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石竹细胞悬浮培养研究

李宗艳

(西南林学院园林学院,云南昆明 650224)

摘 要:石竹细胞继代周期为 7 d 时,悬浮细胞培养系生长最快,生长率最高,而且培养物中胚性细胞较多,并能保持较快的分裂和生长,能促进已形成的大细胞团的生长和分化。转代时接种物与新鲜培养基的体积比以 1∶2 较好,悬浮系细胞生长最快,生长率最高,以 1∶2 和 1∶3 的高倍稀释接种有利于胚性细胞的形成及产生小的胚性细胞团,对悬浮系添加椰乳和水解乳蛋白的混合物,可较大幅度地提高悬浮细胞系的生长速率,单独添加上述两种物质的效果均不如二者的综合效应好。在 6 种不同激素组合中,配方 2(2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L)最好,生长率最高。配方 5(2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 1.0 mg/L)其次;配方 1(2,4-D 1.0 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L)次之。

关键词:石竹;细胞悬浮培养;生长

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Study on cell suspension culture of *Dianthus chinensis*

LI Zong-yan

(*Faculty of Landscape Architecture, Southwest Forestry College*, Kunming, 650224, China)

Abstract: In the present research, the suspension culture clones of *Dianthus chinensis* can keep cell multiplication and growth-rate at high speed when the secondary cycle is arranged for 7 days. Moreover, there exist lots of embryonic cells in the culture fluid that could keep growth and division fast and promote growth and differentiation of the formed big cell groups. When conducting trans-generation, it is better if volume rate between inocula and fresh culture media is 1∶2. Because the cell multiplication and cells' growth keep the fastest under this condition. It is the high dilution rate of 1∶2 or 1∶3 that will contribute to form the embryonic cells and produce the small embryonic cell groups. Provided that a mixture of coconut milk(CK) and lacotalbumin hydrolysate(LH) is added, the growth-rate of suspension clones could be greatly improved. Adding both is better than adding only one of them. The concentration of hormone plays a key role in cells' growth and division. Compared to six different formulas, formula 2(2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L) is the best. Formula 5 comes second (2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 1.0 mg/L) and formula 1(2,4-D 1.0 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L) comes third.

Key words: *Dianthus chinensis*; cell suspension culture; growth

Dianthus chinensis L. is widely distributed in China and an important economic plant. This species is cultivated in the garden not only as an ornamental plant, but also as a medical plant. This perennial herb contains lots of useful chemical compositions in its leaves, stems and roots, such as eu-

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作者简介:李宗艳(1974-),女,云南文山人,硕士,讲师,园林植物专业,主要从事园林植物方向的教学和科研工作。

genol, benzyl benzoate, methyl salicylate, saponins and so on. (Huang TK *et al.*, 2001)

The fluid suspension culture of plant cells is applied for the virus-free and high quality seedlings, the secondary metabolites, producing artificial seeds and building the efficient reproduction system(Tan WD *et al.*, 2001). The present research is carried out to find the best formula by testing the secondary cycle, the proper dilution rate of inocula, additives and concentration of hormone that will improve cells' growth and division rapidly, keep high growth-rate and frequency of the embryonic cells.

1 Materials and Methods

1.1 Materials

Stem apex about 0.4～0.6 mm is cut from a strong branch in greenhouse to be cultured for the test-tub seedlings.

1.2 Inducement of calli

The stem apex taken from test-tub seedling is used as an explantation to be inoculated on the culture medium for the wound callus at 25 ℃ under dark conditions (formula; MS+2,4-D 0.5 mg/L+NAA 1.0 mg/L+6-BA 0.6 mg/L+LH 1 000 mg/L+Cane Sugar 3%). After producing calli, they are transferred to the same culture medium without 2,4-D for extension of propagation. The secondary cycle is 10 to 15 days.

1.3 Establishing suspension culture clones

The calli about 3 or 6 gram are put into a culture bottle with 100 mL MS fluid culture medium when the secondary cells have grown for 5 to 6 days. They are cultured for establishing the suspension lines with shakers functioning at 120 rounds per minute at 25～27 ℃ under the light (Chen W *et al.*, 2002; Gan FY *et al.*, 1997; Zhang QW *et al.*, 1995).

