	15.66*	0.48*	4.57*	0.10
Lycopene	0.98*	0.68*	0.88*	0.00
Total carotenoids	1.03*	0.18*	0.93*	0.00
β-carotene	0.75*	0.01*	0.72*	0.00
Ascorbic acid	268.09*	386.98*	105.90*	2.07
Phenolics	1054211.62*	310609.91*	605985.46*	44.23
Anthocyanin	117.49*	33.95*	27.96*	0.05

CUPRAC – cupric ion reducing antioxidant activity, FRAP – ferric reducing ability of plasma; * – significant at $P \leq 0.01$



Note. CUPRAC – cupric ion reducing antioxidant activity, FRAP – ferric reducing ability of plasma; errors bars indicate standard error of mean and are significant at 5% probability level.

Figure 1. Alteration in different quality traits of A lines (CMS lines) in comparison to B lines (maintainer lines) as influenced by introgression of Ogura cytoplasm in cabbage

ZHB (5.96), RCB (3.92) and RJB (3.19) recorded highest chlorophyll *a* content than the rest of the lines under study.

Chlorophyll *b* content ($\text{mg g}^{-1} \text{FW}$). On introgression of Ogura cytoplasm in 17 lines of cabbage, significant ($P = 0.01$) alteration in chlorophyll *b* content was observed in 14 CMS lines, while it remained unaltered in 3 CMS lines when compared with their respective male fertile lines. In 5 CMS lines chlorophyll *b* content was increased, while it was decreased in 9 lines as compared to their respective B lines (Fig. 1B). The magnitude of increase or decrease in chlorophyll *b* content varied widely among different CMS lines. In the CMS line RRMA (12.79) it was elevated more than 3-fold, while in line 831A (0.26), it decreased to 1/5th on introgression of Ogura cytoplasm. However, A lines RJA (15.51), PMA (14.65) and RRMA (12.79), and B lines ZHB (16.06), RCB (10.32) and RJB (9.22) were found superior for chlorophyll *b* content.

Total chlorophyll content ($\text{mg g}^{-1} \text{FW}$). The concentration of total chlorophyll was influenced significantly ($P = 0.01$) in 14 CMS lines due to introgression of Ogura cytoplasm in 17 lines of cabbage (Fig. 1C). The total chlorophyll content increased in 5 CMS lines, while it decreased in 9 lines as compared

to their respective fertile counterparts (B lines). The magnitude of total chlorophyll content on introgression of Ogura cytoplasm was increased more than 3-fold in the CMS line RRMA (12.07), while it reduced to 1/5th in the CMS line 831A (0.23). Though, the lines RJA (14.85), PMA (13.52) and RRMA (12.07) among A lines, and ZHB (14.83), RCB (9.47) and RJB (8.43) among B lines recorded highest mean values for the trait under study.

Cupric ion reducing antioxidant activity (CUPRAC) content ($\mu\text{mol trolox g}^{-1}$). On introgression of Ogura cytoplasm in 17 lines of cabbage, the amount of CUPRAC in 16 CMS lines altered significantly ($P = 0.01$) (Fig. 1D). It increased in six CMS lines, while it reduced in 10 lines as compared to their respective maintainer lines. A dramatic change in the concentration of CUPRAC on introgression of Ogura cytoplasm was noticed in the CMS lines RRMA (6.42) (29-fold increase) and 9A (0.14) (27-fold decrease). Overall, A lines RRMA (6.42), RCA (5.75) and CH2A (5.17), and B lines 208B (17.31), RCB (12.74) and 835B (10.01) had the highest CUPRAC content.

Ferric reducing ability of plasma (FRAP) content ($\mu\text{mol trolox g}^{-1}$). Introgression of Ogura cytoplasm in different genotypes of cabbage altered the concentration of FRAP significantly ($P = 0.01$) in

15 lines (Fig. 1E). In 6 CMS lines it increased, while in 9 genotypes the amount of FRAP reduced considerably. The FRAP values in the CMS line 5A (1.97) increased drastically to 78-fold, while more than 2-fold decrease was observed in the lines 208A (1.40), MRA (1.65) and ZHA (3.16). The identified genotypes with the highest concentration of FRAP were RJA (7.66), RRMA (7.24) and PMA (6.38) (CMS lines), and RJB (7.49), ZHB (7.47) and RCB (5.99) (fertile lines).

