Influence of Sugar, Ammonium Nitrate and Plant Growth Regulators on *in vitro* Flowering of *Celosia argentea* var. *cristata*

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Abstract: *In vitro* flowering is considered to be a complex process regulated by a vast of environmental and genetic factors. The present study describes the influence of sugar, ammonium nitrate and plant growth regulators on *in vitro* flowering from nodal explants of *Celosia argentea* var. *cristata* cultured for 6 weeks on basal MS medium under aseptic and light conditions. The addition to MS medium different concentrations of sucrose or ammonium nitrate did not influence on morphogenesis of *Celosia* plants. Among the plant growth regulators tested, supplementation with 1.0 mg/l KIN induced maximum number of new shoots (2.52). Flowering occurred on 100% of *in vitro* shoots cultured on modify MS medium supplemented with 16.50 and 8.25 g/l of NH_4NO_3 after 40 days.

Keywords: Ammonium nitrate, inflorescence, morphogenesis, plant growth regulators, sucrose.

INTRODUCTION

Celosia argenta var. *cristata* belongs to the Amaranthaceae family. It is considered to be the herbaceous plant and now becoming more important as ornamental plant. Characteristic pigmentation of flowers makes the plant attractive for planting along the roadsides [1] Attractive *Celosia argenta* flowers similar in shape to a plume contain anthocyanin, a pigment usually responsible for pink, red, purple, violet and blue color in flowering plants. The pigment extracted from flowers of this plant species changes the petal color depending on hydrogen ions concentration in a solution. Therefore, they may be utilized as acid-based indicators [2]. Additionally, *Celosia argentea* plants are source of a spectrum of valuable chemicals such as antiviral proteins or betalains, that can be applied in many beneficial ways, i.e. in Chinese medicine for haemotological and gynaecologic disorders [3, 4]. Due to high concentrations of calcium, phosphorus, potassium, sodium, magnesium, iron, zinc, and copper, *Celosia argentea* leaves are suitable for consumption as vegetables especially in many countries [5].

Because of the rich values and high popularity of *Celosia argentea*, the *in vitro* technique was applied in order to achieve an efficient mass propagation and flower formation of this species. *In vitro* flowering techniques, for example for breeding valuable plants under controlled, high-healthy conditions, is useful method for understanding the physiology of flower induction. Further knowledge on *in vitro* flowered plantlets for the formation of fruit and seeds is highly valuable [6]. Plant's ability to flowering in *in vitro* cultures is affected by numerous factors, both genetic and environmental. The most important factors include: the age of the donor plant, position on the plant from which the explant is excised [7], medium pH [8], and even the concentrations of sugars in the medium [9]. However, addition of exogenous growth regulators is the most important factor exerting the influence on high efficiency of plant's flowering [7]. The timing of the treatment and the concentration of the applied growth regulators are very important factors, depends on plants species. Thus, the selection of type and concentration of a growth regulator should be made individually for a given plant species under study.

The aim of the study was to evaluate the influence of sucrose and ammonium nitrate (NH_4NO_3) concentrations, as well as plant growth regulators (BAP, KIN and 2iP) in MS medium on *in vitro* propagation and flowering of *Celosia argentea* var. *cristata*.

MATERIAL AND METHODS

The research material consisted of 15–20 mm one-node shoots fragments of *Celosia argentea* var. *cristata* (6 weeks-old) obtained from sterile stabilized *in vitro* culture. Explants were placed on modify MS medium [10] supplemented with 20–60 g/l sucrose without plant growth regulators, on modify MS media with 16.50, 8.25 and 4.12 g/l amonium nitrate (NH_4NO_3) and full-strength of MS media supplemented with 1.0 and 2.0 mg/l BAP (6-benzyloaminopurine), KIN (kinetin) and 2iP (2-isopentenyladenine) with normal concentration of sugar (30 g/l of sucrose). Plants placed on MS medium without addition of growth regulators was the

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control group. Each combination included 96 shoots (6 shoots per flask) in sixteen series.

