

RESEARCH PAPER

The effects of picloram and 2,4-dichlorophenoxyacetic acid on induction of red coloured callus from *Celosia plumosa*, an attractive ornamental plant

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Index Terms

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Abstract—*Celosia plumosa* is an attractive ornamental plant having flame-like flowers and is used traditionally as a medicinal herb. This paper deals with the red-coloured callus induction from the root, stem and leaf explants when cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of auxin picloram and 2,4-dichlorophenoxyacetic acid (2,4-D), applied singly. When explants were cultured on MS media supplemented with 6.0 mg/l picloram, the highest amount of red-coloured callus (0.49 ± 0.26 g) was from leaf explants and the lowest (0.09 ± 0.02 g) from stem explants. However, the highest amount of red-coloured callus (0.69 ± 0.13 g) was achieved on Murashige and Skoog (MS) media supplemented with 1.5 mg/l 2,4-D from stem explants, while the lowest (0.13 ± 0.06 g) from root explants was cultured on 0.5 mg/l 2,4-D. Generally, the optimum concentration for red-coloured callus formation using picloram (6.0 mg/l) was higher compared to 2,4-D (1.5 mg/l 2,4-D).

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I. INTRODUCTION

Celosia plumosa is a herbaceous plant and has been classified as a member of the family Amaranthaceae. This annual plant has a distinctive characteristic inflorescence which looks like the crest of a rooster or convoluted brain after it has developed fully [1]. Generally, the flower colours include red, yellow, orange and pink. The *Celosia* plants are erect, branching plants with oval or lance-shaped, strongly veined leaves that are 2-6 inch (5.1-15.2 cm) long and hundreds of tiny flowers packed in dense,

brightly coloured flower heads which usually stand above the foliage.

The flowers have the following characteristics; acrid, astringent, styptic, depurative, uterine sedative, constipating, antibacterial, corrective of urinary pigments, febrifuge and alexeteric. They are useful in the conditions of kapha and pitta, leprosy, burning sensation, skin diseases, diarrhoea, dysentery, fever, headache, hemorrhoids, herpes, internal hemorrhage, leucorrhoea, liver disorders, menorrhagia, ulcers and wounds. Juice of leaves is used in bilious sickness. They are also valued as a stimulant in pregnancy. The seed is hypotensive and ophthalmic. It is used in the treatment of bloodshot eyes, blurring of vision, cataracts and hypertension. The flower

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and seed are astringent, haemostatic, parasiticide and poultice. It is used in the treatment of bloody stool, haemorrhoid bleeding, uterine bleeding, leucorrhoea and diarrhoea.

The aim of the present study is to investigate the effects of picloram and 2,4-dichlorophenoxyacetic acid, and also to determine the optimum medium for in-vitro red coloured callus formation from root, stem and leaf explants.

II. MATERIALS AND METHOD

i) Plant materials and tissue culture initiation

Celosia plumosa is native to South America and widespread in Asia, especially, Malaysia. The seeds were initially washed with tap water and teepol to remove impurities and microorganisms on the seed surface. The seeds were then sterilized with 99% (v/v) sodium hypochlorite and added with two drops of tween-20 for five minutes. The seeds were then soaked in 70%, 50%, 30% and 20% (v/v) sodium hypochlorite for 5 minutes one by one. Then, the seeds were rinsed with distilled water for three times [2]. In laminar flow chamber, the seeds were dipped in 70% (v/v) ethanol for 1 minute and rinsed with sterile distilled water for three times. Seeds were cultured on solid basal germination medium

composed of [3] salts and vitamins supplemented with 30g/L sucrose and 10 g/L agar. One-month-old aseptic seedlings were used as explant sources. The explants were excised into small pieces (3mm²) and cultured on MS media supplemented with picloram and 2,4-dichlorophenoxyacetic acid (2,4D), with 30 replicates for each treatment. Media were adjusted to pH 5.5 and sterilized by autoclaving (15 min, 121°C) and 50 ml aliquots were dispensed into pre-sterilized 290 ml plastic bottles (80 mm diameter x 60 mm high). All cultures were incubated in a growth room at 24°C day and night temperature, with a 16-h photoperiod at 80-85 µmol m⁻² s⁻¹ under cool white fluorescent light.