Lycopene content ($mg\ 100\ g^{-1}$) in all the 17 CMS lines of cabbage altered significantly ($P = 0.01$) due to introgression of Ogura cytoplasm (Fig. 1F). It increased in 7 CMS lines, while substantial reduction was observed in the rest of the 10 lines. A 6-fold increase in lycopene content was recorded in the line 9A (0.62), while a sharp decline (more than 16-fold) was observed in the line CH2A (0.07). However, the genotypes of A lines 5A (1.93), 208A (1.48) and 1A (1.15) and B lines RCB (3.73), RCGB (1.33) and 6B (1.28) were superior for accumulation of lycopene content in cabbage head.

Total carotenoids content ($mg\ 100\ g^{-1}$). On introgression of Ogura cytoplasm, all the CMS lines of cabbage differed significantly ($P = 0.01$) from their respective fertile counterparts for the concentration of total carotenoids (Fig. 1G). The total carotenoids content was increased in 7 CMS lines, while it reduced in 10 lines as compared to their respective B lines. Further, magnitude of total carotenoids content on introgression of Ogura cytoplasm was elevated more than 3-fold in the CMS line 831A (1.21), while it was reduced to 1/5th in the CMS line RRMA (0.11). Overall, CMS lines 208A (1.83), 5A (1.76) and 1A (1.41), and fertile lines RCB (4.05), 6B (1.45) and RCGB (1.39) were superior for total carotenoids.

β -carotene content ($mg\ 100\ g^{-1}$). The amount of β -carotene in all the 17 CMS lines also altered significantly ($P = 0.01$) on introgression of Ogura cytoplasm in cabbage (Fig. 1H). In 7 CMS lines it increased, while in the rest of the 10 lines β -carotene content reduced considerably. The β -carotene content in the CMS line 831A (1.55) increased to 3-fold, while more than 5-fold decrease was noticed in the line RRMA (0.14). Though, the CMS lines 208A (2.36), 5A (2.26) and 1A (1.82), and B lines RCB (2.61), 6B (1.87) and RCGB (1.79) were again found outstanding for the trait under study.

Ascorbic acid content ($mg\ 100\ g^{-1}$). Introgression of Ogura cytoplasm in 17 lines of cabbage altered the concentration of ascorbic acid content significantly ($P = 0.01$) in 10 lines (Fig. 1I). The ascorbic acid content

increased in 3 CMS lines, while it reduced in 7 lines as compared to their respective fertile counterparts. The amount of ascorbic acid content increased significantly up to 1.5-fold in the CMS line 835A (22.05), while it reduced to 1/4th in the line 6A (7.68). Among all the genotypes of A lines RCA (34.23), PMA (31.87) and RJA (26.93), and B lines RCB (37.01), RCGB (35.33) and MRB (35.28) had the highest ascorbic acid content.

Phenolics content ($\mu g\ gallic\ acid\ g^{-1}\ FW$) of 16 CMS lines altered considerably ($P = 0.01$) as compared to their respective fertile analogues on introgression of Ogura cytoplasm in different genotypes of cabbage (Fig. 1J). It increased in 5 CMS lines, while substantial reduction was observed in the rest of the 11 genotypes. A 4-fold increase in phenolics content was recorded in 2 CMS lines, viz. RJA (2353.13) and MRA (1573.13), while a sharp decline of 6-fold was observed in the CMS line 5A (286.88). Overall, the genotypes RJA (2353.13), RCA (1826.25) and RRMA (1587.19) among CMS lines, and RRMB (2098.13), RCGB (1785.01) and 5B (1733.44) among fertile lines were most promising for accumulation of phenolics in cabbage head.