All the media were supplemented with 8 g/l agar (Biocorp) and 100 mg/l myo-inositol and heated cause agar polymerization; 30 ml of media were poured into 300 ml jars and next autoclaved at 121^0C (0.1 MPa) during the time required according to the volume of medium in the vessel. The pH of the media was adjusted to 5.7 before autoclaving. Cultures were incubated in growth room at a temperature of 25^0C under 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

After 40 days of cultures, the mean shoot length (cm), number of shoot per 1 explant, length (cm) and number of root, fresh weight (g) and number of formed flower were determined. The significance of differences was determined by means of variance analysis and Tukey's test, at the level of significance of $\alpha = 0.05$. Homogenous groups between analysed combinations were labeled with successive letters of alphabet.

RESULTS AND DISCUSSION

Sucrose and NH_4NO_3 concentrations in MS medium had no influence on *Celosia argentea* propagation in *in vitro* cultures (Table **1**). The growth regulators (BAP, KIN and 2iP) used in the tests have shown an inhibitive effect on shoot and root length of *Celosia argentea* propagated *in vitro* (Figure **1**). The exception were plants propagated on MS medium supplemented with 1.0 mg/l KIN, the average shoot length of which was 2.52cm (Table **1)**. Furthermore, they produced the longest roots (4.10cm) as compared to plants in other medium combinations. The *Celosia argentea* plants from MS medium with addition of 1.0 mg/l KIN were well-branched as compared to the control and characterized by the highest fresh weight (2.52g). Bakar *et al*. [4] obtained the highest efficiency of *Celosia argentea* propagation on MS medium supplemented with 0.5 mg/l NAA (1-naphthaleneacetic acid) and 1.0 mg/l BAP. While Daud *et al*. [11] suggested that ½MS medium in plant media culture is more response to *Celosia sp*. shoot regeneration. The medium combinations applied in this experiment did not significantly affect the root number, although the largest average number of roots was recorded for *Celosia argentea* grown on the control MS medium and MS with addition of 1.0 mg/l KIN (1.31 and 1.19, respectively) (Table **1**). In studies upon regeneration and acclimatization of selected ornamental plants (*Agapanthus praecox, Jucticia betonica* and *Celosia cristata)* Yaacob *et al*. [12] suggested that combinations of BAP and NAA can result in direct regeneration and rhizogenesis of *Celosia* plantlets.

Many studies have reported the effects of sucrose concentration on induction flowering in *in vitro* culture [13, 9, 14]. According to Vu *et al*. [14] sugars are considered a necessary carbon source in *in vitro* cultures. The addition of optimal carbohydrate source

Table 1: Shoot Production of *Celosia argentea* var. *cristata* in Response to Different Concentrations of Sucrose and NH_4NO_3, and Plant Growth Regulators (BAP, KIN, 2iP)

Medium	Concentration	Plant height (cm)		No of new shoot		Root length (cm)		No of roots		Fresh weight of plantlets (g)	
Control - MS medium		3.97	ab	3.06	ab	0.78	b	1.31	a	2.01	ab
Sucrose	2%	3.87	abc	3.81	ab	0.34	b	0.50	abc	2.06	ab
	4%	3.81	abc	4.75	a	0.53	b	0.63	abc	2.50	a
	5%	3.43	abc	3.31	ab	0.68	b	0.38	abc	2.38	a
	6%	3.50	abc	3.38	ab	0.84	b	0.56	abc	2.16	ab
NH_4NO_3	16.50 g/l	3.75	abc	2.44	b	0.81	b	0.50	abc	1.71	ab
	8.25 g/l	4.06	a	2.69	b	0.56	b	0.31	abc	1.45	ab
	4.12 g/l	2.84	abcd	2.13	b	0.06	b	0.06	bc	0.85	b
BAP	1.0 mg/l	1.79	d	3.00	ab	0	b	0	c	1.79	ab
	2.0 mg/l	1.66	d	3.63	ab	0	b	0	c	1.66	ab
KIN	1.0 mg/l	2.52	c	3.69	ab	4.10	a	1.19	ab	2.52	a
	2.0 mg/l	1.66	d	3.00	ab	0.46	b	0.69	abc	1.66	ab
2iP	1.0 mg/l	1.49	d	2.75	b	0.42	b	0.38	abc	1.50	ab
	2.0 mg/l	1.78	d	3.38	ab	0.06	b	0.13	bc	1.78	ab
$LSD_{0.05}$		1.39		1.76		2.38		1.13		1.34	

