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Analysis of petal pH and metal ions to investigate the mechanism of colour development in *Gerbera hybrid*

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Abstract

Clarification of the mechanism of ion transportation during flower development and the relationship to flower colouration is very important not only from the plant physiological and colour breeding but also from an agricultural demand point of view. Interaction of floral pigments with metal ions can alter the colour of the petals. Metal ions can affect the stability of the final colour in flowers by altering the vacuolar pH and activity of enzymes involved in biosynthesis, destruction, accumulation and transition of pigments. In this study, petals of six *Gerbera* varieties in different colours that were grown in a greenhouse under identical conditions were collected at three stages of colouration and flower development: bud, half-opened and fully opened. Then, the relationships between the parameters of petal colour, pH and changes of metal ions in the petals were analysed and compared during different stages and in different flower colours. According to the results, Fe^{2+} showed a high correlation with colour parameters in all three developmental stages, but no correlation was observed between ions of Zn^{2+} and Cu^{2+} and colour parameters. The most abundant Ca^{2+} amounts in all three stages were found in acyanic flowers (1.97, 2.11 and 2.97 mg g^{-1} FW). Although a significant difference was observed in Mg^{2+} amounts among different varieties, this was not significant between the three different stages for each variety. Ions of Fe^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+} presented more related associations with colour parameters in different varieties of *Gerbera*. The most abundant Fe^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+} amounts in complete colouration were 0.012, 2.97, 1.79 and 0.003 mg g^{-1} FW, respectively.

Keywords: Color variation; CIELAB coordinate; Flower development; Vacuolar pH.
Abbreviations: ANS- anthocyanidin synthase; ATP- adenosine triphosphate; *C*- chroma; CIELAB- commission international de l'eclairage (CIE) *L*a*b** color-space coordinates; FW- fresh weight; *h*- hue angle; *L*- lightness; RHSCC- royal horticultural society color chart; UDP- uridin biphosphate.

Introduction

Flower colour is determined by two major factors: pigments present in the vacuole, and intra-vacuolar environment (vacuolar pH and metal ions). Several reports have demonstrated the importance of vacuolar pH and metal ions in determining flower colour. It has been found that vacuolar pH varies greatly from 4.3 to 7.8 among different species. However, this pH can be altered in different tissues or developmental stages (Tanaka et al., 1998). In higher pH values, the absorption spectra of cyanidin and delphinidin are shifted towards longer wavelengths, causing a darker appearance (Mol et al., 1998). Many metal ions, including Cu^{2+}, Ca^{2+}, Al^{3+}, Fe^{2+}, Mg^{2+}, and Mo^{2+}, have been found to co-exist with pigments (Ellestad, 2006). Such associations usually have a significant impact on flower colour, for instance French marigold (*Tagetes patula* L.) flowers are light yellow naturally, with alum they become golden yellow, chrome gives them a dark orange appearance, and copper gives flowers a brownish tone (Miller et al., 2009). Several studies have examined the effect of different metals on anthocyanin stability in solutions. Mazza & Miniati (1993) have reported that iron, copper, and aluminium ions are capable of forming stable complexes with anthocyanins. Stable ternary complexes containing anthocyanin and magnesium (or magnesium plus ferric ion or aluminium) have also been described (Takeda et al., 1994; Kondo et al., 1992). *Hydrangea* flowers are blue when grown in soils containing aluminium, as aluminium and the pigment delphinidin form a highly stable, blue complex. In low aluminium and high molybdenum soils, the same pigment interacts with the molybdenum ions, causing the flowers to appear light pink. In general, Magnesium, iron, and aluminium ions have all been shown to interact with anthocyanins to shift their absorption spectrum towards blue (Yoshida et al., 2005). Metals can also play important roles in the structure and activity of different enzymes of the phenylpropanoid pathway, leading to the production of different pigments or the formation of ion-pigment complexes (Ellestad, 2006). For instance, the effects of divalent metal ions and metal chelators on glycosyltransferases, a group of enzymes that efficiently control the phenylpropanoid pathway,have been examined (Aksamit-Stachurska et al., 2008). Also, the interference of metal ions in many antioxidant processes has been established and a deficiency of any of these essential ions may impair the function of the overall metabolic system; the role of Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} as co-enzymes has also been determined in the majority of metabolic processes (Razic et al., 2005). Nissan-Levi et al. (2007) studied the role of magnesium in increasing colour in the flowers of several ornamental plants. Adding magnesium led to higher amounts of anthocyanin, even at high temperatures, in *Anigozanthos* with red flowers, *Limonium* with blue bracts, *Gypsophila*

