Research Article

Genotypic Variation for Important Biochemical Constituents in Marigold

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Abstract

Biochemical constituents like carotenes, xanthophylls and chlorophylls were extracted and estimated from ten genotypes of marigold. The experiment aimed to determine the number of pigments retained in discarded marigold flowers, stored at ambient conditions for their further reuse in industries for carotenoid extraction. Genetic variation in various genotypes was also studied and efforts were made to find out some promising genotypes of marigold which could be used for higher recovery of natural color. Total carotene, di-hydroxy pigments and total xanthophylls were found higher at bud burst stage and their maximum content was observed on the first day of storage. Maximum carotenes were obtained in MGH 160-9-2 and mono-hydroxy pigment was present in Pusa Narangi. Chlorophyll 'a' and total chlorophyll were found higher at bud burst stage in contrary to chlorophyll 'b', which was higher at full bloom stage. All types of chlorophylls were found the maximum in genotype MGH 133-3-3. All the pigments decreased with increasing storage period.

Keywords: Carotenoids, Xanthophyll, Chlorophyll, Genotype, Marigold

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Introduction

Marigold (*Tagetes* spp.) is known to be a versatile crop with "golden harvest". Its flowers contain organic pigments like carotenoids, important for human nutrition and health, participating in pro-vitamin A and anticancer activities. Carotenoids are also required by the immune system where they act as detoxifiers neutralizing free radicals before they damage DNA, lipids & proteins [1]. Carotenoid fractions like lutein, carotenes (precursor of Vitamin A), mono-hydroxy pigments, di-hydroxy pigments etc. imparts orange-yellow, yellow, orange and red color respectively for use in food, beverage, poultry and textile industries. These colors are safe for human consumptions, unlike other artificial colors which are carcinogenic in nature. Dry flowers of *Tagetes erecta* and *Tagetes patula* recorded higher carotenoid content (240.25 & 378.08 mg/100g, respectively) than fresh flowers (25.71 & 34.08 mg/100g, respectively) [2]. Xanthophylls inhibit auto-oxidation of cellular lipids, protect against oxidant-induced cell damage, cancer and cardiovascular disease and more importantly, protect against age-related macular degeneration (AMD) [3]. Marigold cultivars with orange color flowers have higher xanthophylls as compared with yellow [4]. Well-preserved flowers exhibit a high yield of Xanthophyll content (105.19 g/Kg) as compared to the unpreserved flowers (54.87 g/Kg), emphasizing the significance of flower preservation in the extraction of xanthophylls [5]. Considering the commercial importance of marigold, present investigation was carried out with the objective to determine pigment contents at different floral developmental stages and during storage in various genotypes of marigold.

Material and Methods

The experimental material for the present investigation consisted of ten genotypes (five each) of two species of marigold (*Tagetes* spp.); *Tagetes erecta* (African type) and *Tagetes patula* (French type) genotypes, obtained from the previously maintained germplasm of the Department of Horticulture, CCS Haryana Agricultural University, Hisar (**Figure 1**). The flowers of ten different genotypes were harvested at two floral developmental stages i.e. bud burst and full bloom and stored at ambient conditions for 1st, 5th, 9th and 13th day and analyzed for different pigments at different time intervals. Fresh flowers were harvested in the morning hours when there were no dew drops present on them. The flowers were then taken to the laboratory and stored in open space at ambient conditions.

