Bluing of *Eustoma* Flowers Induced by Molybdenum in Hydroponics

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Summary

A solution of ammonium molybdate (Mo: 1 mM) was infiltrated into floral disks of several flower plants that contain anthocyanin to study the basis of bluing caused by molybdenum. The floral disk colors of *Eustoma grandiflorum* (Raf.) Shinn. 'Tokinoakari', 'Lination Red' and *Tulipa gesneriana* L. 'Purple Flag' became blue when treated with ammonium molybdate. Those of *Eustoma grandiflorum* 'Exrosa Blue' and *Iris hollandica* hort. 'Blue Magic' became slightly bluish. Molybdenum had no bluing effect on *Tulipa gesneriana* 'Ile de France', *Rosa* spp. 'Carienta' and *Dianthus caryophyllus* L. 'Cain'. The minimum concentrations of molybdenum for bluing of excised flower tissues were 0.5 mM in 'Lination Red' and 1 mM in 'Purple Flag' or 'Exrosa Blue'. Aluminum had no effect on flower colors at any concentrations. The flowers of five varieties which showed bluing by molybdenum contained the anthocyanidin, delphinidin. Molybdenum did not affect the color of flowers containing pelargonidin or pelargonidin and cyanidin. Strong bathochromic shifts with molybdenum were also observed in extracts containing delphinidin glycoside or delphinidin spots on thin layer chromatograms. Furthermore, the flower color of *Eustoma grandiflorum* 'Exrosa Blue Flash', which was grown in hydroponics supplied with ammonium molybdate (Mo:1 mM) before anthesis became bluish.

Key Words: anthocyanin, bluing, *Eustoma grandiflorum*, flower color, molybdenum.

Introduction

The reasons why flowers containing anthocyanin pigments develop blue colors have been investigated by several researchers. One of the theories to explain the bluing involves a metal complex of commelinin in blue flowers in *Commelina communis* L. var. *communis* that has been studied in detail (Hayashi et al., 1958; Kondo et al., 1991; Kondo et al., 1992; Mitsui et al., 1959; Takeda, 1977; Takeda and Hayashi, 1977; Takeda and Mitsui, 1966; Tamura et al., 1986). Commelinin is a stable compound containing Mg^{2+} (Hayashi and Takeda, 1970); however, it is well known that Al contributes to the bluing of hydrangea sepals (Allen, 1932, 1943; Chenery, 1937).

Maekawa et al. (1983) reported that sodium molybdate changed the petal color of the cut flower of *Platycodon grandiflorum* A. DC. 'Samidare' from blue-violet to blue; its effective concentrations were from 2.5 to 5 mM. However, no other flower was affected by molybdenum nor damaged. Thus, molybdenum treatment on cut flowers was considered to be impractical. Recently, another study showed that the stem color of the seedlings of *Brassica* species was changed from red to blue by molybdenum (Hale et al., 2001).

There are not many important blue ornamental flow-

ers. The objective of this study is to investigate the effects of molybdenum to produce new blue flowers. For this purpose, an ammonium molybdate solution was first applied to several fresh purple and red flowers to investigate the bluing phenomenon, that is, effects of molybdenum on blue coloration of their tepals or petals. Subsequently, the relationship between the bluing phenomenon and the anthocyanidin compositions of those flowers was researched because molybdenum brings about a bathochromic shift of the TLC spots of anthocyanins that have adjoining hydroxyls in their B-ring (Ohta et al., 1979). The degrees of bluing caused by molybdenum were compared with the color changes induced by aluminum on *Hydrangea* flowers, as well as bathochromic shifts of anthocyanins in extracts or spots on the thin layer chromatograms. In addition, a final aim to produce a blue flower of *Eustoma grandiflorum* was carried out in a hydroponic culture supplied with molybdenum, to investigate the possibility of its commercial adaptation.

Materials and Methods

Plant materials for in vitro experiment

The following eight varieties of five kinds of flowers were used for the in vitro experiment: *Eustoma grandiflorum* (Raf.) Shinn, 'Tokinoakari', 'Lination Red' and 'Exrosa Blue'; *Tulipa gesneriana* L. 'Purple Flag' and 'Ile de France'; *Rosa* spp. 'Calineta'; *Iris hollandica*

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hort. 'Blue Magic'; *Dianthus caryophyllus* L. 'Cain'. These flowers were purchased at a local market.