Table 1. Description of developmental stages of the florescence.

Description	Days to sampling (after culture)	Length of ray floret corolla (mm)	Stage
Bud stage; Ray floret corolla longer than bracts, the tip of the ray faintly pigmented with anthocyanin.	15	20 - 25	1
The inflorescence is half-opened, the proximal part of the ray floret ligules is pigmented, the tip of the outermost disc floret corollas faintly anthocyanin pigmented.	25 - 30	40 - 45	2
The inflorescence is fully opened, anthers visible in innermost ray florets.	40 - 45	55 - 60	3

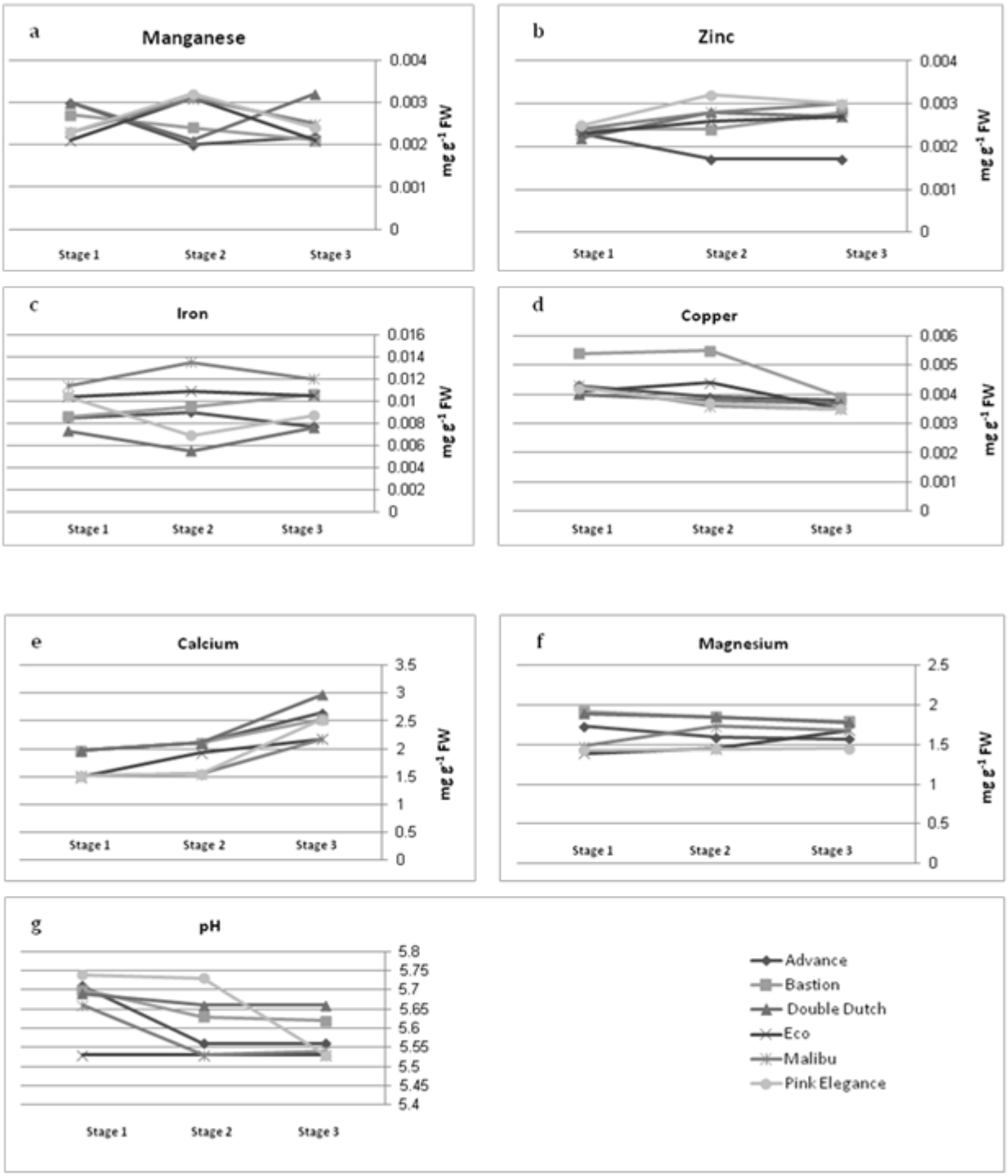

Fig 1. Comparison of Changes of metal ions amounts and pH during flower developmental stages in six *Gerbera* varieties.

with pink flowers and *Aconitum* with blue flowers. In addition, in a study on *Gentiana triflora* flowers, the effect of metal ions on 5-acyltransferase activity showed that Mn^{2+} strongly and Zn^{2+} moderately enhanced enzyme activity, while Ca^{2+} and Mg^{2+} had negligible effects (Fujiwara et al., 1997). Colour is one of the most attractive characteristics of *Gerbera* flowers and contributes dramatically to their market value.In *Gerbera*, similarly to many valuable flowers of a composite family that do not produce blue colours, the production of delphinidin-based anthocyanins is the dream goal of many breeders. Anthocyanins are extremely diverse in flowers; the diversity of anthocyanins mostly reflects modifications of common aglycones by hydroxylation, glycosylation, methylation, and acylation. These modifications are considered unique in different colours of flowers, and enzymes that modify anthocyanins play an important role in the diversity of flower colouration. The activity of these enzymes is affected by different metal ions during the colouration mechanism. Therefore, the detection of changes in the ions during flowering may provide important information on the activity and transportation of metal ions, the activity of effective enzymes on the biosynthesis