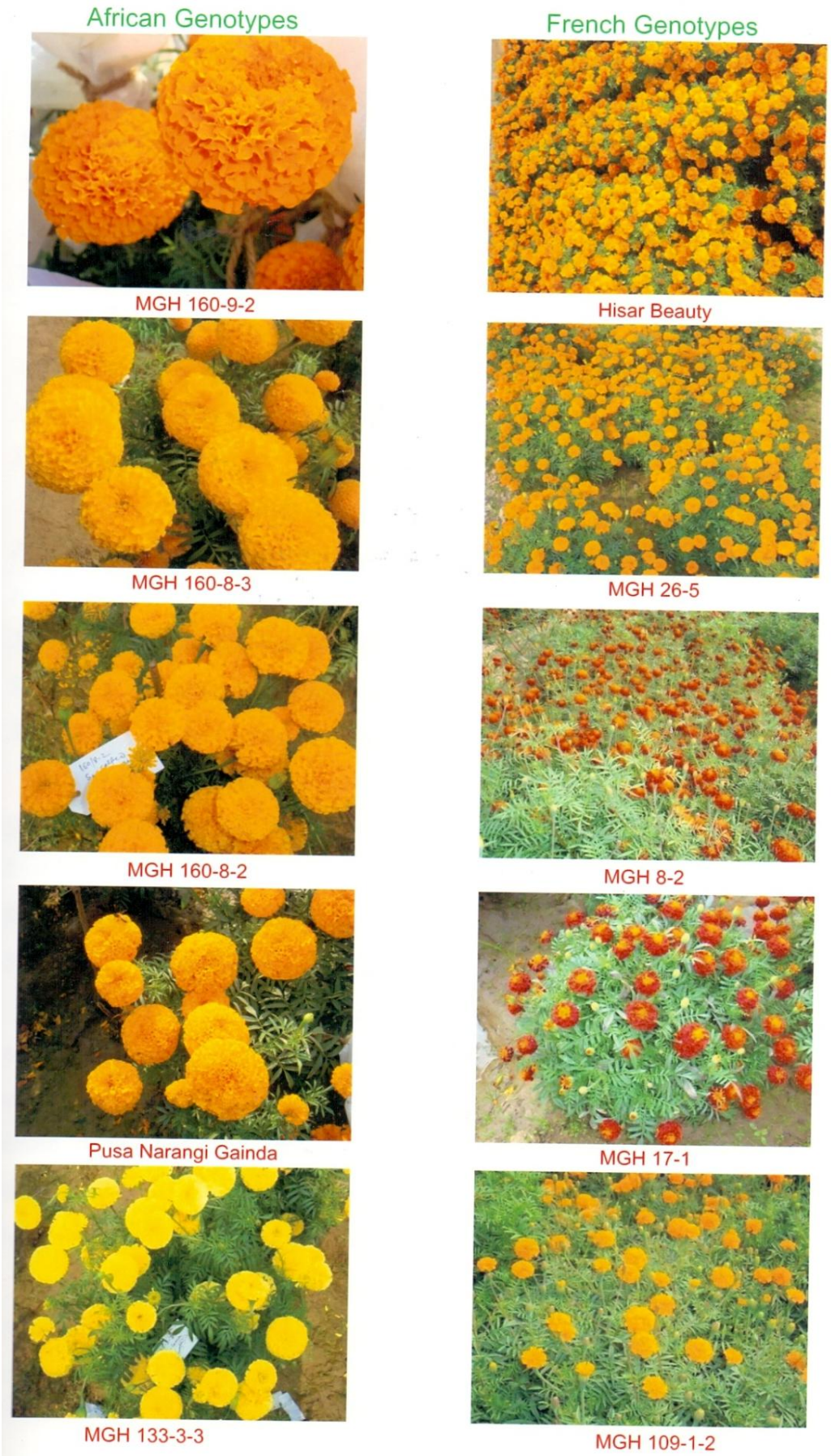

Figure 1 Marigold genotypes in flowering

Reagents used

a) *Extractant*: Hexane-Acetone-Ethanol-Toluene in the ratio of 10:7:6:7 v/v.
b) *Absorbent*: Hyflosupercel and Silica Gel G (Diatomaceous earth) in the ratio of 1:1 w/w.
c) Sodium sulphate (10%)
d) *Solvents*:

Carotenes	: Hexane and Acetone (96:4)
Mono-hydroxy pigments	: Hexane and Acetone (90:10)
Di-hydroxy pigments	: Hexane and Acetone (80:20)
Total xanthophylls	: Hexane, Acetone and Methanol (80:10:10)
Chlorophyll	: Ether and Methanol (50:50)

Method

Identification and separation of carotenoids from marigold flowers involved three steps:

Sample preparation:

Five grams of the sample was taken and homogenized with the help of mortar and pestle. The samples were then put into 100 ml beaker and 40 ml of the extractant was pipette into it. The beaker was then covered with aluminium foil and kept in dark for 24 hours. The extractant was then collected in a conical flask and to assure complete removal of carotenoids, three extractions were carried out and pooled. In order to remove the water and other solvents, carotenoids were filtered through glass wool and transferred to petroleum ether. The remaining traces of water were removed by addition of anhydrous Na_2SO_4 (10%). The process was repeated until no more color was extracted. The experiment sample was further used for chromatography.

Chromatography:

Carotenoids were fractioned into carotenes, mono-hydroxy pigments, di-hydroxy pigments, chlorophylls etc. with the help of open column chromatography method. With the help of a glass rod, an absorbent cotton plug was inserted into the column on the surface of the septa present above the stop cork. The column was packed with 12 cm layer of the mixture of absorbent under vacuum. Approximately 1 cm layer of anhydrous Na_2SO_4 was added on the top of the column to absorb residual water from the sample. A flat instrument such as inverted cork was used to flatten the surface of the absorbent for uniform absorption of the sample. The extraction sample was first added to the column (**Figure 2**). As the sample was absorbed by the absorbent, different colour of the bands were formed which indicated different compounds or fraction of carotenoids (**Figure 3**). The elution of following compounds was then performed by a gradient of solvents with different polarities.