Anthocyanin concentration ($mg\ 100\ g^{-1}$) altered significantly ($P = 0.01$) in 15 CMS lines due to introgression of Ogura cytoplasm in 17 lines of cabbage (Fig. 1K). However, the anthocyanin content was increased in 4 CMS lines, while it decreased in 11 lines as compared to their respective fertile counterparts. The magnitude of anthocyanin content on introgression of Ogura cytoplasm was elevated more than 7-fold in the CMS line 835A (1.74), while it reduced drastically to 1/44th in the CMS line 1A (0.08). However, the genotypes PMA (14.73), RJA (9.21) and RRMA (8.22) among CMS lines, and ZHB (22.88), RJB (18.70) and RCGB (16.29) among fertile lines outperformed for anthocyanin content than the rest of lines under study.

Correlation, principal component and cluster analysis. The Pearson's correlation coefficients among different quality traits in 17 CMS lines and their respective maintainers of cabbage revealed that chlorophyll *a*, chlorophyll *b*, total chlorophyll, FRAP, ascorbic acid, phenolics and anthocyanin content were significantly positively correlated with one another (Table 3). In the meanwhile, FRAP was also found to have significant positive association with CUPRAC, whereas other plant pigments, viz. lycopene, total carotenoids and β -carotene also exhibited significant positive correlation with each other. Based on the PCA, first three components

Table 3. Pearson's correlation coefficients among different quality traits in 17 CMS lines and their respective maintainers of cabbage

Trait	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	CUPRAC	FRAP	Lycopene	Total carotenoids	β -carotene	Ascorbic acid	Phenolics	Anthocyanin
Chlorophyll <i>a</i>	1.00	0.92**	0.93**	0.18	0.84**	-0.14	-0.14	-0.32	0.48**	0.61**	0.65**
Chlorophyll <i>b</i>		1.00	0.98**	0.16	0.89**	0.03	-0.01	-0.17	0.53**	0.63**	0.84**
Total chlorophyll			1.00	0.16	0.89**	0.02	-0.01	-0.18	0.53**	0.64**	0.84**
CUPRAC				1.00	0.38*	0.29	0.32	0.15	0.19	-0.01	0.01
FRAP					1.00	-0.02	0.02	-0.13	0.59**	0.46**	0.78**
Lycopene						1.00	0.96**	0.85**	0.08	0.06	0.04
Total carotenoids							1.00	0.89**	0.13	0.01	-0.01
β -carotene								1.00	-0.02	-0.10	-0.07
Ascorbic acid									1.00	0.57**	0.51**
Phenolics										1.00	0.49**
Anthocyanin											1.00

CUPRAC – cupric ion reducing antioxidant activity, FRAP – ferric reducing ability of plasma; significant ** – at $P = 0.01$, * – at $P = 0.05$

having Eigen values greater than one were retained in the analysis because of the substantial amount of total variations (84.36%) explained (Table 4).

The 1st principal component (PC₁) had the highest positive values for chlorophyll *a* (0.92), chlorophyll *b* (0.97), total chlorophyll (0.98), FRAP (0.92), ascorbic acid (0.67), phenolics (0.70) and anthocyanin (0.84). But, it accounted only for 48.45% of the variability among all lines and was equated with chlorophyll content. While the 2nd principal component (PC₂) was found to be

superior for lycopene (0.96), total carotenoids (0.98) and β -carotene (0.91). It explained 26.68% of total variations and was mainly associated with different plant pigments. On the other hand, 3rd principal component (PC₃) had the highest positive value for CUPRAC (0.85) only and it accounted for 9.23% of total variability among different genotypes of cabbage under study.