or destruction of pigments, and any mechanisms of tolerance in the field of nutrition and colour development. In this study, we investigated the correlations between petal colours, pH and changes of metal ion amounts during the flower's developmental stages in six *Gerbera* varieties, which have provided a comprehensive knowledge on the development of colour in *Gerbera* flowers. The amounts of metal ions have not previously been studied during flower colouration individually for the analysis of possible activities of different enzymes of phenylpropanoid pathway or the complexation of ions with pigment molecules and their effects on visual features of flower colours. Such understanding could facilitate the attainment of novel colours and colour patterns of flowers in breeding programs, and increase the market value of ornamental crops.

Results and discussion

Identification of flower colours by CIELAB colour coordinate and RHSCC

The flower colour was distributed on a CIELAB colour coordinate as follows: the *L** values ranged from 22.88 in 'Eco' to 62.93 in 'Pink Elegance' in stage 1 (bud), from 26.81 to 69.26 in stage 2 (half-opened) and from 32.27 to 76.20 in stage 3 (fully opened) in these very varieties. For all three stages, the highest values of *a** were found to be related to 'Malibu'rather than 'Eco', and the lowest values of *a** related to the 'Double Dutch' variety. The highest values of *b** were found for 'Double Dutch', while the lowest values of *b** were found for 'Pink Elegance'; the highest values of *L** related to 'Double Dutch' and the lowest values of *L** related to 'Eco'. Also, the *C** value ranged from 56.68 in 'Malibu' to 31.48 in 'Pink Elegance' in stage 1; between 76.99 and 33.03 in stage 2; and from 77.99 to 33.03 in these varieties. The visual properties of colour, including colour name and code, as well as colour parameters according to RHSCC and the CIELAB system, during three stages of flowers development are shown in Tables 2, 3 and 4.

