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Relationship of Flower Color Parameters and Metal Ions of Petal Tissue in Fully Opened Flowers of Gerbera

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Abstract

Interaction of pigments and metal ions can change the final color of the petals. Metal ions can affect stability of flowers final color by altering vacuolar pH and activity of enzymes involved in biosynthesis, destruction, accumulation and transition of pigments. In this study, amounts of metal ions of petal tissue and their relationships with parameters of petal color analyzed and compared in stage of full blooming in six varieties Gerbera with different colors. Investigation on metal ions in different varieties statistically did not show significant differences in Cu^{2+} amounts. Results showed that increase Fe^{2+} amount in petals increase redness (*a** value) and Chroma (*C**) and decrease lightness (*L** value). Also, decrease Zn^{2+} amount in petal tissue increase color hue (*h** value). Exactly, the opposite of calcium (Ca^{2+}), Magnesium (Mg^{2+}) showed a significant and positive difference with parameters of *C** and *a**, also a significant and negative correlation observed between Mg^{2+} amount and *L** value. Generally, in this study among evaluated metal ions in different varieties of Gerbera, ions of Fe^{2+}, Ca^{2+} and Mg^{2+} presented more effective relationship with flower color parameters. Also, range of amounts of Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+} limited to 0.0076-0.012, 0.0035-0.004, 0.0017-0.003, 0.0021-0.0032, 2.18-2.97 and 1.45-1.79 mg g^{-1} fresh weight, respectively.

Keywords: chroma, lightness, absorption spectra, metal ions, *Gerbera hybrida*

1. Introduction

Gerbera L. is a genus of ornamental plants from the Composite family. *Gerbera* is very popular and widely used as a decorative garden plant or as cut flowers. *Gerbera* is also important commercially. It is the fifth most used cut flower in the world after flowers of rose, carnation, chrysanthemum, and tulip. *Gerbera* contribute largely to the floriculture industry by properties of yield potential, large flowers, long vase life and color variation. From viewpoint also flower color is defined by two main parameters, pigments present in the vacuole, and intra-vacuolar condition (vacuolar pH and metal ions amount). Several studies confirmed the importance of vacuolar pH and metal ions in fixing flower color. Many metal ions such as Cu^{2+}, Ca^{2+}, Al^{3+}, Fe^{2+}, Mg^{2+}, and Mo^{2+} have been determined to co-operate with pigments (Ellestad, 2006). Such relations usually have a significant effect on flower color. For instance, French marigold (*Tagetes patula* L.) flowers are light yellow naturally, with alum (Al^{3+}) they become golden yellow, chrome (Cr^{+3}) gives dark orange, and copper (Cu^{2+}) gives brownish tone. This is the result of various changes in flavonoids related to quercetin (Miller et al., 2009).

Several studies have analysed the impact of different metals on anthocyanin fixity in solutions. Mazza and Miniati (1993) have reported that iron (Fe^{2+}), copper (Cu^{2+}), and aluminum (Al^{+3}) ions are potent of forming stable complexes with anthocyanins. Stable triple complexes containing magnesium (Mg^{2+}) and anthocyanin (or ferric ion plus magnesium or aluminum) have also been defined (Takeda et al., 1994; Kondo et al., 1992).

Hydrangea flowers are blue when grow in soils containing aluminum (Al^{3+}), as aluminum and the pigment delphinidin form a highly stable, blue complex. If there is less aluminum available in the soil, and more molybdenum (Mo^{2+}), the same pigment interacts with the molybdenum ions, causing the flowers to appear light pink instead. In general, Magnesium (Mg^{2+}), iron (Fe^{2+}), and aluminum (Al^{3+}) ions have all been shown to interact with anthocyanins to shift their absorption spectrum towards blue (Yoshida et al., 2005).