Based on 11 quality traits, the UPGMA dendrogram constructed using neighbour-joining hierarchical cluster analysis classified 34 lines of cabbage into two major groups

Table 4. Eigen vectors for first three principal components (PC) of different quality traits studied in different lines of cabbage

Trait	PC ₁	PC ₂	PC ₃
Chlorophyll a	0.92	-0.12	0.08
Chlorophyll b	0.97	0.03	-0.03
Total chlorophyll	0.98	0.03	-0.03
CUPRAC	0.21	0.40	0.85
FRAP	0.92	0.07	0.25
Lycopene	-0.03	0.96	-0.11
Total carotenoids	-0.05	0.98	-0.05
β-carotene	-0.22	0.91	-0.18
Ascorbic acid	0.67	0.17	-0.08
Phenolics	0.70	0.04	-0.38
Anthocyanin	0.84	0.05	-0.19
Eigen value	5.33	2.94	1.02
Percentage of variance	48.45	26.68	9.23
Cumulative per cent of variance	48.45	75.13	84.36

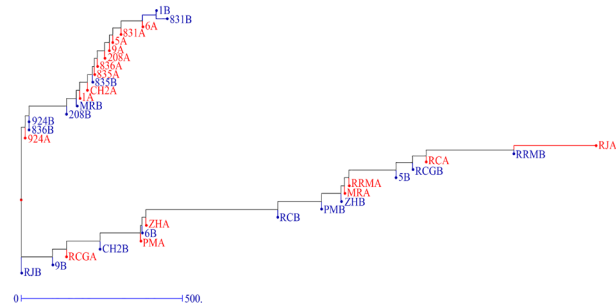
CUPRAC – cupric ion reducing antioxidant activity, FRAP – ferric reducing ability of plasma

and further several sub-groups (Fig. 2). It is clear from Figure 2 that most of the A and B lines with similar nuclear background were clustered separately in different sub-groups. The genotype RJ A and its maintainer line RJB due to their farthest and separate clustering pattern were found very distinct from the rest of the genotypes under study.

Discussion

Brassica vegetables are a good source of different antioxidant compounds (Soengas et al., 2011); therefore, development of nutritionally rich F₁ hybrids is the major objective of all the *Brassica* breeders throughout the world. Presently, Ogura based CMS is commercially used for hybrid seed production in *Brassica* vegetables (Yamagishi, Bhat, 2014). At Indian Agricultural Research Institute Regional Station in Katrain, also had developed Ogura based CMS lines of cabbage by inter-specific hybridization and repeated back-crossing (Parkash et al., 2018). But, introgression of alien cytoplasm in *Brassica* vegetables showed poor agronomic performance (Hoser-Krause, 1989), abnormal style and reduced nectaries (Dey et al., 2011), leaf chlorosis (Ren, Cao, 1990) and poor female fertility (Chamola et al., 2013). These undesirable effects are considered as a consequence of incompatibility between nuclear and cytoplasmic genes (Peletier et al., 1983). Furthermore, Ogura CMS material was improved via asymmetric protoplast fusion between the Ogura based CMS lines with broccoli (*Brassica oleracea* var. *italica* L.), which was widely used for hybrid breeding in cabbage (Wang et al., 2012). Dey et al. (2011) have reported that chloroplast substituted Ogura cytoplasm does not affect vegetative and commercial traits significantly; however, floral and reproductive traits were influenced considerably in cauliflower. The effect of Ogura cytoplasm introgression on agronomic traits of cabbage has already been reported by Melo and Giordano (1994). However, effect of this alien cytoplasm on nutritional quality of cabbage is still unknown. Hence, information on nutritional quality of parental lines (CMS lines with Ogura cytoplasm and their respective maintainer lines) is most critical for quality hybrid breeding in cabbage.

From the present investigation, it was found that effects of genotype, cytoplasm and their interactions were significant for all the traits under study signifying that all the quality traits were influenced greatly due to introgression of Ogura cytoplasm into different nuclear backgrounds of cabbage. The chlorophyll and anthocyanin content were reduced significantly or remained un-altered in most of the cabbage genotypes with introgression of Ogura cytoplasm. The lines RRMA and 835A recorded 3- to 7-fold increase for chlorophylls and anthocyanin pigmentation, respectively. However, the CMS lines RJ A, RRMA and PMA recorded the highest chlorophyll and



Red colour – A lines, blue colour – B lines

Figure 2. UPGMA dendrogram showing clustering pattern of 34 cabbage lines (17 CMS and fertile lines each) constructed using neighbour-joining hierarchical cluster analysis

anthocyanin content. Accumulation of higher chlorophylls and anthocyanin content at marketable stage is desirable in cabbage due to market preference. The CUPRAC and FRAP content was increased drastically in the CMS lines RRMA (29-fold) and 5A (78-fold), respectively. But, the genotypes RRMA, RCA and CH2A (CUPRAC) and RJ A, RRMA and PMA (FRAP) excelled the rest of CMS lines under study. Therefore, CMS lines with high values of CUPRAC and FRAP can be used in the development of F₁ hybrids with higher antioxidant capacity.