1.4 Secondary culture of suspension cells

The secondary culture will be carried out periodically after the calli become dispersed to form a suspension line. When transferred, there are three settings of dilution volume ratio between the inocula and fresh culture fluid, respectively 1∶1, 1∶2, 1∶3.

1.5 Growth determination

1.5.1 Fresh weight determination The culture materials are filtrated through a previously weighed paper when the periodical culture have ended, then weigh it.

1.5.2 Dry weight determination The freshly weighed cells, together with the filtrating paper, will be baked for 12 hours at 60 ℃ in the incubator before weigh. The result is the dry weight.

1.5.3 Relative volume ratio determination When the suspension period is over, suspension materials are put into the scaled-tube and centrifuged for 5 minutes under 800 rounds per minute, then the relative volume ratio is determined(Yu SW *et al.*, 2001).

1.5.4 Increasing rate determination The increasing rate is the ratio between fresh weight and inocula weight.

1.5.5 Net growth rate determination Net growth rate is the net gaining in fresh weight every day.

1.5.6 Relative water content determination Relative water content (RWC) is the ratio between water content and fresh weight. Fresh weight minus dry weight is the water content.

1.6 Suspension cells observation

The microscopic observation is used to analyze the types of suspension cells.

2 Results and Discussion

2.1 Composition and Characters of suspension culture clones

The present study has revealed that there are two types of cells constituting the suspension culture clones by the microscopic observation. One is the embryonic cells, whose volume is small, ovate and elliptical with a thin cell-wall, non-apparently big vacuoles or central vacuoles, dense cytoplasm, a apparent cell nucleus and nucleus. The embryomic cells display in the suspension fluid usually in sin-

gle dispersing or two to several cells which cluster closely together into small embryonic cell groups. The other type is the non-embryonic cell, whose volume is big, stripe-oblong with an apparently big vacuole or central vacuole. These non-embryonic cells gather together and form the sparse, apparent intercellular space and disorderly crumb structure (Zhang LY *et al.*, 1997).

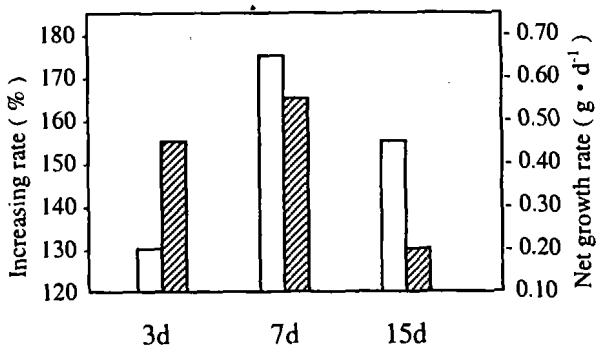

Fig. 1 Effect of three secondary cycle on suspension cells' growth

□ Increasing rate (%); ▨ Net growth rate (g · d⁻¹).

2.2 Effects of the secondary cycle on suspension culture clones

The result (Fig. 1) shows that it is a good time for the secondary cells to grow when the cycle of secondary culture is arranged for 7 days. Its increasing rate reaches to 172%, which is 18% or more higher than those of formula 2. Its net growth-rate is 0.543 g/per day, which exceeds 0.076 than that of 3 days, 0.339 than that of 15 days respectively. Meanwhile, in the culture fluid, the suspension clones can not only keep the growth and division of embryonic cells rapid, but also promote the formed cell groups to grow and differentiate. The reason of the decreases in growth-rate by the 15thday might be that there is not enough nutrition for cells' growth and division, which is resulted from the nutrition of culture medium consumed with the extension of culture time, and is that the accumulations of metabolic products produced by the continuous growth and division of cells restrained the continued growth of cells. However, the reason why the growth-rate is low by the third day might be that the suspension clones can not reach the top of growth and division because it is a short time before the cells just started to divide.

