CALLUS FORMATION AND PLANT REGENERATION OF CHRYSANTHEMUM LEAF DISCS EXPLANTS THROUGH *IN VITRO*

Murgayanti, Erni Suminar, Wieny H.Rizky, and Sri Rustiani

Faculty of Agriculture, The University of Padjadjaran (UNPAD)
Jl. Raya Bandung Sumedang Km 21, Jatinangor Bandung, Indonesia

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Tzevelev.) is one of the most popular ornamental plants in the world, because of its attractive and charming colour. Its popularity has increased not only for its outstanding aesthetic beauty but also for its good potential as cut-flowers.

1. Conventional chrysanthemum propagation can be done trough generative and vegetative ways. However, propagation through both ways relative difficult, generate small quantity, need long period, and produce low quality of flowers.
2. Another alternative which is can be taken is *in vitro* propagation so that can produce plants in large amount quantity, save time and pathogen-free. Chrysanthemum propagation through *in vitro* can be done by direct organogenesis or by callus formation stages.
3. Callus formation and regeneration determined by concentration and ratio of plant regulator hormone, such 2,4-D for auxin also kinetine and BAP for cytokine.

The experiments regarding to determine:

1. The effect of different regulator concentration from 2,4-D and Kinetine, and their interaction on callus formation of chrysanthemum
2. The effect of application 1 mgL^{-1} BAP on regeneration of chrysanthemum callus

MATERIALS AND METHODS

Leaf discs explants of chrysanthemum were cultured on Murashige & Skoog's Medium, with 3% sucrose, 0.8% agar and combination of various growth regulators. The callus formation experiment was conducted using Factorial Completely Randomize Design with three replications. The first factor was three different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) (0.0, 1.0 and 2.0 $mgL^{-1)}$ and the second factor was four different concentrations of Kinetine (0.0, 0.1, 0.5 and 1.0 mgL^{-1}). The calluses which were produced, then regenerated on MS medium with 1 mgL^{-1} BAP.

RESULTS AND DISCUSSION

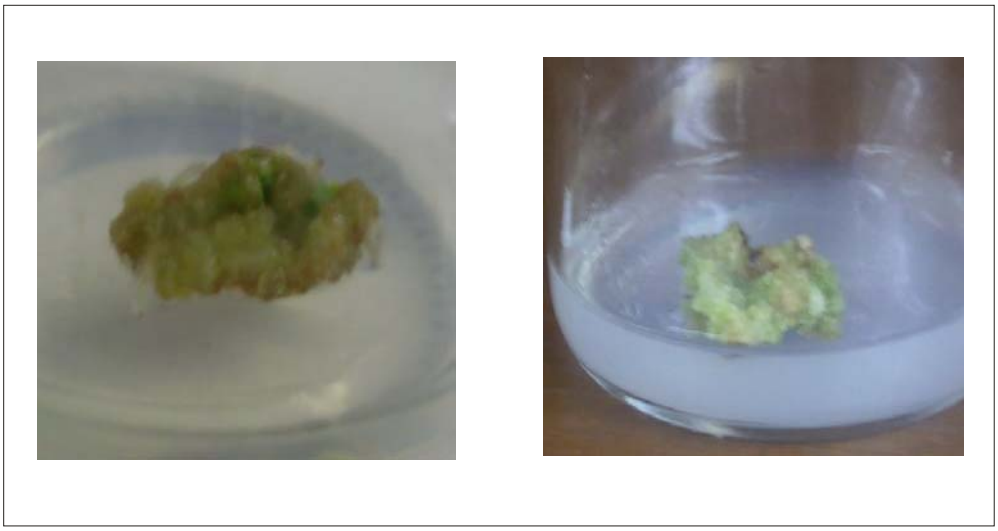
Callus Formation

Table 1. Effect of various 2,4-d and kinetine concentrations on texture and colour chrysanthemum callus 8 weeks after treatments

Treatments	Callus Texture	Callus Colour
A = 0.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	-	-
B = 0.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	-	-
C = 0.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	-	-
D = 0.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	-	-
E = 1.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	granular	yellowish transparent
F = 1.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	compact	green
G = 1.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	compact	green
H = 1.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	compact	green

Treatments	Callus Texture	Callus Colour
I = 2.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	granular	yellowish transparent
J = 2.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	granular	yellowish transparent
K = 2.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	compact	green
L = 2.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	compact	green

(-) means it didn't make callus



a **b**

Figure 1. Texture and colour of chrysanthemum callus
a) Callus texture was granular and yellowish transparent colour
b) Callus texture was compact and green colour

Table 2. Effect of various 2,4-d and kinetine concentrations on wet mass chrysanthemum callus at 8 weeks after treatments

Treatments	Callus Wet Mass (g)
2,4-D	
0.0 mgL^{-1}	0.000 a
1.0 mgL^{-1}	0.701 b
2.0 mgL^{-1}	0.780 b
Kinetine	
0.0 mgL^{-1}	0.672 a
0.1 mgL^{-1}	0.889 a
0.5 mgL^{-1}	0.679 a
1.0 mgL^{-1}	0.718 a

(-) means it didn't make callus

Means followed by the same letter in a column are not significantly different ($p < 0.05$) by Duncan Multiple Range Test.

Regeneration from Callus

Table 3. Number and percentage of shoot from callus after sub-culture with 1 mgL^{-1} BAP adding at 4 weeks after callus formation

Previous Treatments (Callus Formation)	Number of Shoot	Percentage of Shoot (%)
A = 0.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	0.00	0.00
B = 0.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	0.00	0.00
C = 0.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	0.00	0.00
D = 0.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	0.00	0.00
E = 1.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	0.00	0.00
F = 1.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	0.00	0.00
G = 1.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	0.00	0.00
H = 1.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	2.50	44.44
I = 2.0 mgL^{-1} 2.4-D + 0.0 mgL^{-1} Kinetine	0.00	0.00
J = 2.0 mgL^{-1} 2.4-D + 0.1 mgL^{-1} Kinetine	0.00	0.00
K = 2.0 mgL^{-1} 2.4-D + 0.5 mgL^{-1} Kinetine	0.00	00.00
L = 2.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine	1.00	22.22

Figure 2. The shoot regeneration from callus

CONCLUSION

1. There was no interaction for 2,4-D and kinetine concentration on callus formation time and callus wet mass
2. Application of 2.0 mgL^{-1} 2,4 D gave the best wet mass
3. Application of 1 mgL^{-1} BAP could regenerate number of shoot from previous treatment which was 1.0 mgL^{-1} 2.4-D + 1.0 mgL^{-1} Kinetine

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