Treatment of Mo or Al compounds to flower tissues

The anterior parts of fresh densely colored corolla were punched out by using a cork borer (13 mm diam.). Ten disks were floated on 1 mM Mo as ammonium molybdate solution or distilled water (control) in a sterilized petri dish at room temperature.

In addition, disks of 2 varieties of *E. grandiflorum*, 'Lination Red' and 'Exrosa Blue', and *T. gesneriana* 'Purple Flag' were also floated on ammonium molybdate or aluminum chloride solutions to determine the relation between Mo or Al concentration (0.1–10 mM) and color changes. Disks were also floated on acidic water (pH 3.77) adjusted with 1N HCl to investigate the effects of pH on given metal ion solutions on color changes.

The disk colors were measured 72 hr after treatment.

Measurement of flower color

The adaxial colors of the floral disks, treated and untreated with the metal ions mentioned above, were measured by using a spectro photometer (CM-2002, Minolta, Tokyo). Using an $L^*a^*b^*$ color space, the hue angle ($\tan^{-1}(a^*/b^*)$) and the chroma ($((a^{*2}+b^{*2})^{1/2})$) were calculated. When the lightness (L^*) is higher, the color appeared nearer to white. Hue angle is an indicator of the color. An approximate color index of the hue angle is as follows: 0 degree, red; +60 degree, yellow; -60 degree, purple; -120 degree, blue. If the chroma is high, the color is brilliant; when it is low, the color approaches gray. Statistical analysis for the data were performed by using *t*-test or Duncan's new multiple range test.

Anthocyanidins separated from flowers and bathochromic shifts of their spots on TLC

One g of fresh flower pieces was boiled in 10 ml of 2N HCl for 60 min to hydrolyze anthocyanins. After filtration, aglycons were extracted into a small amount of isoamylalcohol from the cooled filtrates. Cellulose TLC plates (FUNACEL SF, 10 × 20 cm, Funakoshi, Tokyo) were used to separate the anthocyanidins. The solvent systems included Forestal, AHW-1 (acetic acid (HOAc):HCl:H₂O = 5: 1: 5, v/v) and iBAW (iso-BuOH:HOAc:H₂O = 8: 2: 3, v/v). Standard aglycons for co-chromatography were prepared from peels of the grape 'Kyoho', which have been shown to contain delphinidin, petunidin, cyanidin, peonidin and malvidin glucosides (Akuta and Matsutomi, 1976). Pelargonidin was obtained from *Pelargonium* petals (Saito, 1967). To identify the anthocyanidin in individual flowers, the R_f value and color of the spots separated by TLC, were compared with those of authentic anthocyanidins. The percentages of individual anthocyanidin spots to the total were calculated from the density of each spot at a

wavelength, $\lambda = 530$ nm by using a Dual-Wavelength Flying-Spot Scanner (CS-900, Shimadzu, Kyoto).

The λ max of red spots of anthocyanidins on chromatograms were also detected before and after spraying with 1 mM Mo as ammonium molybdate solution, using the above apparatus.

Bathochromic shifts of anthocyanin solutions and anthocyanin spots on TLC

Anthocyanins were extracted from *Eustoma grandiflorum* 'Exrosa Blue' petals with 1% HCl to investigate bathochromic shifts of anthocyanins by ammonium molybdate. The extract was filtered through filter paper (ADVANTEC 5C, Toyo Roshi, Tokyo) and the filtrate passed through a SEP-PAK C18 cartridge (Waters Associates, Milford, USA). After washing the column with distilled water several times, the pigments adsorbed in the column were eluted with 1% HCl-methanol. The eluted anthocyanin solution was concentrated by using a rotary evaporator at 35°C under reduced pressure.

After the addition of 2 ml of 10 mM Mo as ammonium molybdate solution or no addition, 2 ml of the concentrated anthocyanin solution was diluted with McIlvaine buffer solution (0.1 M citric acid+0.2 M Na₂HPO₄) to 20 ml. To examine the bathochromic shifts by molybdate at the maximum absorbance wavelength, spectra of the solutions at pH 3.22 and 5.03 respectively were recorded by using UV-visible recording spectrophotometer (UV-160A, Shimadzu, Kyoto).