A. Callus Induction

Celosia plumosa were cultured on MS medium [3], supplemented with various concentrations of plant growth regulators (Picloram and 2,4-D) for red-coloured callus induction and plant regeneration. The callus cultures were maintained at 25±1°C, under 16 hours light of photoperiod with 25 µmol m⁻²s⁻² of light intensity. At regular intervals for all treatments, each callus was harvested carefully by separating from media using spatula, and fresh and dry weight was recorded for each.

TABLE I
EFFECT OF PICLORAM ON THE FORMATION OF CALLUS FROM EXPLANTS (ROOT, STEM AND LEAF) OF CELOSIA PLUMOSA

Picloram Concentration (mg/l)	Explants	Dried weight of callus, g (Mean±SE)	Observation
2.0	Root	-	No callus formation
	Stem	0.08±0.03	Cream-coloured callus formation
	Leaf	0.25±0.11	Red-coloured callus formation
4.0	Root	-	No callus formation
	Stem	0.15±0.07	Red-coloured callus formation
	Leaf	0.24±0.19	Red-coloured callus formation
6.0	Root	0.16±0.10	Red and cream callus formation
	Stem	0.09±0.02	Red and cream callus formation
	Leaf	0.49±0.26	Red-coloured callus formation
8.0	Root	0.10±0.07	Red and cream callus formation
	Stem	0.37±0.19	Red and cream callus formation
	Leaf	0.38±0.07	Red-coloured callus formation

III. RESULTS AND DISCUSSION

[4]Through invitro methods, the effect of the seasons can be eliminated and year-round production can be achieved,

or a new cultivar can be produced commercially at a faster rate. Table 1 shows the effects of picloram on red-coloured callus induction from root, stem and leaf explants. Leaf was the optimum explant with the highest (0.49±0.26 g)

dried weight of red coloured callus cultured on Murashige and Skoog (MS) media supplemented with 6.0 mg/l picloram, followed by MS media supplemented with 8.0 mg/l picloram (0.38 ± 0.07 g). Whilst, the lowest dried

weight of callus (0.09 ± 0.02 g) was obtained from stem explants cultured on MS media supplemented with 6.0 mg/l picloram.

TABLE II
EFFECT OF 2,4-D ON THE FORMATION OF CALLUS FROM EXPLANTS (ROOT, STEM AND LEAF) OF CELOSIA PLUMOSA

2,4-D Concentration (mg/l)	Explants	Dried weight of callus, g (Mean \pm SE)	Observations
0.5	Root	0.13 ± 0.06	Cream-coloured callus formation
	Stem	0.55 ± 0.10	Red and cream callus formation
	Leaf	0.50 ± 0.04	Red-coloured callus formation
1.0	Root	0.27 ± 0.10	Red and cream callus formation
	Stem	0.42 ± 0.10	Red-coloured callus formation
	Leaf	0.45 ± 0.30	Red-coloured callus formation
1.5	Root	0.17 ± 0.03	Red-coloured callus formation
	Stem	0.69 ± 0.13	Red-coloured callus formation
	Leaf	0.39 ± 0.08	Red-coloured callus formation
2.0	Root	0.26 ± 0.14	Red and cream callus formation
	Stem	0.48 ± 0.06	Red-coloured callus formation
	Leaf	0.37 ± 0.17	Red-coloured callus formation

Table 2 shows that the highest (0.69 ± 0.13 g) red-coloured callus formation was achieved on MS media supplemented with 1.5 mg/l 2,4-D from stem explant (Figure 1), while the lowest amount of callus (0.13 ± 0.06) was obtained from

root explants. Generally, 2,4-D gave the better response in forming red-coloured callus from stem explants as compared to leaf explants cultured on picloram, as found in the current study.