The relationships between colour parameters and pH changes

Based on the differences in colour, there was a negative correlation between *L** and *C** values; meaning the brighter the flower colour, the less lightness the flowers had. It has been confirmed that *C**, *b** and *L** parameters are related to vacuolar pH and amounts of metal ions (Reuveni et al., 2001) and the best known effect of metals on flower pigments is due to a change in the hue of the flower colour (Takeda et al., 1994; Kondo et al., 1992). According to data, however, petal pH decreased during flower development in different varieties, but this decrease was only statistically significant for 'Pink Elegance' (Fig.1). Reuveni, et al. (2001) reported that the decrease in vacuolar pH during petunia flower opening and colour development was due to the activity of tonoplast H^+-ATPase (Mitchell et al., 1998). It is believed that similar reasoning can be applied in the present study. In addition, we found that pH changes in *Gerbera* flowers ranged from 5.53 to 5.74 (Table 5). The pH in *pelargonium* flowers was measured between 3.1 and 3.9 (Nielsen et al., 2005), which is well below the range of 4.3– 4.7 found in *Kalanchoe blossfeldiana* flowers (De Vlaming et al., 1983); also, in *petunia* flowers coloured red-purple, the pH ranged from 5.2 to 6.5 (Brouillard, 1980). A comparison of petal pH in different varieties of *Gerbera* revealed that the lowest pH related to 'Malibu' and 'Eco'. Petal pH has been shown to be under the control of genetic factors in a number of species, including *Primula sinensis*, *Papaver rhoeas*, *Tropaeolum majus*, and *Lathyrus odoratus*. Therefore, it is likely that the pH has also affected the colour of the *Gerbera* species investigated in this study. Other studies have confirmed this effect, for instance, in *Petunia* seven loci have been identified which are determinants of petal cell pH (Mol et al., 1998; Brouillard, 1980).

The relationships between pH and metal ions during flower development

In this study, a correlation was found between petal pH and some ions in each stage of flower development (Table 6), confirming that petal pH may directly or indirectly affect some colour parameters. In agreement with these results, it has also been reported that cell vacuole pH can affect petal colour by altering the physical interactions between the electrons in the pigment (Gould et al., 2009). Also, results indicated that the highest amounts of Fe^{2+} were present in 'Eco' and 'Malibu' during all three stages of flower development (in the fully opened stage, this amount was 0.012 mg g^{-1} FW), while the lowest Fe^{2+} amounts (0.008 mg g^{-1} FW) related to 'Advance' and 'Pink Elegance'. However, the highest Cu^{2+} amounts were found in stages 1 and 2 in 'Bastion', but no significant differences were observed in stage 3. A statistically significant difference was observed for Zn^{2+} amounts in stage 1 (Bud). Results showed that the effect of zinc ions on colour development is probably due to the petal pH, so that a significant and positive correlation (R^2=0.55) was found between pH and Zn^{2+} amounts in stage 2 as well (Table 5). In the fully opened stage, the lowest amounts of Zn^{2+}were found in 'Advance' (0.002 mg g^{-1} FW), in comparison to other varieties. The effect of Mn^{2+} amounts on colour parameters was more considerable in stages 1 and 2. However, it seems that in stage 3, Mn^{2+} amounts have closer relationships with colour parameters under the effect of petal pH (Table 5). The most abundant of level of Ca^{2+} ions in all three stages was observed in acyanic flowers. It is likely that Ca^{2+} amounts have closer relationships with the contents of carotenoids (the correlation of Ca^{2+} amounts and *b** in stage 1, 2 and 3 was R^2=0.90; 0.77 and 0.47, respectively). It was also revealed that Ca^{2+} amounts increased with petal development, simultaneously (Table 6).

Table 2. Petal colour parameters of *Gerbera* varieties in bud stage.