A portion of the above concentrated anthocyanin solution was used for two-dimensional TLC, using cellulose plates (FUNACEL SF, 20 × 20 cm). Two-dimensional chromatography was conducted with iBAW as a solvent for the first dimension, and AHW-2 (HOAc:HCl:H₂O = 15:3:82, v/v) for the second dimension. The bathochromic shifts of anthocyanin spots on the chromatogram were detected in the same way as in the investigation of the molybdate shifts of the anthocyanidin spots.

Molybdenum treatment in hydroponics

Hydroponic experiments were carried out with the intent of developing a practice use in horticulture. Young plants of *Eustoma grandiflorum* 'Exrosa Blue Flash' were purchased from Sakata Seed (Yokohama). Six holes (3 cm diam.) were bored in the cover of white plastic containers (38.4 × 59.0 × 32.0 cm high). On May 24, 2002, young plants were transplanted by inserting their bases into cushioned holes and grown hydroponically in a greenhouse. A nutrient solution of 36 liter (total N: 25 ppm, P₀₄: 50 ppm, K: 25 ppm, Mg: 0.2 ppm, Mn: 0.005 ppm, B: 0.025 ppm) was poured into the containers, changed every 10 days with fresh solution and aerated.

The molybdenum treatment was carried out from July 16 to 26, 2002, just before flowering by supplementing ammonium molybdate to make the final concentration of

1 mM Mo. After treatment, 3 flowers were analyzed for the number of petals, diameter of the flowers and petal color.

Results

Effects of ammonium molybdate solution on flower color

Floral disks of some varieties changed colors by floatation in 1 mM Mo solution (Table 1) after 72 hr. Those of *E. grandiflorum* 'Lination Red' became significantly lighter; the hue angle decreased while the chroma faded compared with those of the distilled water treatment, i.e., the molybdate solution turned the purplish red petals of 'Lination Red' to dark blue. Likewise, the hue angle and chroma declined in the tepals of *T. gesneriana* 'Purple Flag', indicating that the tepal color shifted to blue while the brightness of the color faded. The hue angles of *E. grandiflorum* 'Tokinoakari', *Iris hollandica* 'Blue Magic' and *E. grandiflorum* 'Exrosa Blue' decreased to produce more bluish petals.

However, the ammonium molybdate solution had no bluing effect on *T. gesneriana* 'Ile de France', *Rosa spp.* 'Carienta', and *Dianthus caryophyllus* 'Cain' tissues. Thus, the ammonium molybdate solution significantly blued some of the anthocyanin-containing flowers, but not all.

Effects of molybdenum or aluminum concentration on flower color

The effects of molybdenum or aluminum concentrations from 0.1 to 10 mM on the hue angle of petal disks (Table 2) reveal that pH slightly affected floral disk color of three varieties. Ten mM of aluminum chloride solution reduced significantly the lightness of the 'Lination Red' petal with an increased chroma; aluminum did not cause these flowers to turn blue; the hue angle did not change at any concentration. Hue angle of 'Purple Flag' increased with increases in chroma by 10 mM aluminum as the tepal color became yellowish. The petal colors of 'Exrosa Blue' became

Table 1. Effects of ammonium molybdate solution (Mo: 1 mM) on the color of several tepals after 72 hr treatment.

Variety	Lightness		Hue angle		Chroma	
	H ₂ O ^z	Mo ^z	H ₂ O	Mo	H ₂ O	Mo
Lination Red (<i>Eustoma</i>)	50.4	38.7**	-6.9	-78.8**	36.9	22.0**
Tokinoakari (<i>Eustoma</i>)	62.8	54.8	-48.7	-101.3**	26.7	19.9
Purple Flag (<i>Tulipa</i>)	46.6	43.2	-15.9	-48.3**	33.0	26.2**
Blue Magic (<i>Iris</i>)	39.3	33.9	-75.6	-83.6**	37.9	36.7
Exrosa Blue (<i>Eustoma</i>)	59.6	42.0	-49.0	-56.1**	40.1	35.6
Ile de France (<i>Tulipa</i>)	39.4	36.7	30.4	29.7	48.8	39.4
Cain (<i>Dianthus</i>)	56.2	57.4	34.1	38.1	64.3	71.5
Carienta (<i>Rosa</i>)	35.4	37.0	27.5	33.1	39.9	15.3

^z pH of H₂O and Mo treatments were 5.48 and 4.37, respectively.