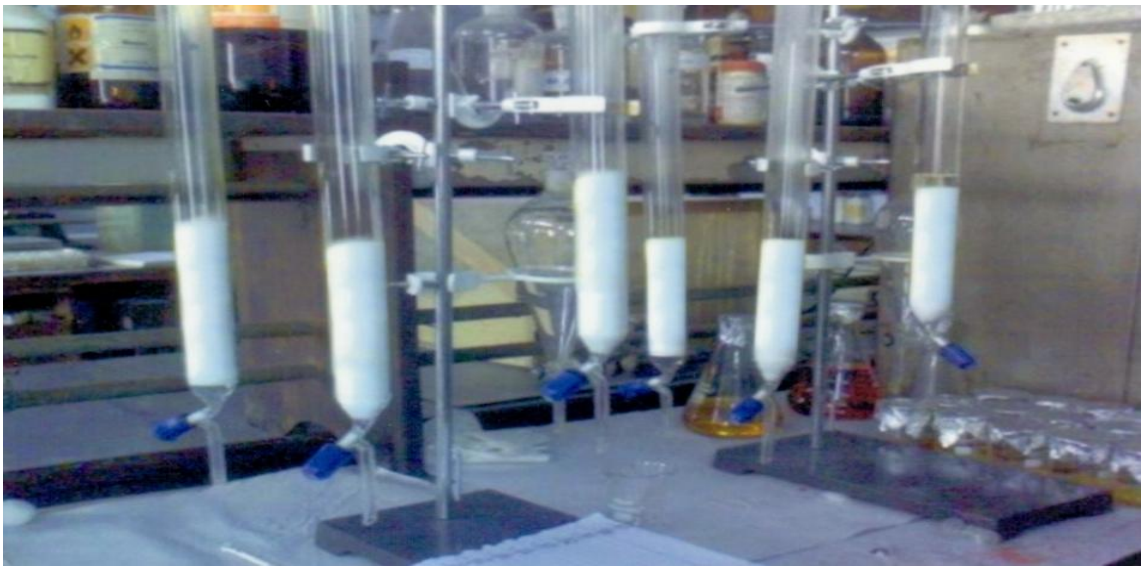

Figure 2 Filling of column chromatography

Figure 3 Separation of various pigments

Total carotenes (mg/100g)
Carotene solvent (Hexane and Acetone, 96:4) was added as the last solution entered the adsorbent and continued until carotene band was collected in the flask. The flask was inverted several times to mix the eluted compound thoroughly before taking its absorption spectra @ 440 nm wavelength.

Mono-hydroxy pigments (mg/100g)

MHP solvent (Hexane and Acetone, 90:10) was added to the column. The band of mono-hydroxy pigments (Zeaxanthin, cryptoxanthin, etc.) or di-esters was collected in a 25 ml volumetric flask and kept in dark. Absorption maxima were recorded at 474 nm.

Di-hydroxy pigments (mg/100g)

As the MHP solvent approached the absorbent surface, DHP solvent (Hexane & Acetone, 80:20) was added which carried the DHP band (Lutein, Zeaxanthin and their easters) through the column and was collected in a different volumetric flask.

Chlorophylls (mg/100g)

The solvent used for chlorophyll extraction was Ether and Methanol (50:50). The absorption maxima was recorded at two different wave lengths i.e. 663 nm and 645 nm for further calculations of chlorophyll 'a', chlorophyll 'b' and total chlorophyll.

Total xanthophylls (mg/100g)

For total xanthophylls, a fresh extraction sample was used. First of all, carotene was eluted by the above method followed by the elution of total xanthophylls with the solvent (Hexane, Acetone and Methanol, 80:10:10).
Calculations: The following carotenoid fractions were calculated according to the method given in AOAC and modified by Singh *et al.* (2008) [6]:

Total Carotenes (mg/100g):

$$\frac{OD_{440} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Mono-hydroxy and Di-hydroxy pigments (mg/100g):

$$\frac{OD_{474} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Total xanthophylls (mg/100g):

$$\frac{OD_{474} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Chlorophyll 'a' (mg/100g):

$$\frac{[(12.7 \times A_{663}) - (2.69 \times A_{645})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Chlorophyll 'b' (mg/100g):

$$\frac{[(22.9 \times A_{645}) - (4.69 \times A_{663})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Total Chlorophyll (mg/100g):

$$\frac{[(20.2 \times A_{645}) - (8.02 \times A_{663})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Results and discussion

Total carotenes (mg/100g)

The maximum carotene was observed in MGH 160-9-2 flowers (47.49 mg/100g) followed by MGH 160-8-2 (42.55 mg/100g) both African types, whereas, minimum (8.52 mg/100g) was observed in MGH 133-3-3 flowers followed by MGH 17-1 (18.65g/100g) (**Table 1A**). This variation in total carotenes among different genotypes may be due to the different genetic makeup of these genotypes. Toiu *et al.* (2008) [2], Singh *et al.* (2008) [6] and Sestras and Boscaiu (2015) [7] obtained similar results in marigold.