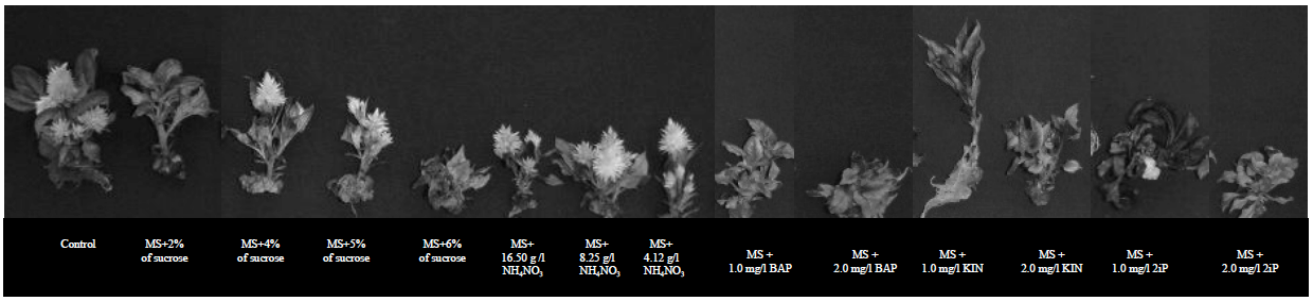

Figure 1: The influence of different concentrations of sucrose and NH_4NO_3, and plant growth regulators (BAP, KIN and 2iP) on flowering of *Celosia* plant propagated *in vitro*.

to the medium induce adventitious shoot or buds and development of flowers. In many studies reported, that the most optimum sucrose concentration in a medium should amount to 3-7% sucrose [13, 14]. In our study the effect of sucrose concentrations on *in vitro* flowering induction was studied by keeping all the other parameters constant. We can state that the best sucrose concentration to obtain maximum of flowering plants (75%) with the greatest number of flower per plant (2.6) was 5% of sucrose (Figures **2**, **3**). Interestingly, Parredy and Greyson [15] who carried out the studies concerning *Zea mays* cv. Oh 43, suggested that the highest flowering efficiency can be achieved applying 0.3M (approximately 100 mg/l) sucrose concentration.

According to Franklin *et al*. [13] and Zhang [16] various levels of ammonium nitrate can induced flowering and fruiting *in vitro*. Generally, ammonium nitrate plays positive role in inducing *in vitro* flowering at lower concentrations and inhibits flowering at higher concentrations. Our studies revealed the largest flowering *Celosia argentea* plants (100%) on modified MS medium containing 16.50 and 8.25 g/l NH_4NO_3 in comparison to the control medium consisted 33.0 g/l NH_4NO_3 (47.4%) (Figure **2**). The decrease in the ammonium nitrate concentration in MS medium to 4.12 g/l had the inhibitive effect on *in vitro* flowering. *Celosia argentea* plants obtained from this medium gave 75% *in vitro* flowering and forming 0.8 flower per plant (Figure **3**). This result confirmed the role of ammonium nitrate on *in vitro* flowering. Reduction of ammonium nitrate to half strength of MS medium resulted in optimum flowering as in the case of *Vigna mungo* [17], green pea [13] and *Perilla frutescens* [16].

Many studies have reported the stimulating effect of cytokinin on *in vitro* floral morphogenesis, but they were species-dependent [8, 18, 14, 19, 20]. *In vitro* flowering in *Celosia argentea* var. *cristata* was first reported by Yamada *et al*. [21]. Similar experiments were performed by Bodhipadma *et al*. [22], who concluded that the induction of *Celosia argentea* var. *cristata* inflorescence *in vitro* from plantlets regenerating from nodal explants occurred on MS medium with or without 0.5 mg/l BA (6-benzyloadenine). In the case of *Celosia argentea* var. *plumosa*, adding the BA and paclobutrazol into MS medium revealed an inhibitive effect on inflorescence formation in shoot tip and nodal explant [22]. In own studies, we also have observed an inhibiting effect of BAP on flowering (Figure **1**). *Celosia* growing on the