** Significantly different from that in H₂O treatment at 1% level (n = 10 disks).

Table 2. Effects of Al and Mo concentration on tepal color of *E. grandiflorum* 'Lination Red' (LR), *E. grandiflorum* 'Exrosa Blue' (EB) and *T. gesneriana* 'Purple Flag' (PF).

Treatment	Concentration of metal ion (mM)	pH	Lightness			Hue angle			Chroma		
			LR	EB	PF	LR	EB	PF	LR	EB	PF
H ₂ O	-	5.43	50.4a ^z	39.6a	46.6ab	-6.9a	-49.0ab	-15.9abc	36.9a	40.1a	33.0b
Acidic water ^y	-	3.77	50.4a	41.6a	47.1ab	-1.8a	-42.0a	-19.2bcd	37.7a	38.7a	32.2bc
AlCl ₃	0.1	4.57	50.7a	40.2a	44.6bc	-7.4a	-50.9a	-17.3bc	37.8a	38.7a	32.7bc
	0.5	4.31	51.6a	40.7a	46.1abc	-7.9a	-53.1bc	-21.6cd	36.7ab	37.1a	32.6bc
	1.0	4.19	51.8a	40.4a	46.2abc	-4.4a	-53.7bc	-18.3bc	34.7ab	36.1a	32.5bc
	5.0	3.91	48.6ab	40.1a	47.0ab	2.4a	-53.3bc	-10.6ab	28.5abc	35.5abc	34.9b
	10.0	3.84	38.8cd	40.2a	49.2a	12.7a	-51.4ab	-6.9a	20.6de	37.4a	39.1a
(NH ₄) ₆ Mo ₇ O ₂₄	0.1	4.39	50.8a	38.1a	45.2bc	-9.5ab	-49.3ab	-19.7bcd	37.1a	40.2a	32.2bc
	0.5	4.27	42.7bc	37.0a	43.2cd	-40.5c	-51.9bc	-28.0d	28.4bcd	35.6abc	29.5cd
	1.0	4.37	38.7cd	41.0a	41.8d	-78.8d	-56.1c	-40.3e	22.0cd	35.8ab	27.3d
	5.0	4.60	32.9de	38.1a	34.4e	-102.3d	-79.5d	-91.8f	14.7ef	28.3bc	27.5d
	10.0	4.71	30.2e	37.9a	32.6e	-93.2d	-100.2e	-105.3g	12.1f	28.1c	26.7d

^z Mean separation within columns by Duncan's new multiple range test, 1% level (n=10 disks).

^y Carried out to investigate the influence of pH of metal compound solutions on tepal color.

Table 3. Anthocyanidin compositions in flower of 8 varieties and bathochromic shifts of anthocyanidin spots by molybdenum on TLC.

Anthocyanidin ^z	Rf value			λ max of spot (nm)		Anthocyanidin composition (%)							
	iBAW ^y	Forestal ^y	AHW-1 ^y	-Mo ^x	+Mo ^x	Lination Red	Tokino-akari	Purple Flag	Exrosa Blue	Blue Magic	Ile de France	Cain	Carienta
Pelargonidin	0.62	0.73	0.60	533	533	26.3	8.3	–	–	–	55.1	100	7.9
Cyanidin	0.42	0.53	0.37	545	556	65.9	44.2	17.5	8.1	–	44.9	–	92.1
Delphinidin	0.26	0.31	0.22	554	560	7.8	47.5	82.5	91.9	100	–	–	–
Peonidin	0.50	0.70	0.51	544	544	–	–	–	–	–	–	–	–
Petunidin	0.34	0.49	0.32	553	560	–	–	–	–	–	–	–	–
Malvidin	0.44	0.66	0.47	553	554	–	–	–	–	–	–	–	–

^z Pelargonidin separated from pelargonium flowers and other anthocyanidins from 'Kyoho' grape peel.^y iBAW: iso-BuOH: HOAc: H₂O = 8: 2: 3, Forestal: HOAc: 36% HCl: H₂O = 30: 3: 10, AHW-1: HOAc: 36% HCl: H₂O = 5: 1: 5 (v/v).^x -Mo: without spraying ammonium molybdate, + Mo: spraying 1mM Mo as ammonium molybdate.

slightly bluish in any concentrations of aluminum.

