Study on the Tissue Culture of *Coleus blumei*

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Abstract: This paper studied on the way of *Coleus blumei*, the leaf was chosen as explants and was inoculated on 1/2 MS medium with different combinations of 6-benzyladenine (6-BA), α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA). The optimal conditions of callus induction from explants were achieved on the medium containing 6-BA (2.0 mg·L^{-1}) and NAA (1.0 mg·L^{-1}). Shoot tips were induced on the medium containing 6-BA (4.0 mg·L^{-1}) and NAA (0.5 mg·L^{-1}). The same media conditions were found suitable for shoot multiplication, we multiplied shoots rooted best on1/2 MS medium supplemented with IBA (0.1 mg·L^{-1}).

Key words: *Coleus blumei*, tissue culture, hormone

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Introduction

Coleus blumei wizard, which belongs to the family Lamiaceae, Java of Indonesia is its provenance. Since 19th century it was found in Java, people started to undertake breeding work in Europe. Now *Coleus blumei* has become an important foliage plant in landscape garden. It is also used for the synthesis of rosmarinic acid[1]. However, because it originates from tropical zone, it couldn't tolerate temperature lower than 10℃ or it would subject to freezing damage, so the low temperature has become the main factor to hinder its application.

Now we are seeking transformation of cold resistant gene through the way of callus to tackle this tough problem, which makes the tissue culture of *Coleus blumei* becoming a urgent issue to be solved. As we need large amount of explants in the process of transformation, the leaf is ideal source of explants. The former documents only focus on introducing callus from nodal segments and shoot tips, but in this paper the leaf was chosen as explants to introduce callus, at the same time we will make further illustration on tissue culture of *Coleus blumei* wizard.

Materials and Methods

Plant materials and pretreatment

We choose *Coleus* wizard that is one cultivated varieties of *Coleus blumei* as experimental material and took its young green leaf which was about 3 cm long as explants, washing it with lotic water at least 1 h, surfacesterilized with 95% alcohol for 15 s, then submersed in 0.1% $HgCl_2$ 1-2 min then rinsed with sterile distilled water 4-5 times[2]. The leaves were dissected under sterile conditions into 4-5 mm^2 tablets, these were placed on agar-solidified introduction medium which contained different level of salinity and growth regulator. Three explants per triangular flask, 10 triangular flasks as a treatment, 3 repetitions.

Condition of cultivation

Illumination length was 12-16 h. Intensity of light was 1 200-1 600 lx. Temperature was 20-25℃. pH 5.6 (medium was adjusted after the addition of agar).

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Category of medium

(1) Callus introduction medium random arrangement experiment: Minimal medium: 1/2 MS; 6-BA levels: 1.0, 2.0, 3.0 mg·L^{-1}; NAA levels: 0.1, 0.5, 1.0 mg·L^{-1}. (2) Differentiation medium: 1/2 MS+6-BA 1.0 mg·L^{-1}+ NAA0.5 mg·L^{-1}; 1/2 MS+6-BA 4.0 mg·L^{-1}+NAA 0.5 mg·L^{-1}; 1/2 MS+6-BA 4.0 mg·L^{-1} +NAA 1.0 mg·L^{-1}. (3) Radicate medium: 1/2 MS+IBA 0.1 mg·L^{-1}; 1/2 MS+IBA 1 mg·L^{-1}; 1/2 MS.

Results and Analysis

Induction of callus

Callus occurred from cut after 20-30 days. In all cultures, the best quality callus was achieved at 2 mg·L^{-1} 6-BA and 1 mg·L^{-1} NAA. The explants partly were necrosis, but the callus grew well, its texture looked green and compact. In the process of induction callus, 6-BA at 2 mg·L^{-1} in combination with NAA resulted in increase in number and height of callus, with increasing in concentration of NAA, leaf explant also showed a similar pattern of growth with various hormonal treatments. Maximum number and height of callus were also achieved at 2 mg·L^{-1} 6-BA combination with 1 mg·L^{-1} NAA (Fig. 1, Table 1). The results are the same as some previous studies, in which 6-BA and NAA are found to be useful in callus induction from leaf explants of various other plants, for example, *Isatis tinctoria*[3]. Similar combinations of plant hormones were also found to be responsible for callus and plantlet initiation in leaf discs of *C. blumei*[4].