Metal ions play also important roles in structure and activity of different enzymes of phenylpropanoid pathway

that lead to production of different pigments or formation of ion-pigment complexes (Ellestad, 2006). For instance, the effects of metal chelators and divalent metal ions on glycosyltransferases, a number of enzymes that efficiently control phenylpropanoid pathway, have been examined (Aksamit-stachurska et al., 2008). For example, it has confirmed that enzyme activity of A3GlcT is completely prevented by 1 mM Cu^{2+}, Mn^{2+}, and Zn^{2+}. It should be mentioned that the prevention effect be the result of substrate pigments destruction by these metal ions (Ford et al., 1998). Also, interference of metal ions in many antioxidant processes has been established and a defect of any of these substantial ions may imperfect the operation of the overall metabolic system, the role of Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} as co-enzymes have been determined in majority of metabolically processes (Razic et al., 2005). Nissan-Levi et al. (2007) investigated the magnesium role in color intensity of flowers in several ornamental plants. Magnesium addition led to more anthocyanin amounts, even at high temperature in red flowers of *Anigozanthos*, blue bracts of *Limonium*, pink flowers of *Gypsophila* and blue flowers of *Aconitum*. Also, in a study on *Gentiana triflora* flowers, effect of metal ions on 5-acyltransferase activity showed that Mn^{2+} strongly and Zn^{2+} moderately enhanced enzyme activity and Ca^{2+} and Mg^{2+} had negligible effects (Fujiwara et al., 1997). Methyltransferases are a class of enzymes of phenylpropanoid pathway that require divalent ions such as Mg^{2+} for the activity (Joshi & Chiang, 1998). Therefore, changes detection of the ions during flowering may give us important information on activity and transportation of metal ions, activity of effective enzymes on biosynthesis or destruction of pigments, mechanisms of tolerance in field of nutrition and color development. In this study, we studied and compared the correlation between petal colors and changes of metal ions amounts in six *Gerbera* varieties.

2. Materials and Methods

2.1 Plant Material

Petals of six *Gerbera* varieties in different colors including; 'Eco' (red), 'Malibu' (purple), 'Pink Elegance' (Pink), 'Advance' (pinkish-orange), 'Double Dutch' (yellow) and 'Bastion' (orange), that were grown under standard and identical condition, in a greenhouse in Pakdasht (lat. 50°41'N, long. 28°35'E), at the height of 1130 meters above sea level located in the south east of Tehran city, Iran, were collected. The collected samples were kept in liquid N for future handling. It should be noted that only ray florets studied in these experiments (Figure 1).

Figure 1. Flower varieties of Gerbera with different colors

2.2 Petal Color Measurement

The colors of fresh petals were first identified according to the Royal Horticultural Society Chart (RHSCC). The RHSCC was published by the Royal Horticultural Society and edited by British Color Council, 2001. Petal color factors in the central section of the upper epidermis were evaluated by colorimetric instrument (CR-400 Minolta

Japan). Colors were explained according to the Commission International de l'Eclairage (CIELAB) color-space coordinates (Nakhumicha Muriithi et al., 2009).

2.3 Analysis of Absorption Spectra of Pigments in Petal Extracts

Absorption spectra of total anthocyanins or red – purple pigments in 0.1% hydrochloric acid-methanol (HCl-MeOH) and total carotenoids or yellow – orange pigments in MeOH: Acetone (1:1) were recorded on a PG Instrumen+T80 spectrophotometer at 400-700 nm. All solvents used were analytical degree (Sigma-Aldrich Company, USA) and were used for extraction of the anthocyanins and carotenoids.

2.4 Analysis of Ions

2.4.1 Reagents and Solutions

All reagents were of analytically reagent grade. Double deionized water was used for all dilutions. All the plastic and glassware were cleaned by soaking in dilute HNO_3 and were rinsed with distilled water prior to use. The standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg^{-l} supplied by Sigma-Aldrich Company, USA.