The 'Lination Red' petals and 'Purple Flag' tepals became dark at and above 0.5 mM molybdenum, whereas the lightness of the 'Exrosa Blue' petals was not influenced by the treatment. Molybdenum at 0.5 mM or higher decreased the hue angles of the petals in 'Lination Red', whereas at 0.5 mM or higher, 'Exrosa Blue' petals and 'Purple Flag' tepals required 1 mM or more. High concentrations of Mo reduced the chroma of those three varieties. For example, the petal color of 'Lination Red' became dark blue with 0.5 mM of molybdenum, resulting from decreased lightness, hue angle, and chroma. In 'Exrosa Blue', the petal color became blue and softened at a high concentration of molybdenum treatment, in which the hue angle and chroma declined, although the lightness was not decreased, even at 10 mM. 'Purple Flag' became dark blue at 1 mM molybdenum.

Anthocyanidin composition in flowers and molybdate shifts of anthocyanidin spots.

Six TLC spots of anthocyanidins contained in the grape peel and pelargonium flowers, separated by three kinds of solvents showed a peculiar Rf value. Also, the λ maxs of the anthocyanidins were distributed from 533 nm of pelargonidin to 554 nm of delphinidin (Table 3). Each λ max of cyanidin, petunidin and delphinidin shifted 11, 7 and 6 nm, respectively, to a longer wavelength by spraying with ammonium molybdate solution. Delphinidin and petunidin spots on the chromatogram were the most bluish, but the λ maxs of the pelargonidin, peonidin and malvidin spots did not change. The background color of the plate didn't change with this treatment.

The anthocyanidin compositions of the fresh flowers and the molybdate shifts of the anthocyanidin spots (Table 3) reveal that in *E. grandiflorum* 'Tokinoakari' and 'Lination Red', the petal hue angles were changed significantly by ammonium molybdate, contained pelar-

gonidin, cyanidin and delphinidin. *T. gesneriana* 'Purple Flag' tepals and *E. grandiflorum* 'Exrosa Blue' petals, which became blue, contained cyanidin and delphinidin. *Iris hollandica* 'Blue Magic' tepals, which showed a slight bluing, had only delphinidin. *T. gesneriana* 'Ile de France' tepals, *Rosa spp.* 'Carienta' petals and *Dianthus caryophyllus* 'Cain' petals that were not affected by the ammonium molybdate solution contained only pelargonidin or both pelargonidin and cyanidin.

Bathochromic shifts of anthocyanin spots on chromatogram and absorption spectrum of anthocyanin solution from *Eustoma grandiflorum* 'Exrosa Blue'

Three spots that were detected on the chromatogram as main anthocyanins from *E. grandiflorum* 'Exrosa Blue' (Table 4) had λ maxs of 555, 555 and 554 nm, which changed by the ammonium molybdate treatment. The degrees of the shifts of those spots were the same as the shifts of delphinidin or petunidin in Table 3. The background color of the plate was not changed. Those were assumed to be anthocyanins binding delphinidin, because petunidin was not present in 'Exrosa Blue' (Table 2).

The effects of molybdenum on the spectra of the extracts from 'Exrosa Blue' (Fig.1) show that below pH 3.22, the λ max of the anthocyanin solution in the presence of Mo ion was 564.5 nm, a shift of 30 nm to a longer wavelength from that of the control. At pH 5.03, it was 589 nm, or a shift of 50 nm to a longer wavelength from that of the control solution. The Mo ion also enhanced the absorbance at the λ max at each pH. The λ max shifts of the anthocyanin solution were larger than those of the anthocyanin spots in Table 4.