No.	Variety	RHSCC [a]		CoordinateCIELAB [b]				
		Code	Colour name	*L**	*a**	*b**	*C**	*h* [c]
1	Advance	12C	Light Yellow	50.50	8.26	40.57	41.40	78.50
2	Bastion	3D	Light Greenish Yellow	52.56	-10.63	45.59	46.81	103.13
3	Double Dutch	154A	Vivid Yellowish Green	57.85	-19.05	55.52	40.70	108.94
4	Eco	45C	Vivid Red	22.88	39.62	27.94	48.48	35.19
5	Malibu	66B	Vivid Purplish	38.29	53.95	17.36	56.68	17.84
6	Pink Elegance	161B	Moderate Yellow	62.93	22.58	21.94	31.48	44.17

[a] RHSCC (The Royal Horticultural Society Colour Chart). [b]*L**: lightness; *a**, *b**: chromatic components; *C**: Chroma, [c] Hue angle (*h*)=arctan(*b**/*a**)(°).

Table 3. Petal colour parameters of *Gerbera* varieties in half-opened stage.

No.	Variety	RHSCC [a]		CIELAB coordinate [b]				
		Code	Colour name	*L**	*a**	*b**	*C**	*h* [c]
1	Advance	29C	Light Yellowish Pink	66.57	22.76	40.05	46.06	60.41
2	Bastion	29A	Brilliant Orange	47.12	30.11	65.62	72.19	65.35
3	Double Dutch	5C	Light Greenish Yellow	68.73	8.03	84.61	62.78	84.58
4	Eco	45D	Strong Red	26.81	54.94	45.26	71.18	39.48
5	Malibu	66C	Deep Purplish Pink	38.29	58.23	23.46	76.99	21.94
6	Pink Elegance	38D	Light Yellowish Pink	69.26	27.32	19.03	33.03	34.86

[a] RHSCC (The Royal Horticultural Society Colour Chart). [b]*L**: lightness; *a**, *b**: chromatic components; *C**: Chroma. [c] Hue angle (*h*)=arctan(*b**/*a**)(°).

Table 4. Petal colour parameters of *Gerbera* varieties in fully opened stage.

No.	Variety	RHSCC [a]		CIELAB coordinate [b]				
		Code	Colour name	*L**	*a**	*b**	*C**	*h* [c]
1	Advance	36A	Light Yellowish Pink	62.97	24.09	37.88	44.89	57.54
2	Bastion	32B	Strong Reddish Orange	42.91	48.62	66.80	82.63	53.95
3	Double Dutch	8A	Vivid Yellow	64.23	23.99	74.13	77.91	52.07
4	Eco	44C	Vivid Reddish Orange	32.27	56.4	51.67	76.49	42.49
5	Malibu	67B	Vivid Purplish Red	34.36	61.08	28.78	87.56	25.30
6	Pink Elegance	39D	Light Pink	76.20	18.84	17.39	25.63	42.70

[a] RHSCC (The Royal Horticultural Society Colour Chart). [b]*L**: lightness; *a**, *b**: chromatic components; *C**: Chroma, [c] Hue angle (*h*)=arctan(*b**/*a**)(°).

In this study, changes of Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+} ranged between 0.005-0.013, 0.003-0.005, 0.002-0.003, 0.002-0.003, 1.49-2.97, 1.39-1.92 mg g^{-1} FW, respectively.
It was determined that Fe^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+} ions had closer correlations with colour parameters in different varieties of *Gerbera*. Mn^{2+} showed the greatest effect on colour parameters in stages 1 and 2. Previous studies were in agreement to our results, stating that this could be due to the formation of specific ion-pigment complexes or interference of specific enzymes in colour biosynthesis during these stages (Miller et al., 2009; Ferrer et al., 2008).

