In Vetro Cell Divission of Pelargonium Pith Tissue

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ABSTRACT

Culture media were used to develop a rapid production system for geranium (Plargonium X hortorum) in vitro. Pith tissue was excised from the stem and induced to divide on White medium supplemented with10^{-6} M 2,4-D and kinetin. Once they have been induced to divide, they maintained on Murashige and Skoog medium supplemented with 2,4-D and kinetin, at 10^{-6} M. Non differentiated organs are produced in the established pelargonium pith callus. Pelargonium pith callus were obtained on medium was very friable. By gentle shaking, free cells were obtained and small cell clumps, which can be used as inocula for plante cultures. The plating efficiency has been brought to about 33% on Murashige and Skoog medium supplemented with 10^{-6} M 2,4-D and 6-dimethylallylaminopurine. Single cells have been observed to divide in the presence of other cells and cell clumps, but never happen in complete isolation

انقسام خلايا خشب الجيرانيوم خارج الجسم الحي

الخلاصة

استخدمت الأوساط الزرعيه خارج الجسم الحي لنبات الجيرانيوم (Plargonium X hortorum) ان نسيج اللب المفصول من ساق الجيرانيوم يمكن تحفيزه على لأنقسام في الوسط الغذائي (وايت) وبإضافة 2,4-D10^{-6} مولاري,والكاينتين. حالما يحفز الأنقسام, يفظل ادامته على الوسط الغذائي (مراشك وسكوك) باضافة2,4-Dوكاينتين, كلاهما بتركيز 10^{-6}مولاري لا توجد اعضاء متميزه في النسيج الأساس للب الجيرانيوم. أن نمو كتلة نسيج لب الجيرانيوم على وسط مغذي صناعي يكون هش جداً . بواسطة التريك الأهتزازي وبلطف, يمكن الحصول على أحد الخلايا الحرة وكتل خلوية صغيرة والتي يمكن استخدامها كأساس للزراعة النسيجية للنباتات. إن كفائة الزراعة في الأطباق قد ارتفعت لما يقارب 33% على الوسط الغذائي (موراشك وسكوك) وبإضافة 10^{-6}2,4-Dمولاري و 6-دايمثيل اليل امينوبيورين. لوحظ حدوث انقسام في خلايا مفردة في حالة وجودها مع خلايا أخرى مفردة وخلايا متكتلة ، ولا يحدث هذا الإنقسام في حالة الإنعزال التام .

INTRODUCTION

Pelargonium is one of the world most important bedding and pot plants. Recent figures show that the annual sales in Europe and North America are worth in excess of 700 million US$, annually [1]. According to Cassels, [2] over 90% of the croup is vegetatively propagated.

The technique of growing excised tissues under sterile conditions has provided a promising tool for investigating the problems of growth and development [3]. This fact strongly supports the view that all the cells of an organism contain the same genetic information, and that the differences in behavior of cells of various types in the organism result from the evocation or suppression of the activity of certain genetic loci by both intrinsic and extrinsic factors [4 , 5]. Nasser, [6] found that 37%of variegated bud culture were less viable than green varieties of *Pelargonium* chimera. The 6-benzylalanine,thidiazuron and kinetin are effective in inducing adventitious shoot production [7], this suggests that cytokinins play a key role in inducing adventitious shoots from young P. x citrosum leaf and petiole explants.

In another study [8] a clonal propagation system of *Pelargonium x sidoides* was developed using explants from mature plants, with particular emphasis on the regeneration potential of N6-benzylamenine(BA) and kinetin(Kin) compared to meta-topolin(mT).

The present study investigated the potential for stem pith cell division and differentiation to a meristematic state from which a variety of cells and tissues or even organs may arise.

MATERIALS AND METHODS

The basal media were those of White [9], Murashige and Skoog, [10] designated White and Murashing media respectively, to which were added the following organic materials; thiamin (0.1 mg/l), pyridoxine (0.5 mg/l), nicotinic acid (0.5 mg/l), glycen (2.0 mg/l), myo-inositol (100 mg/l), and sucrose (3%). The pH of the medium was adjusted to 5.0 with KOH before autoclaving. The media were then supplemented with 1%agar and was autoclaved at 1.04 kg/cm^2 pressure for 15 minutes. The pith in the Pelargonium stem was about 6 mm in diameter, and consisted entirely of living parenchyma cells about 100 to 200µ in diameter, frequently containing numerous prominent starch grains. Pith samples for culture were obtained as follows: stem were harvested, leaves and buds removed. The internodes were then cut into segments about 2.5 cm in length, sterilized in a beaker containing 15% commercial Clorox for 30 minutes, and washed 6 times with sterile distilled water. The pith was then excised and cut with a scalpel into rectangular sections (3x3x4 mm), which were used as the inocula.