Effect of molybdenum on flowers of *Eustoma grandiflorum* 'Exrosa Blue Flash' in hydroponics

The artificial bluing of flowers was conducted for *E.*

grandiflorum 'Exrosa Blue Flash' plants to investigate if this technique is adaptable for commercial production of blue flowers. From the results of the above experiments, the optimum concentration of molybdenum was 1 mM. The molybdenum treatment was conducted just before anthesis. A 10 day treatment produced blue flowers with a steep hue angle shift (Table 5). The color of the petals discolored became deeper blue 10 days after the molybdenum was added. The Mo treatment neither changed the diameters of the flowers nor the number of petals; it

Table 4. Bathochromic shifts of anthocyanin spots of *Eustoma grandiflorum* 'Exrosa Blue' by molybdenum on TLC.

Anthocyanin spot	Rf value		λ max of spot (nm)	
	iBAWz	AHW-2 ^z	-Mo ^y	+Mo ^y
1	0.16	0.50	555	560
2	0.17	0.60	555	560
3	0.26	0.68	554	560

^z iBAW: iso-BuOH:HOAc:H₂O=8:2:3, AHW-2: HOAc: 36% HCl:H₂O=15:3:82 (v/v).

^y -Mo: without spraying ammonium molybdate, +Mo: spraying 1mM Mo as ammonium molybdate.

Table 5. Effects of molybdenum on flower color of *Eustoma grandiflorum* 'Exrosa Blue Flash' in hydroponics (Growing period: May 24 - July 26, 2002).

Treatment	Hue angle of petals	Number of petals / flower	Diameter of flowers (cm)
Control	-49.6	20	6.5
Mo ^y	-70.8	19	6.5

^y Ammonium molybdate was supplemented to solution (Mo: 1 mM) at July 16, just before flowering.

did not damage any flowers.

Discussion

Ammonium molybdate solutions turned some of the anthocyanin-containing flowers significantly blue, but not all varieties, indicating a presence of certain factors that shift flower color in the direction of blue.

Commelinin is an example of a metal ion participating in the blue color development of flower petals. It is one of the natural metallo-anthocyanins of delphinidin binding Mg²⁺ and flavone in its structure. The noticeable shifts in λ max by molybdenum indicate that other possibilities exist for the bluing mechanism of flowers which differ from that of commelinin. Moreover, aluminum ion had little bluing effect on floral disks at 10 mM or less. In contrast, floral disks floated on the solution with a high concentration of aluminum chloride turned slightly yellowish, because the hue angle increased in *E. grandiflorum* 'Lination red' or *T. gesneriana* 'Purple Flag'. It was deduced that the bluing phenomenon of these flowers was different from the bluing of hydrangea reported by Allen (1932, 1943) and Chenery (1937).

Maekawa et al. (1983) reported that cut flowers of *Platycodon grandiflorum* 'Samidare', in which bluing was caused by molybdenum, contained delphinidin glycoside. In our experiment, flowers containing delphinidin were also turned blue by molybdenum. Thus, it is plausible that delphinidin plays a specific role with Mo in making the color of flower petals to turn blue.

A strong acid solution of saturated ammonium molybdate brings about a bathochromic shift on TLC with spots of anthocyanins having adjoining hydroxyls in their B-ring (Ohta et al., 1979). In our experiment, bathochromic shifts with 1 mM of molybdenum were detected in the cyanidin, delphinidin and petunidin spots

Fig. 1. Changes in absorption spectra of the extract from tepals of *Eustoma grandiflorum* 'Exrosa Blue' as affected by adding 1 mM Mo as ammonium molybdate under pH 3.22 or 5.03.

on chromatogram. Bathochromic shifts, caused by B-ring structures of anthocyanidin, seemed to be a contributing factor to the bluing phenomenon by molybdenum in fresh flower tissues. The flowers used in our experiment did not have petunidin. A petunidin spot on the TLC showed the same strong bathochromic shift as delphinidin by ammonium molybdate, suggesting that flowers which contain petunidin glycoside may also react with molybdenum.

However, if the bluing phenomenon with molybdenum in flowers occurred in only the B-ring structures of anthocyanidin, tissues containing delphinidin or cyanidin showed similar tendencies. However, not all flowers containing cyanidin change their color by this treatment, especially *Rosa spp.* 'Carienta' having more than 90% of cyanidin in petals remained unchanged. Although the bathochromic shift of the cyanidin spots on TLC by ammonium molybdate was the largest, its shift of λ max was to a shorter wavelength than that of delphinidin or petunidin. A low concentration of molybdenum in weak acidity solution in our experiment had only small visual effect on cyanidin. Cyanidin may have the potentialities to participate in the bluing phenomenon, but it has no strong bluing effect independently.