Table 1 'Total carotene' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage Interval ➡ Genotype ↓	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
	1	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
						Bud burst	Full bloom	
MGH 109-1-2	69.83	54.03	28.99	13.90	**41.69**	43.09	40.29	**41.69**
MGH 26-5	62.63	42.13	22.71	9.77	**34.31**	35.78	32.85	**34.31**
Hisar Beauty	47.41	33.84	19.67	7.58	**27.13**	28.94	25.31	**27.13**
MGH 17-1	34.46	23.55	11.82	4.78	**18.65**	19.42	17.89	**18.65**
MGH 8-2	36.84	24.22	14.26	5.27	**20.14**	21.95	18.34	**20.14**
MGH 160-9-2	77.36	58.80	34.95	18.86	**47.49**	50.12	44.86	**47.49**
MGH 160-8-2	66.85	51.38	35.27	16.72	**42.55**	45.64	39.47	**42.55**
Pusa Narangi	57.71	40.92	25.19	13.35	**34.29**	34.30	34.28	**34.29**
MGH 160-8-3	39.96	23.98	12.33	4.36	**20.16**	22.25	18.06	**20.16**
MGH 133-3-3	21.60	10.32	2.14	0.00	**8.52**	8.76	8.27	**8.52**
Mean	**51.47**	**36.31**	**20.73**	**9.46**		**31.03**	**27.96**	
CD (P= 0.05)	1.02					2.28		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	53.47	37.98	22.28	10.36	**31.03**	Genotypes		1.14
Full Bloom	49.46	34.65	19.18	8.56	**27.96**	Stages of harvest		0.51
Mean	**51.47**	**36.32**	**20.73**	**9.46**		Storage intervals		0.72
CD (P= 0.05)	1.61							

The carotene content decreased with increasing period of storage and on the 13th day of storage carotene content reduced significantly in all the genotypes, however, carotene content was significant up to the fifth day of storage (Table 1A). Maximum carotene content (51.47 mg/100g) was observed on the 1st day of storage and minimum (9.46 mg/100g) was recorded on the 13th day of storage irrespective of genotypes. The decrease in carotene content may be due to the reduction of active carotenoids or due to their transformation into *cis* isomers. In case of interaction among genotypes and storage intervals, maximum carotene content was (77.36 mg/100g) was found in MGH 160-9-2 followed by MGH 109-1-2 (69.83 mg/100g) on first day of storage, while minimum (0.05mg/100g) in MGH 133-3-3 followed by MGH 160-8-3 (4.36 mg/100) on 13th day of storage (Table 1A). Total carotene content was higher (31.03 mg/100mg) in bud burst stage than full bloom stage (27.96 mg/100g) (Table 1B). Genotypes × Stages interaction reveal that maximum carotene content (50.12 mg/100g) was observed in MGH 160-9-2 at bud burst stage and minimum (8.27 mg/100g) was found in MGH 133-3-3 at full bloom stage (Table 1B). The interaction between stages of flower harvest and storage interval show that maximum carotene content (55.37 mg/100g) was found in flowers harvested at bud burst stage and on the first day of storage, whereas, minimum carotene content (8.56 mg/100g) observed at full bloom stage on the 13th day of storage (Table 1C).

Mono-hydroxy pigments (mg/100g)

The maximum mono-hydroxy pigments (81.17 mg/100g) were observed in Pusa Narangi flowers followed by MGH 160-8-3 (69.40 mg/100g), both African types, while minimum (18.37 mg/100g) in MGH 133-3-3 followed by MGH 26-5 (28.25 mg/100g) (**Table 2A**). This variability in mono-hydroxy pigments is mainly due to different genetic nature, growing environmental conditions and cultural practices in marigold. Similar findings were recorded by Singh *et al.* (2008) [6] in marigold. Mono-hydroxy pigments decreased with increasing period of storage interval (Table 2A).

Table 2 'Mono-hydroxy pigment' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage interval ➡ Genotype ↓	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
	1	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
						Bud burst	Full bloom	
MGH 109-1-2	68.94	43.74	22.84	7.88	**35.85**	37.89	33.80	**35.85**
MGH 26-5	57.46	33.43	16.35	5.76	**28.25**	31.11	25.39	**28.25**
Hisar Beauty	84.84	57.44	31.54	11.74	**46.39**	47.52	45.25	**46.39**
MGH 17-1	97.18	75.84	45.56	19.09	**59.42**	62.65	56.19	**59.42**
MGH 8-2	104.99	87.21	49.04	21.20	**65.61**	65.97	65.25	**65.61**
MGH 160-9-2	89.67	67.28	45.46	20.03	**55.61**	59.85	51.37	**55.61**
MGH 160-8-2	72.42	52.12	29.78	13.16	**41.87**	45.46	38.31	**41.87**
Pusa Narangi	121.18	100.24	71.21	32.06	**81.17**	83.28	79.06	**81.17**
MGH 160-8-3	103.64	87.51	60.47	25.97	**69.40**	72.39	66.40	**69.40**
MGH 133-3-3	44.39	21.95	6.69	0.45	**18.37**	19.18	17.56	**18.37**
Mean	**84.47**	**62.68**	**37.89**	**15.73**		**52.53**	**47.86**	
CD (P= 0.05)	3.10					1.39		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud burst	87.69	65.18	39.85	17.40	**52.53**	Genotypes		1.55
Full bloom	81.25	60.17	35.94	14.06	**47.86**	Stages of harvest		0.69
Mean	**84.47**	**62.68**	**37.89**	**15.73**		Storage intervals		0.98
CD (P= 0.05)	2.19							