The technique of Bergman [11] was employed for plant culture. Cell suspensions, obtained from Pelargonium pith callus by shaking in freshly prepared liquid medium for 5 minutes, were filtered through stainless steel mesh (1x1mm). Aliquots of the filtrate, containing free cells and small cell clumps, were used as the inocula. At inoculation, 2ml of cell suspensions were pipetted into a sterile plastic dish (100X15ml) and mixed with 2 ml of melted and cooled (30^o to 35^o C) agar medium. The dishes were then sealed with cellulose tape to prevent contamination and desiccation. All manipulations were carried out in a sterile cabinet equipped with ultraviolet sterilizing lamp which was turned off only during handling of tissues. Cultures were kept in continuous light, at a temperature of 26^o C . In the light, all cultures were kept under continuous illumination of florescent sources yielding about 4300 lux intensity.

The Plating efficiency was calculated as follows: . initial number of living free cells was 173, cell clumps 158, and total living units 331per plate. Plating efficiency = (number of colonies produced per plate)/(number of living units per plate) X 100

RESULTS

Pith cells of Pelargonium were induced to divide when removed from the intact plant and cultured on White medium plus 2, 4-D and kinetin, both at 10^{-6}M Figure (1). When 2, 4-D or kinetin was omitted from the medium, no cell division was observed. The optimal concentration of 2,4-D and kinetin was 10^{-6} M. Cell division began after 5 to 6 days of cultivation, as indicated by anatomical sections Figure (2). Only those cells near the cut also found among the proliferated cells. After 3 weeks of culture, an obvious callus was formed Figure (1). Fresh weight increased 2 to 3 fold over the original inoculums. Pith callus was excised from such cultures and sub cultured on White or Murashige medium supplemented with 10^{-6} M 2, 4-D and kinetin.

Results showed Table (1) that Murashige medium permitted more growth of callus than White medium, although the reverse was true for induction of growth in initial pith explants. Pelargonium pith callus was isolated on mid August. Since then, cultures were periodically transferred to fresh Murashige medium plus 4-D and kinetin at 10^{-6} M, and are maintained under continuous light at 26^{o}CPelargonium pith callus was so friable that cell suspensions can be prepared by gentle shaking without difficulty. When cell suspensions were mixed in Petri dishes with Murashige medium plus 10^{-6}2,4-D and 6-dimethylallylaminopurine, it was found that a large number of colonies was formed. The plating efficiency was brought to about 33% in an average of 8 cultures Table (2).

To investigate whether the visible colonies were produced from free cells or small cell clumps, several hundred free cells in plant cultures were circled with a marking pencil and observed periodically under a cut ceased after one or a few divisions. They never grew continuously to form macroscopically visible colonies. This implied that all visible smallest inoculums known to have grown to a macroscopically visible colony had 4 to 5 living cells Figure (3). After 4 weeks of cultivation, most colonies reached an average size of 3 to 5 mm in diameter, and could be isolated and grown further.

DISCUSSION

The ready growth of excised pith tissues on artificial media raises the question; why pith cells not divide in the intact plant? One possibility is that the plant may produce certain controlling bacterial contaminants, which were present in small numbers and undetectable can actively multiply, stored in the pith cells, were they interfere with the operation of some systems required for growth and cell division [12].

If the growing pith callus is dissociated into small cell clumps and free cells, and plated on agar medium in a Petri dish, a more effective kinetin, such as 6-dimethylallylaminopurine, is required in order to promote colony formation. The plating efficiency was about 33% Table (2). Similar results were obtained [13] when only 30% of the shoots produced an organogenic callus which was capable of being continuously sub cultured, using shot tips of *P.* Zonale cultivars. Occasionally, Free cells in plate cultures are observed to divide in the presence of other cells and small cell clumps. The established cultures are pale with a slightly yellowish cast, friable, and occasionally with a few tracheids found among the rapidly growing cells. No organs have ever been formed. This is in agreement with Mayer [14] who showed that calli and roots but not shoots could be induced to develop from stem segments of Pelargonium zonale.

However, no continuous cell division was noted in individual free cells cultured in plates containing a chemically defined medium. This failure indicates that cells may be able to divide continuously only in an extremely complex created by mass of tissue [15].

The experimental results have also revealed the different requirements of nutrition for growth in Pelargonium pith cells. For the indication of cell division in pith explants on White medium containing auxin and kinetin is required. Up on sub culture, Murashige medium permits a much more rapid growth than White medium Table (1). Since Murashige medium contains higher concentrations of inorganics than White medium. The decrease in growth of callus on White medium must be the result of the limitation of certain in organic constituents, such as nitrogen and potassium [10]. Plant ages are also important variables affecting tissue culture and plant regeneration [16]. This displays real difference between pith cells and stem buds explants on their growing behaviors.