2.4.2 Sample Preparations

Petals were dried at 70ºC for 24 h in a forced air oven and then powered in a cutting mill fitted with a 20mesh sieve. 1 g of dried and ground petals was transferred to porcelain crucible and placed in oven for dry ashing. The furnace temperature was slowly increased from room temperature to 450ºC in 1 h. The samples were ashed for approximately 4 h until a white or grey ash residue was obtained (Tuzen, 2003). The residue after cooling to room temperature was dissolved in 5 ml of 2 N HCl Solution and the mixture was heated slowly on a hot plate at about 80ºC to dissolve the residue. The final residue was filtered, transferred to a 25 ml volumetric flask and volume was made up with deionized water. Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, and Cu^{2+} in the digests were determined using a Varian Spect AA 220FS atomic absorption spectrophotometer.

2.5 Statistical Analysis

For statistical analysis of data, a completely randomized design with three replications was used. Significant differences were found at $P < 0.05$ using Duncan's Multiple Range Test. Also variance analysis of data and correlation of color parameters with other factors were made using SPSS software.

3. Results and Discussion

The visual properties of color including color name and code have been presented according to RHSCC in Table 1. The flower color distribution on a CIE 1976 (*L**, *a**, *b**) color space (or CIELAB) was as follows; the *L** values limited from 76.20 in 'Pink Elegance' to 32.27 in 'Eco', *a** values between 61.08 in 'Malibu' and 18.84 in 'Pink Elegance', *b** values from 66.80 in 'Bastion' to 17.39 in 'Pink Elegance', *C** values from 87.56 in 'Malibu' to 25.63 in 'Pink Elegance' and *h* values between 57.54 in 'Advance' and 25.30 in 'Malibu' (Table 2).

Table 1. Colors and names for the six varieties of Gerbera

No.	Variety	Munsell [a]	RHSCC [b]	
			Color Code	Color Name
1	Advance	2.5 R 8/4	36A	Light Yellowish Pink
2	Bastion	10 R 5/14	32B	Strong Reddish Orange
3	Double Dutch	2.5 Y 8/12	8A	Vivid Yellow
4	Eco	7.5 R 4/14	44C	Vivid Reddish Orange
5	Malibu	7.5 RP 3/10	67B	Vivid Purplish Red
6	Pink Elegance	10 RP 7/8	39D	Light Pink

[a] Munsell numbers: hue, value and chroma; In colorimetry, the Munsell color system is a color space that specifies colors based on three color dimensions: hue, value (lightness), and chroma (color purity). It was created by Professor Albert H. Munsell in the first decade of the 20th century and adopted by the USDA as the official color system for soil research in the 1930s.

[b] RHSCC: The Royal Horticultural Society Color Chart.

Table 2. Petal color parameters of six Gerbera varieties

No.	Variety	CIELAB Coordinate				
		L*	***a****	***b****	***C****	***h***
1	Advance	62.97	24.09	37.88	44.89	57.54
2	Bastion	42.91	48.62	66.8	82.63	53.95
3	Double Dutch	64.23	23.99	47.13	77.91	52.07
4	Eco	32.27	56.4	51.67	76.49	42.49
5	Malibu	34.36	61.08	28.78	87.56	25.3
6	Pink Elegance	76.2	18.84	17.39	25.63	42.7

*L**: lightness; *a**, *b**: chromatic components; *C**: Chroma.

Hue angle (*h*) = arctan(*b**/*a**).

$C^* = (a^{*2}+b^{*2})^{1/2}$

In investigation on the amount of ions in different varieties, no significant difference statistically observed in copper (Cu^{2+}) amount. The most of Iron (Fe^{2+}) amount was present in 'Malibu' and the least of Iron (Fe^{2+}) amount in 'Advance' (Table 3). Also, any significant difference in zinc (Zn^{2+}) amount not found in various varieties except 'Advance' contained the lower zinc (Zn^{2+}) amount rather than other varieties. Manganese (Mn^{2+}) amount except 'Double Dutch' yellow-colored was very lower than other varieties. Similarly, calcium (Ca^{2+}) amount in this variety was in the most amounts and in red – purple; cyanic varieties were in the least amount.

