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Effects of Medium Constituents on the In Vitro Flowering Response in *Celosia argentea* L.

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Abstract

The effects of medium constituents on the growth and flowering in *Celosia argentea* L. were investigated. At first, the influence of the difference in medium strength was examined. The flowering rate was almost 100 % from 0 MS to 1/8 MS, but no flowering was observed at concentrations higher than 1/4 MS. As the medium strength was lowered, the number of true leaves formed until flowering decreased gradually, and on the 0 MS medium, inflorescence was formed without the formation of true leaf after cotyledons unfolding. Inflorescence formation without true leaf formation was observed only when macro-nutrients in the MS medium constituents were not added. Furthermore, it became clear that inflorescences formed without true leaf formation occurred only when NH_4NO_3 and KNO_3 in the macro-nutrients were not added. All of 16 cultivars tested formed inflorescence without true leaf formation on the 0 MS medium. When the time of transplantation from 0 MS medium to 1/30 MS medium was changed, we confirmed the NH_4^+ and NO_3^--free period required for the phenomenon of inflorescence formation without true leaf formation was at least 3 weeks

INTRODUCTION

Tissue culture techniques have been used to precisely analyze the effects of environment such as light and temperature and/or the effects of chemical substances such as plant growth regulators on the growth and flowering in plants. Many studies using tissue culture techniques reporting the in vitro flowering response of plants and proposed several in vitro flowering systems (Van Staden and Dickens, 1991). For example, tissue culture

systems using stem thin cell layer (TCL) explant of *Nicotiana tabacum* L. (Tran Than Van, 1973, 1999), internodal stem explant of *Torenia fournieri* Linden ex E. Fourn. (Tanimoto and Harada, 1981a, 1981b; Tanimoto et al., 1985) and shoot apex explant of *Chrysanthemum morifolium* Ramat., *Ipomoea nil* (L.) Roth (Harada, 1967; Ishioka et al., 1990) and *Kalanchoe blossfediana* Poelln. (Dickens, 1987; Yang et al., 1999), have revealed the influences of medium constituents, culture environment conditions and physiological conditions of mother plants for promoting in vitro flowering (Van Staden and Dickens, 1991). We have accidentally discovered that *Celosia argentea* L. easily flowered in vitro through the series of experiments which the growing of sterile plants in many plant species (Yamada et al., 1997). In this report, we have examined in detail the effects of medium constituents on the in vitro flowering in *Celosia argentea*.

MATERIALS AND METHODS

The following experiments were performed using commercial cultivar seeds of *Celosia argentea.* The seeds were immersed in 70% ethanol and 3% sodium hypochlorite solution containing 0.01% Tween 20 for 30 seconds and for 10 minutes respectively, and then washed three times with sterilized pure water. The culture vessel was a glass flat-bottom test tube (40 × 150 mm). Murashige and Skoog (1962) medium (MS) added 30 gL^{-1} sucrose and 8 gL^{-1} agar and the pH was adjusted to 5.8 as a basal medium. Each of test tube dispensed 30 mL of respective media. The test tubes were closed with aluminum foil, and autoclaved (120°C, 15 minutes) before use.

After sowing 3 seeds in each test tube, placed them in the dark at 24°C for the first 3 days for dark germination. From the 4th day, they were illuminated with white fluorescent lamps (FL40SS-N/37, Toshiba Lighting & Technology Co., Ltd., Yokosuka city, Kanagawa prefecture) for 16 hours (30 µmol m^{-2} s^{-1} PPFD) / 8 hours in the dark at 24°C for 12 weeks after sowing. Two weeks after sowing, thinning was performed to obtain one seedling in a test tube, and 10 seedlings were used in each treatment plot.

Effects of medium constituents on flowering reaction

Seeds of *Celosia argentea* 'Castle Yellow' were used. At first, effect of medium strength on flowering was examined. All constituents of MS were diluted to 0 (means no addition), 1/1000, 1/500, 1/100, 1/50, 1/15, 1/8, 1/4, 1/2 and 1/1 concentrations. Secondly, using 1/15 MS, we divided the MS constituents into 3 groups, macro-nutrients, micro-nutrients and organic components such as vitamins, and set up 8 treatment plots combined with and without them to examine the effect on flowering (Table 1).