Changes of metal ions during flowers development in different varieties

According to results, however, a significant and positive correlation was found between Mn^{2+} and *a** values in stages 1 and 2, and a negative correlation between Mn^{2+} and *b** values in different varieties as well (Table 6).However, in comparison with the three developmental stages, a converse trend was observed between cyanic varieties: 'Pink Elegance', 'Eco' and 'Malibu', and acyanic varieties: 'Double Dutch' and 'Advance' (Fig.1; a-Manganese). Also, during different developmental stages for all varieties, the amounts of Zn^{2+} were lower in 'Advance' than other varieties; the amounts in the bud stage were also higher than in the other two stages (Fig.1; b-Zinc). The range of changes of Fe^{2+} was broader in stage 2 than other stages (0.055-0.013 mg g^{-1} FW) (Fig.1; c-Iron). However, a considerable decrease was observed in Cu^{2+} amounts in 'Bastion' than in other varieties during the different developmental stages (Fig.1; d-Copper), and Ca^{2+} amounts increased correspondingly with petal development (Fig.1; e-Calcium). It has been confirmed that anthocyanins are biosynthesised simultaneously with the development of petal tissue and under the regulation of flower growth and development (Martin and Grates, 1993). The increase in Ca^{2+} may have an effect on colouration, petal development and tissues firmness. A significant and positive correlation was also found between Ca^{2+} and *h* value in all three developmental stages (Table 6). Although a significant difference in Mg^{2+} amounts was found in different varieties, these differences were not considerable between the three developmental stages of petals in each of variety (Fig.1; f-Magnesium). It is hypothesised that magnesium can stabilise all of the different pigments involved. Nissan-Levi et al. (2007) reported that different plants with different types of anthocyanins can show increased colour (15–70%) with increased magnesium. Also,another study confirmed that methyltransferases, a class of enzymes of phenylpropanoid pathway, require divalent ions such as Mg^{2+} for their activity (Joshi and Chiang, 1998). In this study, a significant and positive correlation was found between *C** and Ca^{2+} amounts during stages 2 and 3 (Table 6). Fe^{2+} showed a closed relationship with colour parameters in all of the developmental stages. No correlation was observed between Zn^{2+} and Cu^{2+} ions and colour parameters (Table 6).It is confirmed that in the last stage of the colour fixation mechanism, glycosylation of anthocyanidins makes the molecules more stable and increases their solubility in the vacuole (Ferrer et al., 2008). In the cyanic flowers of *Dahlia variabilis* (Asteraceae), an enzyme which catalysesthe transfer of a glucosyl group from UDP-glucose to the 5’

Table 5. Comparison of analytical results for *Gerbera* petals in different varieties during different developmental stages. Table continued below.

No.	Variety	Fe^{2+} (mg g^{-1} Fresh petal)			Cu^{2+} (mg g^{-1} Fresh petal)			Zn^{2+} (mg g^{-1} Fresh petal)			pH		
		Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
1	Advance	0.008 c	0.009 d	0.008 d	0.004 b	0.003 c	0.004 a	0.002 a	0.002 b	0.002 b	5.71 b	5.56 d	5.56 c
2	Bastion	0.008 c	0.010 c	0.010 b	0.005 a	0.005 a	0.004 a	0.002 a	0.002 b	0.003 a	5.70 b	5.63 c	5.62 b
3	Double Dutch	0.007d	0.005 f	0.008 d	0.004 b	0.003 c	0.004 a	0.002a	0.003 a	0.003 a	5.69 b	5.66 b	5.66 a
4	Eco	0.010 b	0.011 b	0.010 b	0.004b	0.004 b	0.003 a	0.002 a	0.003 a	0.003 a	5.53 d	5.53 e	5.53 d
5	Malibu	0.011a	0.013 a	0.012 a	0.004b	0.003 c	0.003 a	0.002 a	0.003 a	0.003 a	5.66 c	5.54 e	5.54 d
6	Pink Elegance	0.010 b	0.007 e	0.009 c	0.004 b	0.003 c	0.004 a	0.002 a	0.003 a	0.003a	5.74 a	5.73 a	5.53 d