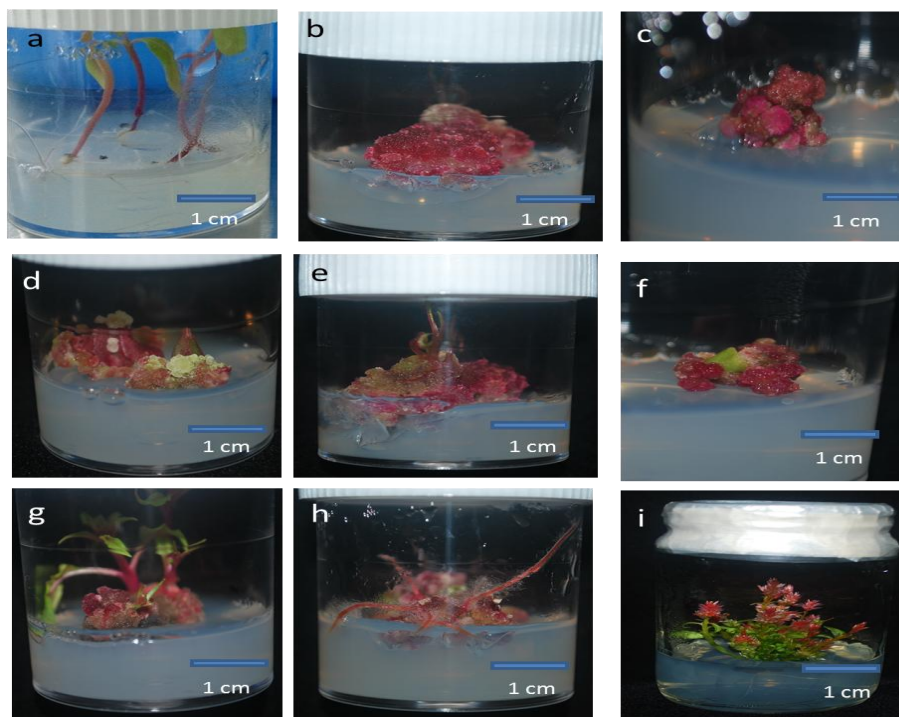


Fig 1. Formation of red-coloured callus on Murashige and Skoog (MS) media.

Supplemented with 1.5 mg/l 2,4-D from stem explants. a) one-month old in-vitro seed germination b) red-coloured friable callus formation after 3 weeks c) red-coloured compact callus after 8 weeks d) red micro-shoot formation after 10 weeks e) elongated red micro-shoots f) green micro-shoots) elongated multiple shoots h) root formation i) in-vitro flowering after 10 months.

[5] Reported that explants from stem segments of *Celosia* spp. formed callus when cultured on media containing either half strength MS or without MS combined with 100 g/l each gelling agent and 70 ml/l coconut water. Also, in-vitro flowering of *Celosia cristata* was reported by [6]. [7] The highest amount of reddish callus was obtained when stem explants were cultured on MS media supplemented with 0.5–1.0 mg/l 1-Naphthaleneacetic acid (NAA) and 0.5-1.0 mg/l 6-Benzylaminopurine (BAP) after 3 weeks. *Celosia* spp. are amenable in tissue culture and all *C. crispata* explant sources could regenerate or were able to produce shoots in-vitro [2] whereas, in V. Faber, it is comparatively more difficult to induce callus and regeneration in-vitro [8].

IV. CONCLUSION

The highest amount of red-coloured callus (0.69 ± 0.13 g) was achieved on MS media supplemented with 1.5 mg/l 2,4-D from stem explants. Whilst, the highest amount of red-coloured callus (0.49 ± 0.26 g) was achieved on MS media supplemented with 6.0 mg/l picloram from leaf explants.

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