Thirdly, using 1/15 MS, 8 treatment plots combined with or without the addition of N (NH_4NO_3, KNO_3), P (KH_2PO_4) and K (KNO_3, KH_2PO_4) in the macro-nutrients were set up, and the effect of each macro-nutrient on flowering was examined (Table 2). When N and P were not added, the amounts of potassium equivalent to KNO_3 and KH_2PO_4 were supplemented with KCl. When K was not added, the amounts of nitrogen and phosphoric acid equivalent to KNO_3 and KH_2PO_4 were supplemented with NH_4NO_3 and $NaH_2PO_4 \cdot 2H_2O$, respectively.

Cultivar differences in flowering response on 0 MS medium

Since there are many strains and cultivars in *Celosia argentea* (The Royal Horticultural Society, 1992), 16 cultivars were tested for the flowering response on the 0 MS medium. The strains used were 10 cultivars of Plumosa group, 5 cultivars of Kurume group and one cultivar of Cristata group (Table 3).

Table 1. Effects of MS medium strength on growth and flowering in *Celosia argentea* 'Castle Yellow'.

1/15 MS medium			Flowering	No. of	Days to	Plant height
Macro-nutrients*	Micro-nutrients*	Vitamins*	(%)	true leaves	flowering	(mm)
−	−	−	88.8	0c	49.0	7.2d
−	+	−	100	0c	45.0	7.5d
−	−	+	100	0c	35.8	6.9d
−	+	+	100	0c	55.3	7.0d
+	−	−	100	10.8a	46.7	42.1b
+	+	−	100	9.8b	42.9	44.1b
+	−	+	100	10.4a	52.9	55.8a
+	+	+	100	9.0b	56.0	37.5c

* Macro-elements: NH_4NO_3, KNO_3, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, KH_2PO_4, Micro-elements: Fe-Na-EDTA, H_3BO_3, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, KI, $NaMoO4 \cdot 2H_2O$, $CuSO4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, Vitamins: myo-inositol, glycine, pyridoxine-HCl, thiamine-HCl, nicotinic acid

Table 2. Effects of N, P, K addition or absence on the growth and flowering in *Celosia argentea* 'Castle Yellow'.

1/15 MS medium			Flowering	No. of	Days to	Plant height
N*	P*	K*	(%)	true leaves	flowering	(mm)
−	−	−	100	0d	49.7	6.8d
−	+	−	87.5	0d	52.0	6.9d
−	−	+	100	0d	52.1	6.7d
−	+	+	88.8	0d	49.9	6.9d
+	−	−	33.3	2.0c	46.7	10.0b
+	+	−	12.5	3.0c	35.0	8.8c
+	−	+	100	4.3b	47.8	37.5a
+	+	+	100	6.1a	49.0	41.4a

* N: NH_4NO_3, KNO_3, P: KH_2PO_4 , K: KNO_3, KH_2PO_4. When N and P were not added, the amounts of potassium equivalent to KNO_3 and KH_2PO_4 were supplemented with KCl. When K was not added, the amounts of nitrogen and phosphoric acid equivalent to KNO_3 and KH_2PO_4 were supplemented with NH_4NO_3 and $NaH_2PO_4 \cdot 2H_2O$, respectively.

Effect of N application time on flowering reaction

In order to reveal the relationship between the N (NH_4^+ and NO_3^-) containing period in the medium and the phenomenon of flowering without the formation of true leaf, the following experiment was performed. Seeds of *Celosia argentea* 'Castle Yellow' were used. The seeds were sowed in 0 MS medium, and then transplanted to 1/30 MS medium at 1, 2 and 3 weeks after sowing. In addition, a plot without transplantation (all culture period used 0 MS) and another plot which was sowed to 1/30 MS from the beginning were set up (Table 4).

Table 3. Cultivar differences in the growth and flowering responses of *Celosia argentea* on the 0 MS medium.