Table 3. Comparison of Analytical results of ions for *Gerbera* petals in different varieties

No.	Variety	Iron	Copper	Zinc	Manganese	Calcium	Magnesium
1	Advance	0.0077 d	0.0038 a	0.0017 b	0.0022 b	2.64 b	1.57 c
2	Bastion	0.0106 b	0.0040 a	0.0028 a	0.0021 b	2.53 c	1.79 a
3	Double Dutch	0.0076 d	0.0037 a	0.0027 a	0.0032 a	2.97 a	1.78 a
4	Eco	0.0105 b	0.0035 a	0.0027 a	0.0021 b	2.18 b	1.68 b
5	Malibu	0.0120 a	0.0035 a	0.0030 a	0.0025 b	2.18 b	1.68 b
6	Pink Elegance	0.0087 c	0.0035 a	0.0030 a	0.0024 b	2.52 c	1.45 d

Within a column, values with the identical letter are not significant by Duncan's multiple range test ($P<0.05$).

Also, the most of Magnesium (Mg^{2+}) amounts observed in yellow and orange varieties; 'Bastion' and 'Double Dutch' and the least of amounts found in 'Advance'. Whereas between Fe^{2+} amounts and parameters of *a** and *C** there was a positive and significant correlation and between Fe^{2+} amounts and parameters of *L** and *h* observed a significant and negative correlation (Table 4). This means that corresponding to increase Fe^{2+} amount in petals, *a** and *C** values increase and *L** value decrease, so that the most of *C** and *a** values observed in 'Malibu' with the most of Fe^{2+} amount (0.012 mg g^{-1} FW) and the highest absorption spectra of total anthocyanins (Figure 1). Regression equations of some parameters affecting flower color has presented in Table 5. Whereas it has been confirmed *C** parameter correlate to anthocyanins amounts, *b** to carotenoids and *L** to flavonoids (Jia et al., 2008; Seroczyńska et al., 2006; Uddin et al., 2004). Also the main known effect of metals on pigments in flowers has been stated to be a change in flower color hue (Takeda et al., 1994; Kondo et al., 1992).

Table 4. The correlation coefficients of evaluated factors in Gerbera varieties

h	*C**	*b**	*a**	*L**	Mg^{2+}	Ca^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}	Fe^{2+}	
-0.72 **	0.59 *	-0.15	0.93 **	-0.84 **	0.27	-0.84 **	-0.42	0.53 *	-0.16	1	**Fe^{2+}**
0.52	0.09	0.38	-0.13	0.07	0.3	0.34	0.08	-0.3	1		**Cu^{2+}**
-0.57 *	0.27	-0.05	0.33	-0.20	0.16	-0.27	0.09	1			**Zn^{2+}**
0.02	0.07	0.26	0.39	0.39	0.19	0.60 **	1				**Mn^{2+}**
0.71 **	-0.22	0.47 *	- 0.79 **	0.72 **	0.15	1					**Ca^{2+}**
0.12	0.90 **	0.87 **	0.47 *	-0.55 *	1						**Mg^{2+}**
0.46	-0.80 **	-0.22	- 0.98 **	1							***L****
-0.59 *	0.71 **	0.09	1								***a****
0.52 *	0.62 **	1									***b****
-0.3	1										***C****
1											***h***