First day of storage retained maximum mono-hydroxy pigments (84.47 mg/100g) and minimum (15.73 mg/100g) were found on the 13th day of storage. Interaction between genotype and storage intervals shows that maximum mono-hydroxy pigments (121.18 mg/100g) were found in Pusa Narangi followed by MGH 8-2 (104.99 mg/100g) on the 1st day of storage, whereas, minimum (0.45 mg/100g) in MGH 133-3-3 followed by MGH 26-5 (5.76 mg/100g) on 13th day of storage (Table 2A). Mono-hydroxy pigments were found higher (52.53 mg/100g) in the bud burst stage than full bloom stage (47.86 mg/100g) (Table 2B). Genotypes×Stages interaction reveals that maximum mono-hydroxy pigments (83.28 mg/100g) were present in Pusa Narangi at bud burst stage and minimum (18.37 mg/100g) in

MGH 133-3-3 at full bloom stage (Table 2B). Maximum mono-hydroxy pigments (87.69 mg/100g) were found in flowers harvested at bud burst stage and on the 1st day of storage, while minimum (14.06 mg/100g) were observed on 13th day of storage at full bloom stage (Table 2C).

Di-hydroxy pigments (mg/100g)

The maximum di-hydroxy pigments were recorded in MGH 160-9-2 (165.02 mg/100g) followed by MGH 160-8-3 (140.50 mg/100g), both African marigold genotypes, while minimum (58.17 mg/100g) in MGH 133-3-3 followed by MGH 8-2 (66.49 mg/100g) (**Table 3A**). Singh *et al.* (2008) [6] recorded similar results in African marigold.

Di-hydroxy pigments decreased with increasing period of storage intervals. Maximum di-hydroxy pigments (156.88 mg/100g) were recorded on the 1st day of storage and minimum (50.71 mg/100g) on the 13th day of storage. Within interactions, maximum di-hydroxy pigments (229.18 mg/100g) found in MGH 160-9-2 followed by 160-8-2 (193.89 mg/100g) on the 1st day of storage, while minimum (14.99 mg/100g) in MGH 133-3-3 followed by MGH 8-2 (22.06 mg/100g) on the 13th day of storage (Table 3A). Di-hydroxy pigments were higher (113.39 mg/100g) in the bud burst stage, than full bloom stage (99.40 mg/100g) (Table 3B). Similar results have also been observed by Singh *et al*. (2008) [6] in marigold. In the interaction between stages and genotypes, the maximum di-hydroxy pigments (173.15 mg/100g) were recorded MGH 160-9-2 flowers at bud burst stage and minimum (60.63 mg/100g) in MGH 133-3-3 at full bloom stage (Table 3B). Content of di-hydroxy pigments was maximum (161.69 mg/100g) on the first day of storage and in the bud burst stage of marigold flowers, while the minimum (45.19 mg/100g) was found in full bloom stage on last day of storage as revealed by the data shown in Table 3C.

Table 3 'Di-hydroxy pigment' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage Interval ➞ Genotype ↓	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
	1	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
						Bud burst	Full bloom	
MGH 109-1-2	171.55	139.42	95.22	52.78	**114.74**	120.40	109.07	**114.74**
MGH 26-5	141.87	112.11	75.49	39.70	**92.29**	97.24	87.36	**92.29**
Hisar Beauty	127.22	97.88	65.38	33.00	**80.87**	85.28	76.46	**80.87**
MGH 17-1	110.41	81.83	51.67	24.44	**67.09**	71.23	62.95	**67.09**
MGH 8-2	112.16	83.22	48.53	22.06	**66.49**	76.78	56.20	**66.49**
MGH 160-9-2	229.18	193.27	143.12	94.50	**165.02**	173.15	156.89	**165.02**
MGH 160-8-2	193.89	152.79	106.92	61.19	**128.70**	137.05	120.35	**128.70**
Pusa Narangi	189.84	159.75	129.05	81.74	**140.09**	150.51	120.34	**140.09**
MGH 160-8-3	192.61	162.99	123.67	82.72	**140.50**	141.68	139.32	**140.50**
MGH 133-3-3	100.06	76.72	40.89	14.99	**58.17**	60.63	55.71	**58.17**
Mean	**156.88**	**126.00**	**87.99**	**50.71**		**111.39**	**99.40**	
CD (P= 0.05)	2.03					4.53		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	161.69	133.01	94.64	56.23	**111.39**	Genotypes		2.26
Full Bloom	152.07	118.98	81.34	45.19	**45.19**	Stages of harvest		1.01
Mean	**156.88**	**126.00**	**87.99**	**50.71**		Storage intervals		1.43
CD (P= 0.05)	3.20							