Cultivar name (Strain)	Plant form	Earliness	Flowering (%)	No. of true leaves	Days to flowering	Plant height (mm)
Kimono Red(P)	Very dwarf	Very early	100	0	32.2d	9.0b
Kimono Yellow(P)	Very dwarf	Very early	100	0	36.4d	12.0b
Scarlet Charm(P)	Very dwarf	Early	100	0	43.4cd	13.0b
Castle Scalet (P)	Very dwarf	Early	66	0	49.0c	13.3b
Castle Pink (P)	Very dwarf	Early	100	0	46.2c	12.5b
Castle Yellow (P)	Very dwarf	Early	100	0	42.0c	11.9b
Red Kewpie (P)	Dwarf	Early	100	0	43.4cd	20.5a
Yellow Kewpie (P)	Dwarf	Early	80	0	49.0c	18.0a
Century Red (P)	Medium tall	Early	100	0	60.2ab	8.0b
Century Yellow (P)	Medium tall	Early	100	0	64.8a	6.9c
Fire Glow (K)	Medium tall	Very early	100	0	52.5b	10.0b
Golden Glow (K)	Tall	Very early	60	0	60.7ab	8.0b
Early Rose (K)	Tall	Early	40	0	42.0cd	8.5b
Kurume Kagayaki (K)	Tall	Early	100	0	40.6cd	8.5b
Kurume Gold (K)	Tall	Late	80	0	71.8a	9.0b
Jewel Box (C)	Very dwarf	Very early	100	0	30.8d	8.0b

Table 4. Effects of nitrogen supply period on the growth and flowering in *Celosia argentea* 'Castle Yellow'.

Media		Transplant timing	Flowering (%)	No. of true leaves	Days to flowering	Plant height (mm)
First	Second					
0 MS		No	100	0c	56.0	9.0c
0 MS	→ 1/30 MS	After 1 week	100	6.0a	49.3	36.0a
0 MS	→ 1/30 MS	After 2 weeks	100	2.1b	49.0	17.4b
0 MS	→1/30 MS	After 3 weeks	100	0c	48.5	10.4c
1/30 MS		No	90	4.8a	45.4	33.8a

RESULTS AND DISCUSSION

The flowering rates were almost 100% from 0 MS to 1/8 MS, but no flowering was seen at concentrations higher than 1/4 MS (Fig. 1). As the medium strength was lowered, the number of true leaves formed until flowering decreased linearly (Fig. 1), and on the 0 MS medium, inflorescence was formed without the formation of true leaf after cotyledons unfolding (Fig. 2). Inflorescence formation without true leaf formation was observed only when macro-nutrients in the MS medium constituents were not added (Table 1 and Fig. 3). Furthermore, it became clear that inflorescences formed without true leaf formation occurred only when N (NH_4NO_3 and KNO_3) in the macro-nutrients was not added (Table 2 and Fig. 4).