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

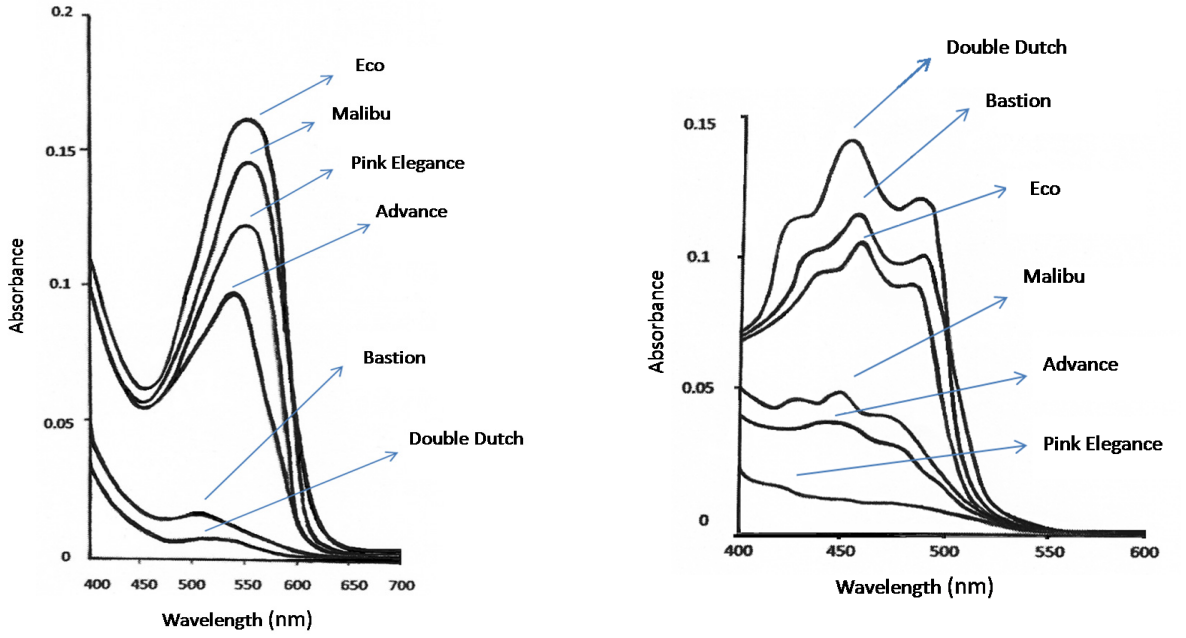
Table 5. Regression equations of some important factors evaluated in Gerbera varieties

No.	Regression equations	R^2
1	$Fe^{2+} = - 8.33\ L^* + 0.014$	0.7
2	$Fe^{2+} = 8.92\ a^* + 0.006$	0.86
3	$Fe^{2+} = 4.27\ C^* + 0.007$	0.35
4	$Fe^{2+} = h^* + 0.015$	0.52
5	$Zn^{2+} = - 2.64\ h^* + 0.004$	0.33
6	$Ca^{2+} = 0.1\ L^* + 1.88$	0.52
7	$Ca^{2+} = - 0.13\ a^* + 2.97$	0.62
8	$Ca^{2+} = 0.006\ b^* + 2.21$	0.23
9	$Ca^{2+} = 1.68\ h + 0.2$	0.5
10	$Mg^{2+} = - 0.004\ L^* + 1.86$	0.3
11	$Mg^{2+} = 1.53\ a^* + 0.003$	0.22
12	$Mg^{2+} = 1.42\ b^* + 0.005$	0.76
13	$Mg^{2+} = 1.35\ C^* + 0.005$	0.81

A negative correlation observed between Zn^{2+} amount and *h* value, on the other hand, corresponding to decrease Zn^{2+} amount in petal tissue, *h* value increase. So that, 'Advance' contained the most of *h* value and the least of Zn^{2+} amount (Table 4). Also, according to obtained results a positive and significant correlation found between *b** and *h* values and a negative and significant correlation confirmed between *a** and *h*. this means that corresponding to increase *b** and decrease *a**, *h* increase. In respect to absorption maximum of anthocyanins in 530 nm and absorption maximum of carotenoids in 450 nm, figure 2 confirm the presence and the positive correlation of anthocyanins with redness (*a** value) and so, presence and the positive correlation of carotenoids with yellowish (*b** value). Figure 2 showing Eco and Malibu varieties, with the highest *a** value have the most absorption in 530 nm and Double Dutch and Bastion varieties, with the highest *b** value have the most absorption in 450 nm (Figure 2). Although the interaction of petal pigments affecting final absorption maximum.