No.	Variety	Ca^{2+} (mg g^{-1} Fresh petal)			Mg^{2+} (mg g^{-1} Fresh petal)			Mn^{2+} (mg g^{-1} Fresh petal)		
		Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
1	Advance	1.97 a	2.11 a	2.64 b	1.73 b	1.59 c	1.57 c	0.003 a	0.002 b	0.002 b
2	Bastion	1.97 a	2.11 a	2.53 c	1.92 a	1.85 a	1.79 a	0.003 a	0.002 b	0.002 b
3	Double Dutch	1.97 a	2.11 a	2.97 a	1.89 a	1.85 a	1.78 a	0.003 a	0.002 b	0.003 a
4	Eco	1.49 b	1.93 b	2.18 d	1.39 c	1.45 d	1.68 b	0.002 b	0.003 a	0.002 b
5	Malibu	1.52 b	1.55 c	2.18 d	1.48 b	1.73 b	1.68 b	0.002 b	0.003 a	0.002 b
6	Pink Elegance	1.51 b	1.55 c	2.52 c	1.43 bc	1.44 d	1.45 d	0.002 b	0.003 a	0.002 b

Within a column, values with the same letter are not significantly different by Duncan's multiple range test ($P<0.05$)

Table 6. The Correlation coefficients of evaluated factors in *Gerbera* varieties.

Stage 1		Stage 2		Stage 3	
Factors	R^2	Factors	R^2	Factors	R^2
$Fe^{2+} – L$*	- 0.61 *	$Fe^{2+} – a$*	0.91 *	$Fe^{2+} – L$	- 0.84 **
$Fe^{2+} – a$*	0.78 **	$Fe^{2+} – b$*	- 0.48 *	$Fe^{2+} – a$*	0.92 **
$Fe^{2+} – b$*	- 0.96 **	$Fe^{2+} – C$	0.60 **	$Fe^{2+} – C$*	0.58 *
$Fe^{2+} – C$	0.47 *	$Fe^{2+} – h$	- 0.69 **	Fe^{2+} - h	- 0.72 **
$Fe^{2+} – h$	- 0.94 **	$Ca^{2+} – a$*	- 0.72 **	$Ca^{2+} – L$*	0.72 **
$Ca^{2+} – a$*	- 0.79 **	$Ca^{2+} – b$*	0.77 **	$Ca^{2+} – a$*	- 0.78 **
$Ca^{2+} – b$*	0.90 **	$Ca^{2+} – h$	0.86 *	$Ca^{2+} – b$*	0.47 *
$Ca^{2+} – h$	0.92 **	$Mg^{2+} – L$	0.63 **	$Ca^{2+} – h$	0.70 **
$Mg^{2+} – a$*	- 0.69 **	$Mg^{2+} – b$*	0.67 **	$Mg^{2+} – L$	- 0.54 *
$Mg^{2+} – b$*	- 0 69 **	$Mg^{2+} – C$*	0.52 **	$Mg^{2+} – a$*	0.47 *
$Mg^{2+} – h$	0.092 **	$Mn^{2+} – L$	- 0.49 *	$Mg^{2+} – b$*	0.87 **
$Mn^{2+} – a$*	0.67 **	$Mn^{2+} – a$*	0.66 **	$Mg^{2+} – C$*	0.90 **
$Mn^{2+} – b$*	0.75 **	$Mn^{2+} – b$*	0.64 **	$pH – Ca^{2+}$	0.77 **
$Mn^{2+} – h$	0.76 **	$pH – Fe^{2+}$	- 0.62**	$pH – Mg^{2+}$	0.71 **
$pH – Mn^{2+}$	0.48 *	$pH – Zn^{2+}$	0.55 *	$pH – Mn^{2+}$	0.52 *