The concentration of lycopene, total carotenoids and β-carotene was found to be increased in seven CMS lines (2- to 6-fold) on introgression of Ogura cytoplasm. Though, the best performing CMS lines 208A, 5A and 1A reported an increase of 2- to 3-fold only. Thus, these lines can be used as female parent in the development of hybrids with higher concentration of carotenoids pigments. The ascorbic acid content was increased significantly in three CMS lines only (up to 1.5-fold), while in the rest of the lines it was either reduced significantly or remained un-altered on Ogura cytoplasm introgression. However, the lines RCA, PMA and RJ A recorded the highest ascorbic acid content.

The phenolics content was increased in five CMS lines and a 4-fold elevation was recorded in the CMS lines RJ A and MRA. But, the lines RJ A, RCA and RRMA performed best for the trait under study. These findings confirm that introgression of Ogura cytoplasm in different nuclear backgrounds of cabbage enhanced nutritional worth of some lines, while considerable reduction was noticed in others. It means that these effects are genotype specific, suggesting the possible role of nuclear-cytoplasmic gene interactions for the expression of different quality traits under study (Dey et al., 2017 a). This is because of the fact that refined chloroplast substituted Ogura cytoplasm (Earle et al., 1992) still has the mitochondria from Japanese radish. Hence, the positive interactions / compatibility between nuclear genome and mitochondrial DNA might have resulted in enhanced nutritional quality of some lines, while negative interactions / incompatibilities between the two led to considerable reduction in the concentration of important vitamins, plant pigments and antioxidant compounds in other lines. Mitochondrial-nuclear incompatibilities can disrupt transcription and translation of the mitochondrial genome, leading to compromised development and loss in fitness. Cytoplasmic male sterility (CMS) is also the result of an incompatibility between the nucleus and mitochondrial genomes, such that male pollen is either aborted or does not develop properly (Srivastava, 2004). Therefore, best performing CMS lines, viz. RRMA, RJ A, PMA, RCA, 208A and 5A with higher concentration of different nutritional compounds can be utilized in future breeding programs for quality F₁ hybrid development in cabbage. The significance of heterosis breeding for quality hybrid development has also been reported

earlier in cabbage (Singh et al., 2010; Parkash et al., 2017), cauliflower (Dey et al., 2014; Ram et al., 2018) and Chinese cabbage (Xie et al., 2018).

The correlation studies indicated that chlorophylls, FRAP, ascorbic acid, phenolics and anthocyanin content had strong positive correlation with one another. In the meanwhile, FRAP was also positively associated with CUPRAC, whereas other plant pigments, viz. lycopene, total carotenoids and β -carotene also exhibited significant positive correlation with one another. This illustrated that these plant pigments have similar pathway of their synthesis in plant species (Dey et al., 2017 a). Hence, positive correlations among different quality traits offer the chances for selection of genotypes with superior multiple quality traits in cabbage. The outcome of PCA indicated that chlorophyll *b*, total chlorophyll, CUPRAC, FRAP, ascorbic acid, phenolics and anthocyanin are the main components of divergence between 34 lines of cabbage, whereas contribution of the rest of the traits under study was less divergent. Hence, main emphasis should be placed on these traits for quality improvement in cabbage. Further, neighbour-joining UPGMA dendrogram revealed that based on specific quality trait to be improved; distantly placed genotypes can be selected as parental lines for the development of heterotic quality hybrids in cabbage. Dey et al. (2017 a; b) and Parkash et al. (2018) have also used hierarchical method of clustering to discriminate different cultivars of cauliflower and cabbage, respectively.