Fig. 1 The information of callus induction on MS medium containing 6-BA (2 mg·L^{-1}) and NAA (1 mg·L^{-1})

Table 1 The results of statistical analysis for different treatment of induction of callus

Growth regulators (mg·L^{-1})		Inductivity (%)
NAA	6-BA	
0.1	1.0	7.33c
0.1	2.0	27.67bc
0.1	3.0	9.94c
0.5	1.0	45.00b
0.5	2.0	34.91b
0.5	3.0	26.44bc
1.0	1.0	51.94b
1.0	2.0	89.11a
1.0	3.0	81.11a

The differentiation of adventitious bud

Adventitious bud initiation occurred from callus, all cultures maximum number of shoots were achieved at 4 mg·L^{-1} 6-BA and 0.5 mg·L^{-1} NAA(Fig. 2, Table 2). The high level of 6-BA was suitable for differentiation of *Coleus blumei* and other plants[5], it may neutralize the high level growth hormone in *Coleus blumei*.

Fig. 2 The information of adventitious bud differentiation on MS medium containing 6-BA (2 mg·L^{-1}) and NAA (1 mg·L^{-1})

Table 2 The results of statistical analysis for different treatment of the differentiation of adventitious bud

Medium	6-BA (mg·L^{-1})	NAA (mg·L^{-1})	Average of adventitious bud
1/2 MS	1.0	0.5	0
1/2 MS	4.0	0.5	5
1/2 MS	4.0	1.0	2

Successive transfer medium of callus

In the successive transfer culture, 4 mg·L^{-1} 6-BA and 0.5 mg·L^{-1} NAA were found to be not suitable for secondary culture, because when callus was put on it after 30-40 days, the callus always differentiateed root, even the callus had't growth adventitious bud (Table 3)[6]. Through the further study, we have made another successive transfer medium.

Table 3 The results of statistical analysis for different treatment of successive transfer medium

Medium	6-BA (mg·L^{-1})	NAA (mg·L^{-1})	Height of callus
1/2 MS	4.0	0	1.37a
1/2 MS	4.0	1.0	0.93b
1/2 MS	2.0	1.0	0.94b

Table 3 showed that 6-BA 4.0 mg·L^{-1}+NAA 0 mg·L^{-1} had the best impact on the secondary cultures and we gained the best quality and maximum height.

Root media

Coleus blumei generated root easily, even though it was put in 1/2 MS media without any hormone. So if there is micro amount of IBA, we often gain high level rate of root[7]. This also indicates that the *Coleus blumei* has a high level growth hormone, but when the IBA arrives at higher level, it would suppress the generation of root[8]. This experiment showed that 1/2 MS+IBA 0.1 mg·L^{-1} was the best for introducing root (Fig. 3, Table 4).

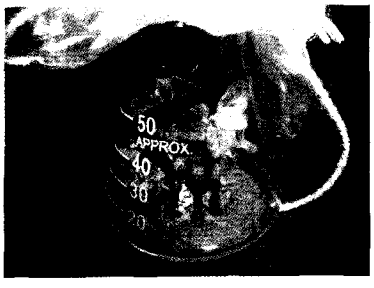

Fig. 3 Rooting of shoots on MS medium containing IBA (0.1 mg·L^{-1})

Table 4 The results of statistical analysis for different treatment of root media

Medium	IBA (mg·L^{-1})	Radiating rate (%)
1/2 MS	0.1	100.00
1/2 MS	1.0	75.24
1/2 MS	0	68.50

Root plantlet transplanting

Triangular flask with root plantlet was put in natural light, after 2-3 days. The final encapsulation was opened, making the plantlet adapted the natural circumstances, then rooted plantlets were established in soil with 100% survival (Fig. 4).

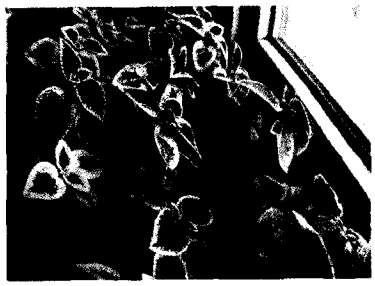

Fig. 4 Transplanted plant in pot

Discussion

In the study on the tissue culture of *Coleus blumei*, we found that in the process of introduction of callus, finally we often gained three kinds of calli, the first one looked semitransparent and its texture was loose, the second looked green and its texture was tight, the last one looked little white and its texture was hard. We made three kinds of calli in the same condition based on believe that it may correlate physiological condition of every cell in the explant, since different cells have different physiological conditions even though they are in the same leaf[9]. This study showed that the second and the last kinds of calli more easily generated adventitious bud, while what kind of physiological condition of plant cell could generate health callus, we need to make further study.

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