Total xanthophyll (mg/100g)

Genotype MGH 160-9-2 obtained the maximum xanthophyll content (285.11 mg/100g) followed by MGH 160-8-3 (264.64 mg/100g) both African marigold genotypes, while minimum (110.05 mg/100g) was found in MGH 133-3-3 followed by MGH 8-2 (159.65 mg/100g) (**Table 4A**). This variation in xanthophyll content may be due to the different genetic makeup of these genotypes. These findings are in line with the reports of Shivakumar *et al.* (2014) [8], Karuppaiah *et al.* (2011) [9] and Ahmad *et al.* (2011) [10] in marigold. Total xanthophyll content decreased with increasing period of storage from 1st day to 13th day of storage (Table 4A). Maximum xanthophyll content was observed 303.7 mg/100g on the 1st day of storage and minimum (103.5 mg/100g) on the 13th day of storage. Singh *et al.* (2008) [6] reported similar variation in marigold. Interaction between genotype and storage intervals reveals that

maximum total xanthophylls content (403.44 mg/100g) was found in MGH 160-9-2 followed by MGH 160-8-3 (375.09 mg/100g) on 1st day of storage, whereas, minimum in MGH 133-3-3 (34.25 mg/100g) followed by MGH 8-2 (65.91 mg/100g) on 13th day of storage (Table 4A). Total xanthophyll was found higher (216.48 mg/100g) in the bud burst stage as compared to full bloom stage (188.79 mg/100g). Among the interactions, maximum total xanthophylls were observed in MGH 160-9-2 (296.36 mg/100g) at bud burst stage and minimum in MGH 133-3-3 (92.11 mg/100g) at full bloom stage (Table 4B). Interaction between stages of flower harvest and storage interval shows that maximum total xanthophylls content (317.89 mg/100g) was found in flowers harvested at bud burst stage and on 1st day of storage, whereas, minimum total xanthophylls content (90.60 mg/100g) was observed at full bloom stage on 13th day of storage (Table 4C).

Table 4 'Total Xanthophyll' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage interval ➡	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
Genotype ↓	**1**	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
						Bud burst	Full bloom	
MGH 109-1-2	305.48	228.55	143.93	84.73	**190.67**	196.72	184.62	**190.67**
MGH 26-5	265.13	195.98	140.34	93.87	**173.83**	189.37	158.29	**173.83**
Hisar Beauty	269.19	207.37	152.91	89.44	**179.73**	196.24	163.22	**179.73**
MGH 17-1	261.45	201.68	122.51	70.30	**163.99**	178.76	149.22	**163.99**
MGH 8-2	247.02	202.01	123.64	65.91	**159.64**	170.09	149.21	**159.65**
MGH 160-9-2	403.44	332.32	245.18	159.48	**285.11**	296.36	273.86	**285.11**
MGH 160-8-2	357.43	277.99	207.71	131.63	**243.69**	252.63	235.32	**243.69**
Pusa Narangi	358.76	288.27	220.87	152.17	**255.02**	275.71	234.33	**255.02**
MGH 160-8-3	375.09	301.16	228.51	153.81	**264.64**	281.52	247.77	**264.64**
MGH 133-3-3	194.92	134.73	76.33	34.25	**110.05**	128.00	92.11	**110.05**
Mean	**303.79**	**237.01**	**166.19**	**103.56**		**216.48**	**188.79**	
CD (P= 0.05)	8.05					17.99		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	317.89	250.13	181.39	116.52	**216.48**	Genotypes		NS
Full Bloom	289.69	223.89	150.99	90.60	**188.79**	Stages of harvest		4.02
Mean	**303.79**	**237.01**	**166.19**	**103.56**		Storage intervals		5.69
CD (P= 0.05)	12.72							

Chlorophyll 'a' (mg/100g)

The maximum chlorophyll 'a' (2.48 mg/100g) was observed in MGH 133-3-3 followed by Hisar Beauty (2.39 mg/100g) and minimum in MGH 160-8-3 (1.62 mg/100g) followed by MGH 160-9-2 (1.72 mg/100g) (**Table 5A**). Similar results were observed by Sestras and Boscaiu (2015) [7] in marigold. Chlorophyll 'a' decreased with increasing period of storage interval from 1st day to 13th day. Maximum chlorophyll 'a' (3.25 mg/100g) was found on the first day of storage and minimum (0.57 mg/100g) on the 13th day of storage. Among the interactions between genotypes and storage intervals, maximum chlorophyll 'a' was found in MGH 133-3-3 (3.96 mg/100g) followed by Hisar Beauty (3.69 mg/100g) on the first day of storage, while minimum (0.46 mg/100g) in MGH 26-5 on the 13th day of storage (Table 5A). Mean chlorophyll 'a' was found higher in full bloom stage (2.08 mg/100g) than bud burst stage (1.96 mg/100g). In case of interaction between genotypes and stages, maximum chlorophyll 'a' (2.53 mg/100g) was observed in MGH 133-3-3 at bud burst stage and minimum in flowers of MGH 160-8-3 (1.57 mg/100g) at full bloom stage (Table 5B). The interaction between stages and storage intervals depicts that maximum chlorophyll 'a' (3.30 mg/100g) was recorded at bud burst stage and on the 1st day of storage, while, minimum (0.55 mg/100g) was observed on 13th day of storage (Table 5C).