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

position of anthocyanidin 3-*O*-glucoside and 3-*O*-malonylglucoside was slightly activated by Mg^{2+} and Ca^{2+}, but strongly inhibited by Zn^{2+}, Cu^{2+}, Mn^{2+}, and Fe^{2+} (Ogata et al., 2001). A significant and positive correlation was observed between Ca^{2+},Mg^{2+} and colour parameters, especially in the final stages. On the other hand, it was confirmed that Cu^{2+} and Zn^{2+}didnot interfere with thecolour fixation mechanism. It has been reported that two other important enzymes from the phenylpropanoid pathway are involved in pigment biosynthesis. Flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase, are members of the cytochrome P450 family and divalent metal ions playan important role in their activity (Seitz et al., 2007). Activity of these enzymes in *Petunia* flowers varies during different developmental stages and colour development (Menting et al., 1994). However, in ray florets of *Osteospermum hybrida*, these enzymes are active in all of the developmental stages (Seitz et al., 2007). Interference of Fe^{2+} ions in the activity of Anthocyanin synthase has been confirmed in *Petunia* (Nakajima et al., 2001). According to a study on *Gerbera* flowers, the enzymatic dimerisation of catechin monomers by anthocyanin synthase plays an important role for ANS beyond the oxidation of leucocyanidins and the role of Fe ions on the activity of this enzyme was apparent (Wellmann et al., 2006).

Materials and methods

Plant materials

Petals of six *Gerbera* varieties in different colours including; 'Eco' (red), 'Malibu' (purple), 'Pink Elegance' (Pink), 'Advance' (pinkish-orange), 'Double Dutch' (yellow) and 'Bastion' (orange) grown in a greenhouse under standard and identical conditions (lat. 50°41' N, long. 28°35' E) were collected at three stages of flower development and colouration (Table 1) (Helariutta et al., 1993). The collected samples were kept in liquid N until use. It must be mentioned that only ray florets were studied in theseexperiments.

Petal colour measurement

The colours of fresh petals were first identified according to the Royal Horticultural Society Chart (RHSCC). Petal colour parameters in the middle portion of the upper epidermis were measured with a colourimetric instrument (CR-400 Minolta Japan). Colours were expressed according to the Commission International de l'Eclairage (CIE) *L*a*b** colour-space coordinates. CIELAB coordinate scale measured related to:
*L** (Lightness) Dark-Bright (0 = black, 100 = white)
*a** Green-Red (negative value = more green, positive value = more red)
*b** Blue-Yellow (negative value = more blue, positive value = more yellow)
*C** (Chroma) Colour intensity (the higher the more intense)
h (Hue angle) Degree of brownness (the higher the more brown)
(Nakhumicha Muriithi et al., 2009).

Petal pH measurement

Fresh petals (ca. 0.3 g) were ground with a mortar and filtered using double-layered gauze. The pH of the obtained press-juice was recorded with a pH meter.

Analysis of ions

Reagents and solutions

All reagents were of analytical grade. Double deionised water was used for all dilutions. All of the plastics 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^{-1} supplied by Sigma and Aldrich.

Sample preparations

Petals were dried at 70°C for 24 h in a forced air oven and ground in a cutting mill fitted with a 20-mesh sieve. 1 g of dried petals was transferred into a porcelain crucible and placed in an oven for dry ashing. The furnace temperature was slowly increased from room temperature to 450°C over a period of1 h. The samples were ashed for approximately 4 h until a white or grey ash residue was obtained. After cooling to room temperature, the residue was dissolved in 5 ml of a 2M HCl solution and the mixture was heated slowly on a hot plate at 80°C to dissolve the residue. The final residue was filtered, transferred into a 25 ml volumetric flask and diluted to 25 ml with deionised 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.

Statistical analysis

For the statistical analysis of data, a completely randomised design with three replicates was used. Significant differences were found at $P<0.05$ using Duncan's Multiple Range Test. Analysis of variance of data and correlations between petal colour parameters and other factors were made using SPSS software. The graphical representation was produced by Microsoft Office Excel 2007.

Conclusions

According to the results of this study, Fe^{2+} showed a high correlation with colour parameters in all of the developmental stages. No correlation was observed between Zn^{2+} and Cu^{2+}ions and colour parameters. The most abundant Ca^{2+} contents were found in acyanic flowers.in all three developmental stages. In general, the pH of *Gerbera* flowers ranged from 5.53 to 5.74 during different developmental stages. Also, changes of Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+}were limited to 0.005-0.013, 0.003-0.005, 0.002-0.003, 0.002-0.003, 1.49-2.97, 1.39-1.92 mg g^{-1} FW, respectively.

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