Table 1 Effects of volume rate between inocula and culture fluid on suspension cells' growth

Volume rate	Weight of inocula(gram)	Fresh weight (gram)	Dry weight (gram)	Increasing rate percentage(%)	Net growth rate (Fw · g · d⁻¹)	RWC (%)
1 : 1	4.553	6.515	0.931	143.09	0.280	85.71
1 : 2	3.644	6.232	0.956	171.02	0.370	84.65
1 : 3	3.861	5.253	0.796	136.05	0.199	84.85

Note: 1. The secondary cycle: 7 days; 2. Culture formula: MS+2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L+CM 5%.

Table 2 Effects of different additive on suspension cells' growth

Additive	Weight of inocula (gram)	Fresh weight (gram)	Dry weight (gram)	Increasing rate percentage (%)	Net growth rate (Fw · g · d⁻¹)
CM	5.254	9.067	1.285	172.58	0.545
LH	5.102	7.801	1.134	152.90	0.386
CM+LH	4.575	9.059	1.315	198.01	0.641
CK	5.251	6.450	0.942	122.83	0.172

Note: 1. The secondary cycle: 7 days; 2. Culture formula: MS+2, 4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L; 3. Dilution rate: 1 : 2.

2.3 Effects of different volume rate on suspension culture clones

According to the results, the increasing rate and the cells' net growth rate of suspension culture clones keep the highest under rate 1 : 2. Its increasing rate is 171% of the increasing rate and cells' net growth is 0.370 gram of fresh weight per day(Fw · g · d⁻¹) in net growth rate under this condition. The increasing-rate of rate 1 : 1 is similar to that of rate 1 : 3. At the same time, the microscopic observation shows that high dilution rate of 1 : 2 and 1 : 3 will be beneficial to form embryonic cell groups and produce the small embryonic cells. However, low rate of 1 : 1 will be good to

form many non-embryonic and big cell groups. Results of relative water content(RWC) have proved that.

2.4 Effects of coconut milk and lacotalbumin hydrolysate on suspension culture clones

The net growth rate and increasing rate will be apparently improved if either of two additives is added in the fluid culture medium. Using a mixture of CM and LK is better than using only one of them. Its increasing rate and net growth rate keep the highest. Its increasing rate is 198% of the increasing rate, which is 45% higher than that of LH, 23% higher than CM respectively. Its net growth rate reaches 0.641 unit($Fw \cdot g \cdot d^{-1}$). Moreover, there is a higher ratio of embryonic cells in the fluid culture medium. Compared to lacotalbumin hydrolysate(LH), coconut milk(CM) will be better.

2.5 Effects of hormone on suspension culture clones

The variety and concentration of hormone have a key effect on the growth and division of suspension cells. According to Table 3, when NAA and 6-BA have the same concentration, the proper concentration of 2,4-D is 1.5 mg/L, whose increasing rate can reach 256% and growth rate is 0.922. The proper concentration of 6-BA is 0.5 mg/L. Formula 2 is the best for promoting the growth and division of suspension cells (2,4-D 1.5 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L). Its growth-rate is more than 69% higher than that of other 5 formula. Formula 5 comes second, follow by formula 1(2,4-D 1.0 mg/L+NAA 0.5 mg/L+6-BA 0.5 mg/L).

Table 3 Effects of hormone on suspension cells' growth

Hormone component concentration (mg/L)	Weight of inocula (gram)	Fresh weight (gram)	Dry weight (gram)	Growth-rate percentage(%)	Net growth rate ($Fw \cdot g \cdot d^{-1}$)
2,4-D 1.0+NAA 0.5+6-BA 0.5	4.511	8.343	1.225	184.95	0.547
2,4-D 1.5+NAA 0.5+6-BA 0.5	4.123	10.578	1.525	256.55	0.922
2,4-D 2.0+NAA 0.5+6-BA 0.5	3.837	6.572	0.948	171.28	0.391
2,4-D 1.0+NAA 0.5+6-BA 1.0	3.751	5.982	0.863	159.47	0.319
2,4-D 1.5+NAA 0.5+6-BA 1.0	4.157	7.773	1.125	186.99	0.517
2,4-D 2.0+NAA 0.5+6-BA 1.0	4.324	5.425	0.783	125.46	0.157

Note: 1. The secondary cycle: 7 days; 2. Culture formula: MS+CM 5%+LH 1 000 mg/L; 3. Dilution rate: 1 : 2.

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