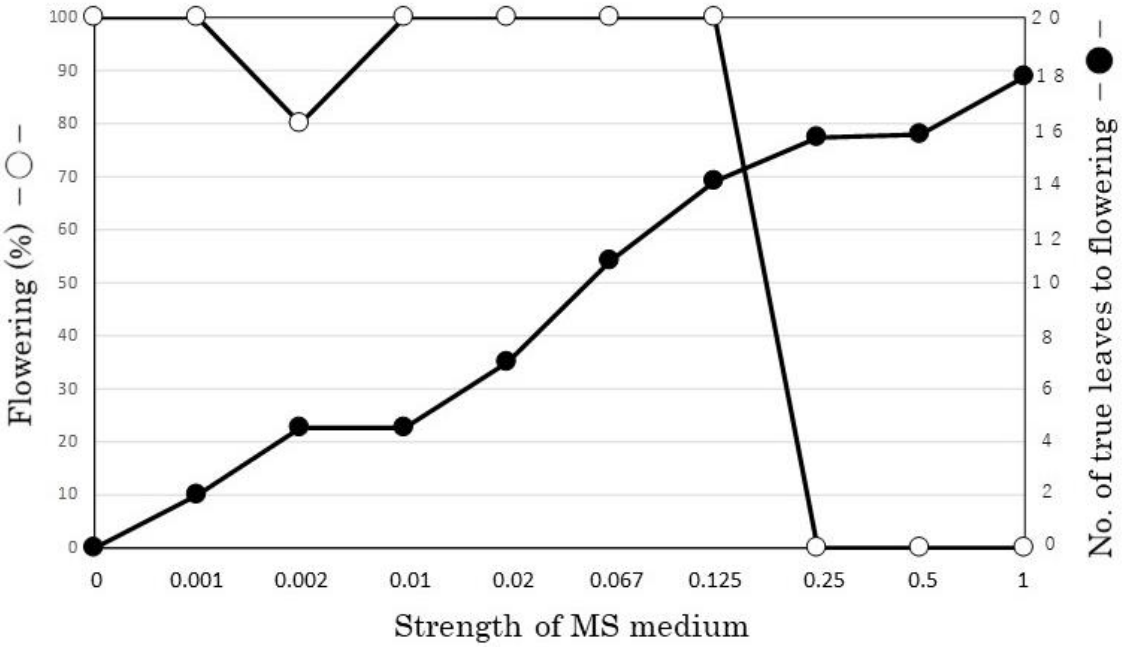

Figure 1. Effects of MS medium strength on the in vitro flowering rate and number of true leaves to flowering in *Celosia argentea* 'Castle Yellow'. All seedlings were cultured for 12 weeks.

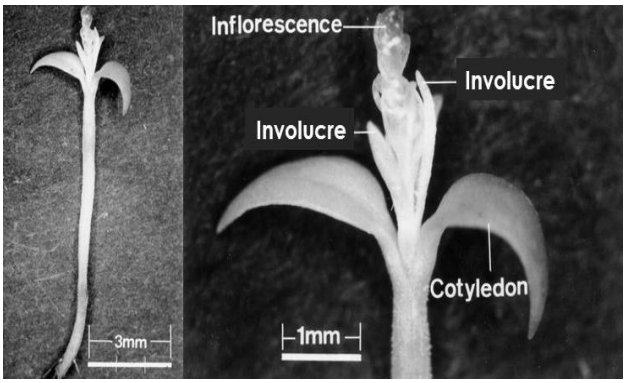

Figure 2. Photographs of seedlings of *Celosia argentea* 'Castle Yellow' cultured in vitro on the 0 MS (only added 30 gL^{-1} sucrose and 8 gL^{-1} agar) medium for 12 weeks. Left: Whole seedling. Right: Enlarged view around the inflorescence.

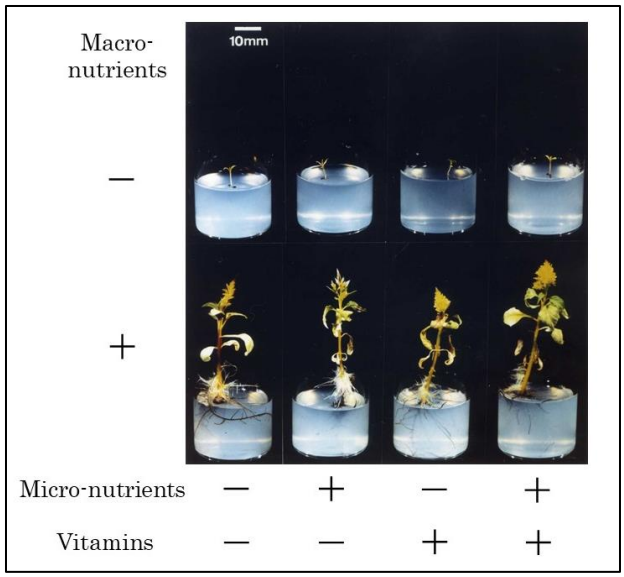

Figure 3. Effects of deletion of macro-nutrients, micro-nutrients and/or vitamins from 1/15 MS medium on the growth and flowering in *Celosia argentea* 'Castle Yellow' (12 weeks after sowing).

When K (KNO_3 and KH_2PO_4) was not added, the number of true leaves decreased to 2.0 - 3.0, but at the same time the flowering rate was significantly reduced, that is, potassium deficiency markedly inhibited the both of vegetative and reproductive growth of *Celosia argentea* (Table 2 and Fig. 4).