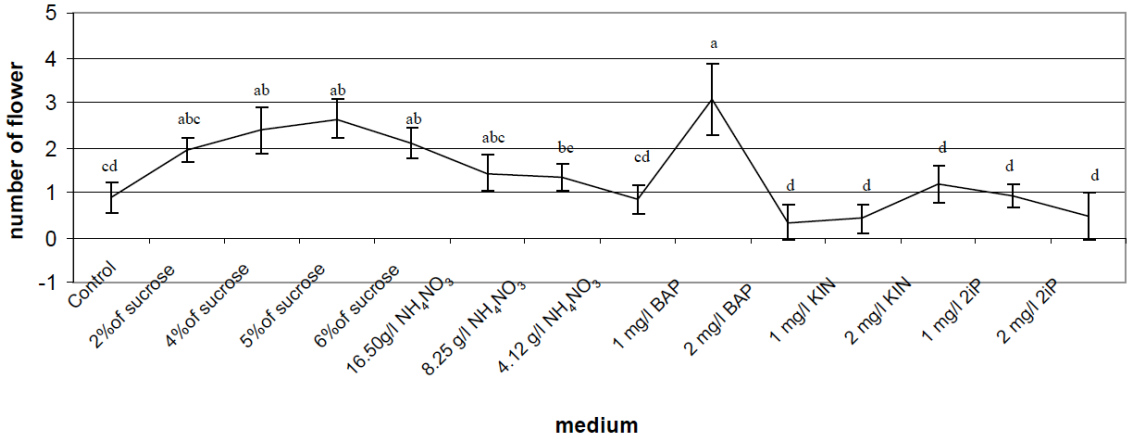

Figure 2: Number of flowers per *Celosia* plant propagated on different MS media compositions.

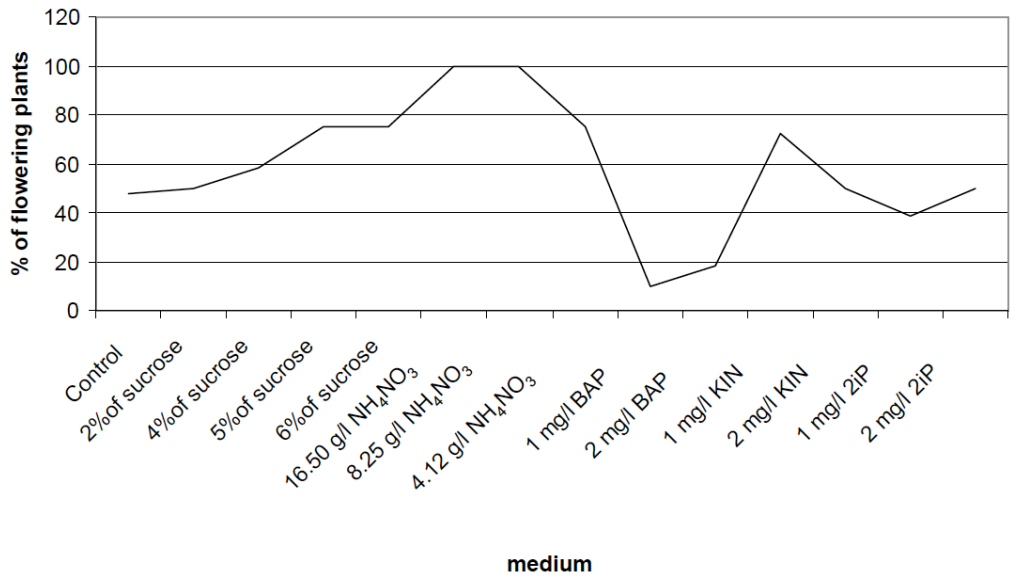

Figure 3: Percent of flowering *Celosia* plant propagated on different MS media compositions.

MS medium supplemented with 1.0 mg/l BAP produced only 10% of flowering plants (Figure **2**). On the other hand, *Celosia argentea* plants from this medium produced the largest average number of flowers per plant (3) (Figure **3**). From the other cytokinins used, the largest percentage of flowering plants (72.2%) was observed on MS medium supplemented with 1 mg/l KIN (Figure **2**).

It can be suggested, flowering is a complex process regulated by a vast of environmental and genetic factors. *In vitro* flowering process is affected not only by the type and concentration of plant growth regulators, but also the sucrose and ammonium nitrate concentrations. The MS medium with reduced content of ammonium nitrate (16.50 and 8.25 g/l) appeared to be the best, for it allowed achieving the largest percentage (100%) of *Celosia argentea* var. *cristata in vitro* flowering. Nevertheless, plants from MS medium containing 5% of sucrose flowered in 75% and developed 2.6 flowers per plant. Among tested growth regulators, the highest flowering effectiveness (72.2%) was recorded when 1.0 mg/l KIN was supplementes to the MS medium.

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