Chlorophyll 'b' (mg/100g)

Maximum chlorophyll 'b' (4.14 mg/100g) was obtained in MGH 133-3-3 followed by Hisar Beauty (3.86 mg/100g), while minimum in MGH 160-8-3 (2.71 mg/100g) followed by MGH 160-9-2 (2.97 mg/100g) (**Table 6A**). Similar results were recorded by Sestras and Boscaiu (2015) [7] in marigold. Chlorophyll 'b' decreased with increasing

period of storage interval (Table 6A). Maximum chlorophyll 'b' (5.54 mg/100g) was obtained on the 1st day of storage and minimum (0.94 mg/100g) on the 13th day of storage.

Table 5 Chlorophyll 'a' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
Interval ➞	**1**	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
Genotype ↓						Bud burst	Full bloom	
MGH 109-1-2	3.38	2.70	1.76	0.58	**2.10**	2.20	2.01	**2.10**
MGH 26-5	3.17	2.45	1.66	0.46	**1.93**	1.98	1.89	**1.94**
Hisar Beauty	3.69	3.02	2.11	0.75	**2.39**	2.49	2.31	**2.39**
MGH 17-1	3.53	2.88	2.04	0.67	**2.28**	2.37	2.19	**2.28**
MGH 8-2	3.08	2.46	1.62	0.48	**1.91**	1.96	1.87	**1.91**
MGH 160-9-2	2.87	2.39	1.13	0.49	**1.72**	1.76	1.69	**1.72**
MGH 160-8-2	3.18	2.71	1.31	0.55	**1.94**	1.98	1.91	**1.94**
Pusa Narangi	3.00	2.53	1.21	0.53	**1.82**	1.86	1.78	**1.82**
MGH 160-8-3	2.68	2.23	1.06	0.50	**1.62**	1.66	1.57	**1.62**
MGH 133-3-3	3.96	3.44	1.80	0.72	**2.48**	2.53	2.44	**2.48**
Mean	**3.25**	**2.68**	**1.57**	**0.57**		**2.08**	**1.96**	
CD (P= 0.05)	0.08					0.05		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	3.30	2.75	1.66	0.59	**2.08**	Genotypes		0.04
Full Bloom	3.20	2.61	1.48	0.55	**1.96**	Stages of harvest		0.02
Mean	**3.25**	**2.68**	**1.57**	**0.57**		Storage intervals		0.02
CD (P= 0.05)	0.03							

Table 6 Chlorophyll 'b' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
interval ➞	**1**	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
Genotype ↓						Bud burst	Full bloom	
MGH 109-1-2	5.49	4.80	2.26	0.95	**3.38**	3.27	3.48	**3.38**
MGH 26-5	5.34	4.45	2.30	0.70	**3.20**	3.28	3.12	**3.20**
Hisar Beauty	6.01	5.14	3.02	1.26	**3.86**	3.77	3.95	**3.86**
MGH 17-1	6.05	5.21	2.57	1.07	**3.72**	3.66	3.79	**3.72**
MGH 8-2	5.26	4.42	1.99	0.82	**3.12**	3.04	3.20	**3.12**
MGH 160-9-2	5.04	4.22	1.83	0.80	**2.97**	3.00	2.95	**2.97**
MGH 160-8-2	5.56	4.69	2.34	1.09	**3.42**	3.40	3.44	**3.42**
Pusa Narangi	5.24	4.38	2.07	0.89	**3.14**	3.19	3.10	**3.14**
MGH 160-8-3	4.69	3.93	1.63	0.59	**2.71**	2.74	2.68	**2.71**
MGH 133-3-3	6.71	5.66	2.94	1.25	**4.14**	4.24	4.04	**4.14**
Mean	**5.54**	**4.69**	**2.30**	**0.94**		**3.36**	**3.38**	
CD (P= 0.05)	0.17					0.12		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	5.44	4.77	2.24	0.97	**3.36**	Genotypes		0.09
Full Bloom	5.64	4.60	2.35	0.91	**3.38**	Stages of harvest		NS
Mean	**5.54**	**4.69**	**2.30**	**0.94**		Storage intervals		0.05
CD (P= 0.05)	0.07							