A negative and significant correlation observed between *a** value and Ca^{2+} amount, also a positive and significant correlation found between *L**, *b** values and Ca^{2+} amount. This indicates that there is a closed relationship between Ca^{2+} amount and carotenoid amounts, so that 'Double Dutch' with the most of *b** value and

the most of Ca^{2+} amount according to Figure which presented a more spectra absorption. Also, a positive and significant correlation found between Ca^{2+} and Mn^{2+} amounts.



A B

Figure 2. (A) Absorption spectra of total anthocyanins and (B) total carotenoids in six Gerbera varieties

Mg^{2+} similar to Ca^{2+} showed a positive and significant correlation to *b** value, but differently, the correlations between Mg^{2+} amount and parameters of *L**, *a** and *C** were different to correlations between these parameters with Ca^{2+} amounts. On the other hand, a positive and significant correlation observed to *C** and *a**, also a negative and significant correlation found to *L** and (*C** and *a**). So that 'Advance' and 'Pink Elegance' with the least of *C** and *a** values and the most of *L** contained the least of Mg^{2+} amounts. Nissan-Levi et al. (2007) confirmed that different plants containing different anthocyanins with increased magnesium could show increased color (15-70%).

A positive correlation observed between Ca^{2+} amounts and *h* value, whereas no correlation observed between this parameter and Mg^{2+} amounts (Table 5). So that, results showed a negative correlation between *a** and *C** parameters with *L** parameter, it means that lighter petals have lower lightness and hue.

Ions of Fe^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+} showed more relationship with color parameters in different varieties of *Gerbera*. This can be due to the formation of specific complexes of ion-pigment or interference of specific enzymes involved in biosynthesis, destruction, accumulation and transition of pigments that play a determinant role in final color fixation of petals (Miller et al., 2009; Goto & Kondo, 1991; Gould & Davis, 2009). The last stage of color fixation mechanism; glycosylation of anthocyanidins makes the molecules more stable and increase the solubility in the vacuole (Ferrer et al., 2008). In the cyanic flowers of *Dahlia variabilis* (Composite), an enzyme which catalyzes a glucosyl group transfer from UDP-glucose to the 5 position of anthocyanidin 3-*O*-glucoside and 3-*O*-malonylglucoside was demonstrated slightly is activated by Mg^{2+} and Ca^{2+} but strongly is inhibited by Zn^{2+}, Cu^{2+} (Ogata et al., 2001), as in these experiments, observed a significant and positive correlation between Ca^{2+}, Mg^{2+} and color parameters, but Cu^{2+} and Zn^{2+} indicated no interference in color fixation mechanism in full bloom stage of flowers.

Two other important enzymes from phenylpropanoid pathway involved in pigments biosynthesis, flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase, are members of the cytochrome P450 family and divalent metal ions have important role in their activity (Seitz et al., 2007).

Also interference of Fe^{2+} ions in activity of Anthocyanin synthase confirmed in *Petunia* (Nakajima et al., 2001). According to a study in *Gerbera* flowers for the enzymatic dimerization of catechin monomers by Anthocyanin

synthase (ANS) suggested a role for ANS beyond the oxidation of leucocyanidins and Fe^{2+} ion role was apparent in activity of this enzyme (Wellman et al., 2006), Fe^{2+} presence and its significant correlation with color parameters in this study is defensible.

4. Conclusion

According to the obtained results revealed for color, parameters have tight relationships to metal ions. Results showed that flower color parameters is affected by amounts of metal ions and other factors, so that variations in these parameters can led to changes even though minute in flower colors. Nowadays modification of parameters affecting color can facilitate attainment to specialty and novel colors of flowers during breeding programs. In this work, relationships of parameters affecting flower color and amounts of metal ions of petals studied, moreover ranges of metal ions determined in petal tissues under optimum conditions of culture, also amounts of these ions compared in different colors of Gerbera flowers.

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