Furthermore, the degree of decrease in hue angles of the floral disks was not proportional to the percentage amount of delphinidin in the flower tissues. *E. grandiflorum* 'Lination Red' petals, which changed to deep blue by molybdenum, delphinidin made up only 7.8% of the total anthocyanidins. Delphinidin is one of the important component necessary for the bluing of flowers by molybdenum, but other factors must be further investigated.

In *E. grandiflorum* 'Exrosa Blue', the bathochromic shifts of petals extracts by molybdenum were strong compared with those of the anthocyanin spots on the chromatogram. This might be ascribed to the presence of chemical compounds like flavonoids in extracts which act on enhancing bathochromic shifts as co-pigments of anthocyanin. In *Hydrangea macrophylla*, 3-*p*-coumaroylquinic acid and 3-caffeoylquinic acid were reported as co-pigments for delphinidin glycoside (Takeda et al., 1985a, b, 1990). Thus, some of complex organic acids may be involved in the bathochromic shift by molybdenum.

The lowest level of molybdenum providing a significant bluing seemed to be in the range between 0.5 mM and 1 mM in the absorption from the cut end of floral disks of several varieties. This level was linked to the bluing phenomenon of *E. grandiflorum* 'Exrosa Blue Flash' under the hydroponics supplemented with 1 mM of molybdenum through the roots.

Maekawa et al. (1983) reported that 2.5–5 mM sodium molybdate solution changes the petal color of the cut flowers of *Platycodon grandiflorum* 'Samidare' from blue-violet to blue, but that 10 mM was toxic.

Thus, color change of cut flowers by Mo treatment is rather difficult in practice. Stems of seedling of *Brassica spp.* turned from red to blue on agar medium containing ammonium molybdate, but the growth of treated roots in molybdenum (60 mg liter⁻¹) was inhibited (Hale et al., 2001). In our trial, a satisfactory result was obtained with 1 mM of molybdenum given 10 days before flowering.

The application of molybdenum compounds in sufficient amounts to induce bluing phenomenon to soils may cause soil pollution if the plants are not able to absorb the entire compounds. In hydroponic culture is used, molybdenum can be eliminated using a technology for waste water treatment before draining, in consideration of the environment.

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養液栽培条件下でのモリブデンによるトルコギキョウの花色の青色化

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摘 要

アントシアニンを含む数種の花き花卉に関して、モリブデンによる花色の青色化を調査した。花卉切片をモリブデン酸アンモニウム水溶液に浸したところ、チューリップ‘パープルフラッグ’とトルコギキョウ‘季の明’、‘リネーションレッド’で花色が青色に変化した。トルコギキョウ‘エクローサブルー’とダッチアイリス‘ブルマジック’ではわずかに青色化した。チューリップ‘イルドフランス’、バラ‘カリエンタ’、カーネーション‘カイン’では変化しなかった。モリブデンとアルミニウムの濃度が花色の青色化に与える影響を調査するために、モリブデン酸アンモニウムと塩化アルミニウムの水溶液に3種の花き‘リネーションレッド’、‘エクローサブルー’、‘パープルフラッグ’を浸したところ、

青色化が生じる最低のモリブデン濃度は‘リネーションレッド’で0.5 mM、‘エクローサブルー’、‘パープルフラッグ’では1 mMであった。アルミニウムの青色化効果はほとんどなかった。モリブデンで花色が青色化した5品種はアントシアニンとしてデルフィニジンを含んでいた。シアニンとペラルゴニン、あるいはペラルゴニンのみを含む品種はモリブデンの影響を受けなかった。モリブデンによる深色作用はデルフィニジンを含む花卉からの抽出液及び薄層クロマトグラム上のスポットでも観察された。また、トルコギキョウ‘エクローサブルーフラッシュ’の養液栽培において、開花直前に養液にモリブデンを1 mM添加したところ、花色が青色化した。