These differences in behavior suggest that yet described control mechanisms may exist. The experimental results have also revealed the different requirements of media for growth in Pelargonium pith callus needs more investigation in subsequent publication.

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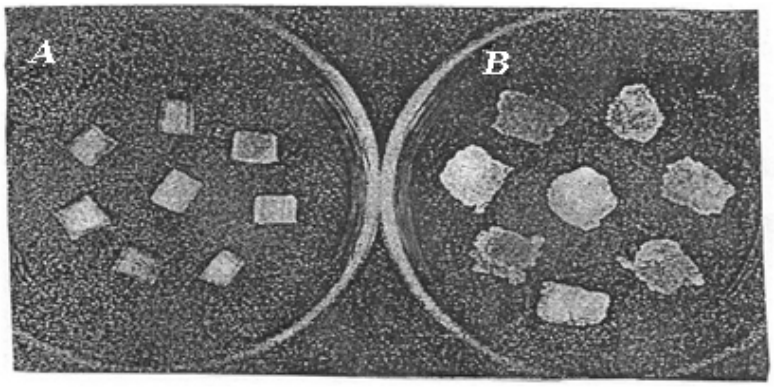
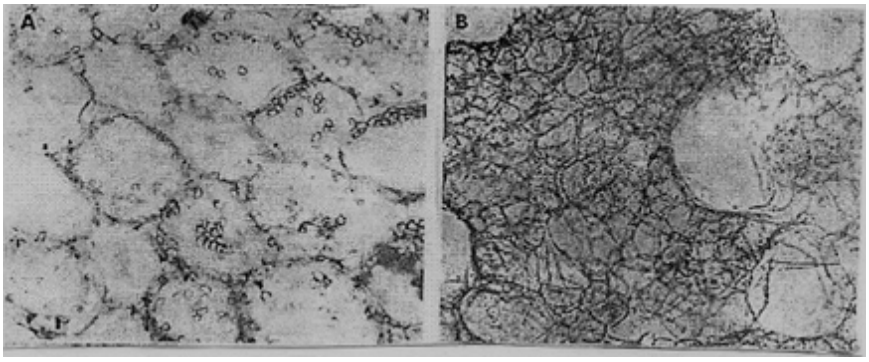
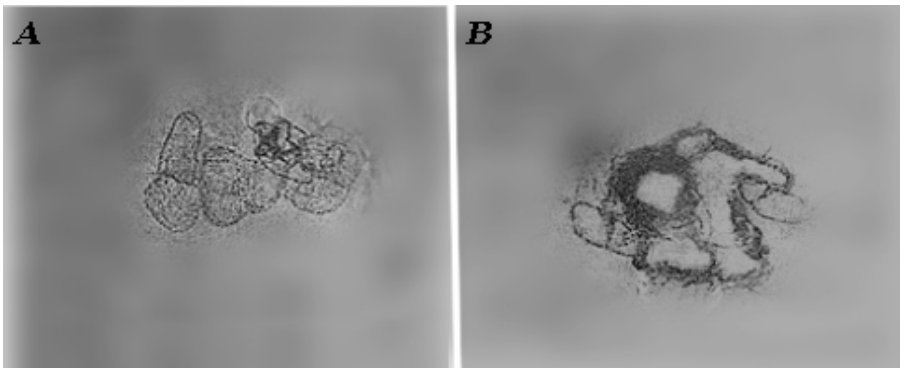
Figurs and Tabels.



Figur (1) Pelargonium pith cultures (a) before culture (b) after culture on W medium plus, for 3 weeks.



Figur(2) Pelargonium pith cultures (a) before (b) after cell division. magnifications (220X).



Figur (3)Pelargonium pith cell division in plated culture (a) 5 cell clumps (b) this clump become a visible colony(220X)

Table (1) Comparison of the effect of White medium and Murashige medium on the growth of Pelargonium pith and pith callus. Both media were supplemented with 2,4-D and kinetin at 10^{-6}M. Initial fresh weight of pith was 75 mg and pith callus 90 mg. Duration for culture; 4 weeks.

Medium	*Increase in fresh weight in mg*	
	Pith	*Pith callus*
White	*167*	*155*
Murashige	*311*	*677*

***T*able(2) Growth in plates of 3 weeks old cultures of pelargonium pith callus cells on the M medium supplemented with 2,4-D and 6-dimethylallylaminopurineat 10^{-6}M. initial number of living free cells was 173, cell clumps 158, and total living units 331per plate..**

Plate	*No. of colonies per plate*	*´ Plating efficiency*
1	*200*	*60.4*
2	*104*	*31.4*
3	*160*	*48.3*
4	*100*	*30.2*
5	*58*	*17.5*
6	*65*	*19.6*
7	*67*	*20.2*
8	*110*	*33.2*
Mean	*108*	*32.6*

Plating efficiency = (number of colonies produced per plate)/(number of * living units per plate) X 100