Conclusion

It is evident from the results that introgression of Ogura cytoplasm in different genotypes of cabbage enhanced the nutritional value of some lines, while considerable reduction was noticed in others. Therefore, best performing cytoplasmic male sterile (CMS) lines, viz. RRMA, RJA, PMA, RCA, 208A and 5A with higher concentration of different nutritional compounds can be utilized in future breeding programs for quality F_1 hybrid development in cabbage. Further, positive correlations among different quality traits offer the chances for selection of genotypes with superior multiple quality traits.

The results of principal component analysis (PCA) indicated that chlorophyll *b*, total chlorophyll, concentration of cupric ion reducing antioxidant capacity (CUPRAC), FRAP, ascorbic acid, phenolics and anthocyanin are the main components of divergence among 34 lines of cabbage. Hence, main emphasis should be put on these traits for quality improvement in cabbage. Further, based on the clustering pattern of UPGMA dendrogram, distantly placed genotypes can be selected as parental lines for the development of quality F_1 hybrids in cabbage. Hence, present findings will be instrumental for the quality breeding in cabbage as well as other *Brassica* vegetables throughout the world.

Acknowledgements

The authors are highly thankful to the Indian Council of Agricultural Research, New Delhi for funding this research programme.

Received 31 07 2018

Accepted 01 02 2019

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ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 273–280

DOI 10.13080/z-a.2019.106.035

Ogura citoplazmos introgresija į gūžinį kopūstą keičia jo mitybinę vertę ir antioksidacinį aktyvumą

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Santrauka

Ogura citoplazmos sukkelto vyriško sterilumo (CMS) sistema yra plačiai taikoma kopūstinių daržovių hibridų selekijoje. Iki šiol nėra žinoma faktų apie neigiamą Ogura citoplazmos introgresijos įtaką gūžinio kopūsto mitybinei vertei. Tyrimo tikslas – ištirti Ogura citoplazmos introgresijos įtaką 17-os gūžinio kopūsto (*Brassica oleracea* var. *capitata* L.) linijų įvairiems kokybiniams požymiams.

Tyrimo rezultatai parodė, kad Ogura citoplazmos introgresija reikšmingai pakeitė įvairius kokybinius gūžinio kopūsto požymius. Kai kuriose linijose įvairių mitybinių junginių koncentracija padidėjo 3–5 kartus, o kitose linijose ji 4–5 kartus sumažėjo. Tačiau *vario jonų* redukcijos antioksidantinės gebos (CUPRAC) ir geležies jonų redukcijos antioksidacinės gebos (FRAP) koncentracijų reikšmingas padidėjimas buvo nustatytas CMS linijose RRMA (29 kartus) ir 5A (78 kartus). Kita vertus, CUPRAC, antocianino ir likopeno kiekis sumažėjo CMS linijose 9A (27 kartus), 1A (44 kartus) ir CH2A (16 kartų). Šie įvairių kokybinių požymių pokyčiai gali būti paaiškinami suderinamų / nesuderinamų nukleocitoplazminių ir mitochondrinių genų sąveikomis, susijusiomis su svetimos citoplazmos introgresija. Todėl, siekiant pagerinti gūžinio kopūsto kokybę, perspektyviausios CMS linijos: RRMA, RJA, PMA, 208A ir 5A, su padidėjusia įvairių mitybinių junginių koncentracija gali būti panaudotos ateities selekcinėse programose. Tarp įvairių kokybinių požymių nustatytos teigiamos koreliacijos suteikia galimybę atrinkti genotipus su daugybe geresnių kokybinių požymių. Be to, pagrindinių komponentų analizė (PCA) ir klasterinė analizė padės atrinkti tinkamas tėvines linijas kuriant gūžinio kopūsto F₁ hibridus.

Reikšminiai žodžiai: antioksidaciniai junginiai, augalų pigmentai, *Brassica oleracea* var. *capitata*, Ogura citoplazma, vitaminai.