In case of interaction among genotypes and storage intervals, maximum chlorophyll 'b' was found in MGH 133-3-3 (6.71 mg/100g) followed by MGH 17-1 (6.05 mg/100g) on the 1st day of storage, while minimum in MGH 160-8-3 (0.59 mg/100g) and MGH 26-5 (0.70 mg/100g) on 13th day of storage (Table 6A). Chlorophyll 'b' was higher (3.38 mg/100g) in full bloom stage than bud burst stage (3.36 mg/100g). In the interactions between stages and genotypes, maximum chlorophyll 'b' (4.24 mg/100g) was recorded in flowers of MGH 133-3-3 at bud burst stage and minimum in flowers of MGH 160-8-3 (2.68 mg/100g) at full bloom stage (Table 6B). The interactions shows that chlorophyll 'b' content (5.64 mg/100g) was maximum at full bloom stage on the 1st day of storage, whereas, minimum chlorophyll 'b' (0.91 mg/100g) was found in full bloom stage on the 13th day of storage (Table 6C).

Total Chlorophyll (mg/100g)

The maximum total chlorophyll was recorded in MGH 133-3-3 (6.72 mg/100g) followed by Hisar Beauty (6.26 mg/100g), whereas, minimum in MGH 160-8-3 (4.33 mg/100g) followed by MGH 160-9-2 (4.70 mg/100g) (**Table 7A**). Total chlorophyll decreased with increasing period of storage interval from 1st day to 13th day. Maximum total chlorophyll was observed 8.79 mg/100g on the 1st day of storage and minimum (1.52 mg/100g) was observed on 13th day of storage. Interaction between genotypes and storage intervals reveals that maximum total chlorophyll was found in MGH 133-3-3 (10.58 mg/100g) followed by Hisar Beauty (9.71 mg/100g) on the 1st day of storage, while minimum in MGH 160-8-3 (1.10 mg/100g) followed by MGH 26-5 (1.16 mg/100g) on 13th day of storage (Table 7A). Total chlorophyll was found higher (5.43 mg/100g) in bud burst stage than full bloom stage (5.35 mg/100g) (Table 7B). Among the interactions, maximum total chlorophyll was recorded in MGH 133-3-3 (6.72 mg/100g) at bud burst stage and minimum (4.26 mg/100g) in MGH 160-8-3 at full bloom stage. The interaction between stages and storage intervals indicates that total chlorophyll content (8.85 mg/100g) was maximum at full bloom stage on the first day of storage and minimum total chlorophyll content (1.47 mg/100g) was found at full bloom stage on the 13th day of storage (Table 7C).

Table 7 'Total Chlorophyll' content (mg/100g) at different stages of flower harvest and storage of various genotypes of marigold

Storage Interval → Genotype ↓	**Genotypes × Storage intervals (A)**					**Genotypes × Stages (B)**		
	1	**5**	**9**	**13**	**Mean**	**Stages ↓**		**Mean**
						Bud burst	Full bloom	
MGH 109-1-2	8.87	7.50	4.02	1.54	**5.48**	5.47	5.50	**5.48**
MGH 26-5	8.52	6.90	3.96	1.16	**5.14**	5.27	5.01	**5.14**
Hisar Beauty	9.71	8.17	5.13	2.02	**6.26**	6.26	6.26	**6.26**
MGH 17-1	9.58	8.09	4.62	1.74	**6.01**	6.03	5.98	**6.01**
MGH 8-2	8.35	6.88	3.62	1.31	**5.04**	5.00	5.07	**5.04**
MGH 160-9-2	7.92	6.62	2.96	1.29	**4.70**	4.76	4.63	**4.70**
MGH 160-8-2	8.75	7.40	3.66	1.64	**5.36**	5.38	4.63	**5.36**
Pusa Narangi	8.25	6.91	3.29	1.42	**4.97**	5.05	4.89	**4.97**
MGH 160-8-3	7.37	6.16	2.70	1.10	**4.33**	4.40	4.26	**4.33**
MGH 133-3-3	10.58	9.10	4.74	1.98	**6.60**	6.72	6.48	**6.60**
Mean	**8.79**	**7.37**	**3.87**	**1.52**		**5.43**	**5.35**	
CD (P= 0.05)	0.17					0.12		
Stages ↓	**Stages × Storage intervals (C)**				**Mean**	**C.D. (P = 0.05)**		
Bud Burst	8.74	7.53	3.91	1.57	**5.43**	Genotypes		0.09
Full Bloom	8.85	7.22	3.84	1.47	**5.35**	Stages of harvest		0.04
Mean	**8.79**	**7.38**	**3.87**	**1.52**		Storage intervals		0.05
CD (P= 0.05)	0.08							

Conclusion

From the present investigation it may be concluded that flowers of African marigold genotype MGH 160-9-2 were found superior for carotenoids extraction at bud burst stage and their maximum content was observed on the first day of storage.

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