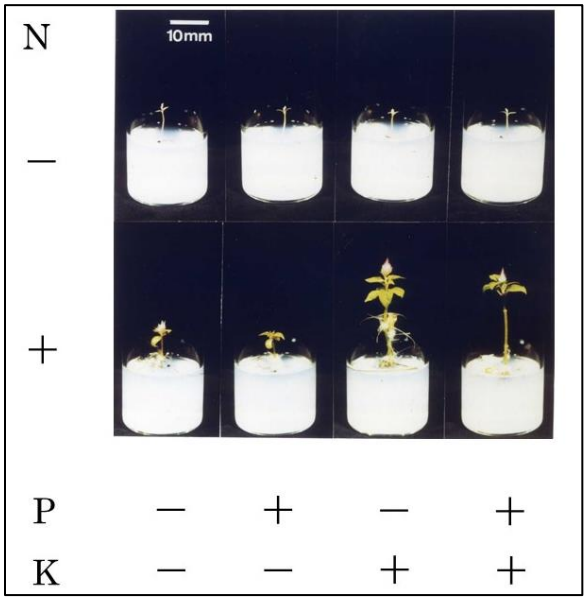

Figure 4. Effects of deletion of N (NH_4NO_3, KNO_3), P (KH_2PO_4) and/or K (KNO_3, KH_2PO_4) in the macro-nutrients from 1/15 MS medium on the growth and flowering in *Celosia argentea* 'Castle Yellow' (12 weeks after sowing).

When N and P were not added, the amounts of potassium equivalent to KNO_3 and KH_2PO_4 were supplemented with KCl. When K was not added, the amounts of nitrogen and phosphoric acid equivalent to KNO_3 and KH_2PO_4 were supplemented with NH_4NO_3 and $NaH_2PO_4 \cdot 2H_2O$, respectively.

From the above results, it has been clarified that the phenomenon of inflorescence formation without true leaf formation was caused by the no addition of inorganic nitrogen salts (N) to the medium. As a result of investigating the differences between cultivars in the phenomenon, all of 16 cultivars tested formed inflorescence without true leaf formation on the 0 MS medium (Table 3). The number of days from sowing until the visible inflorescence (Days to flowering) varied greatly between cultivars (from 30.8 to 71.8 days), but about 30 days for 'Kimono Red', 'Kimono Yellow' and 'Jewel Box' (Table 3). It has long been known as the Carbon to Nitrogen ratio (C/N) theory that the flowering response is promoted by reducing the inorganic nitrogen salts application rate (Corbesier et al., 2002). Past studies on the in vitro flowering using *Torenia fournieri* (Tanimoto and Harada, 1981a) and *Ipomoea nil* (Ishioka et al., 1991; Wada and Shinozaki, 1985) also reported that in vitro flowering is promoted by lowering the NH_4^+ and NO_3^- concentration and increasing the sugar concentration in media. However, *Celosia argentea* is unique compared to other plants reported in the past that it reacts very sensitively to the NH_4^+ and NO_3^- concentration in the medium and does not form true leaves without the addition of NH_4^+ and NO_3^- .

When the time of transplantation from 0 MS medium to 1/30 MS medium was changed, the NH_4^+ and NO_3^--free period required for the phenomenon of inflorescence formation without true leaf formation was examined. The NH_4^+ and NO_3^--free period of at least 3 weeks was required (Table 4). Therefore, it is considered that *Celosia argentea* was physiologically converted to reproductive growth phase without vegetative growth phase within 3 weeks after sowing on the NH_4^+ and NO_3^--free medium. If 'Kimono Red', 'Kimono Yellow' and 'Jewel Box' which are have about 30 days to flowering would be used, there is a possibility that the nitrogen-ions content in soil, media and freshwater such as rivers, ponds, lakes, etc. can be evaluated by the number of formed true leaves. In other words, there is a possibility that *Celosia argentea* can be used as an indicator plant for

evaluating eutrophication. In addition, since the number of leaves can be controlled by the amount of nitrogen applied, the plant size also can be regulated. In fact, an item named "Celosia candle cake" of pot-plants that have been cultivated like a colorful decoration cake have been commercialized by seeding of dozens mixed flower- color seeds on potting media with reduced fertilizer and applying growth retardant in Japan.

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