

CALLUSES FROM TOMATO CV. TYMOTI AND THEIR MORPHOLOGICAL CHARACTERISTICS AS SUPPORTING MATERIAL FOR PLANT TISSUE CULTURE LESSON

W.D. Savitri, A.B. Ferina, Y. Octavia, E. Muliawan, E.E. Effendi

Department of Biology, Faculty of Biotechnology, University of Surabaya
Jalan Raya Kalirungkut Surabaya 60292, East Java, Indonesia. Phone/fax: (031) 2981399/1278
winasavitri@staff.ubaya.ac.id

ABSTRACT

Plant tissue culture is one of interesting and important lessons in Department of Biology, Faculty of Biotechnology, University of Surabaya. Unfortunately, many students do not understand enough of what actually callus is. They also do not have sufficient understanding about callus types. The problem probably arises from the lack information about callus types in a real photograph. This experiment was conducted by students, aimed to produce callus from leaf explants of tomato cv. Tymoti by using benzylaminopurine (BA) and thidiazuron (TDZ) (0.5-2.5) ppm, each of which was combined with 0.1 ppm indoleacetic acid (IAA). After five weeks, the various types of callus were morphologically characterized and the photographs were collected. The photographs will be used as supporting materials in plant tissue culture lesson.

Keywords: callus types, tomato cv. Tymoti, BA, TDZ, IAA

INTRODUCTION

As one of the interesting applied subjects in Department of Biology, Faculty of Biotechnology, University of Surabaya, plant tissue culture is needed to be understood by students. Unfortunately, students oftenly have difficulty in characterizing callus morphology, since the information about callus types in a real photograph is limited. To date, the sources of callus photograph were mostly provided from journals and text books.

Callus is unorganized mass of proliferating cells found in in vivo and in vitro plants. In a normal condition (in vivo), plants develop callus due to the wound healing process, while in in vitro condition, it is only produced if growth regulator is added to the culture medium. In vitro callus has some purposes, i.e. to produce cell culture, as research materials for plant transformation and produce new plants through indirect regeneration pathway.

In plant biotechnology, tomato is a model organism which frequently used because of its short life cycle (Nath et al, 2014) and have already known genetically (The tomato genome consortium, 2012). In this research tomato is used because its easiness to regenerate and induce by using plant growth regulator (PGR). The attempts to induce callus have been conducted from different tomato explants and various PGR, namely 0.5 ppm 2,4-D from nodal segments (Jehan and Hassanein, 2013), 0.5 ppm NAA from hypocotyls

(Osman et al, 2010), and 1 ppm BAP + 1 ppm NAA from anther explants (Brasileiro,1999).

This experiment objected to compare callus types induced by BAP, TDZ and IAA. Moreover, this also aimed to characterize the callus based on its morphological feature. The information will be very useful as supporting material for plant tissue culture lesson.

MATERIALS AND METHODS

Preparation of plant material

Nodal parts of tomato cv. Tymoti were grown in Murashige and Skoog (MS) basal medium containing 1 ppm indole acetic acid (IAA) for 10 weeks. The leaves were then collected as plant materials. Under sterile condition, they were excised 0.5x0.5 cm² in size. The leaf discs were subsequently cultured on to culture media.

Culture media

MS basal medium containing 3% sucrose and 100 mg myoinositol solidified with 0.8% agar (basal mixture) was used. As much as 1 ppm IAA was added to the basal mixture for preparing medium to grow the nodes, whereas for the treatments, the basal mixture was enriched with BAP (0.5, 1.0, 1.5, 2.0, 2.5) ppm + 0.1 ppm IAA and TDZ (0.5, 1.0, 1.5, 2.0, 2.5) ppm + 0.1 ppm IAA. The pH was adjusted at 5.8 and then autoclaved at 121°C, 1.5 atm for 20 minutes.

In vitro culturing and callus development

Every treatment was conducted in a series of 4 bottles and each bottle contained 3 explants. Each treatment was divided into two culture conditions, i.e. dark condition (24h dark) and light condition (16h light period per day). Incubator was set at $23 \pm 2^\circ\text{C}$ and 80% humidity. The light intensity was regulated at 1.000 lux with 16/8 h (light/dark) cycle. The development of callus

were observed and photographed after 5 weeks of incubation.

Photograph collection and characterization of callus

Calluses were photographed with Canon EOS 60D 18-55 mm. The photographs were then characterized based on the morphological features, i.e. compact, friable, embryonic, and organogenic (shooty and rooty).

RESULTS

Table 1. Effect of Various Plant Growth Regulator on Callus Development

Treatments (ppm)	Callus Formation (%)		Callus Type*		Callus Color		Callus Score**	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
0.5 BAP + 0.1 IAA	100	83.3	f,o	c,o	brownish	green	4	2
1.0 BAP + 0.1 IAA	100	100	f,o	f,o	brownish	green	4	2
1.5 BAP + 0.1 IAA	100	100	f,o	c,o	brownish	green	4	1
2.0 BAP + 0.1 IAA	100	100	f,o	c,o	brownish	green	4	2
2.5 BAP + 0.1 IAA	100	66.7	f,o	c,o	brownish	green	4	1
0.5 TDZ + 0.1 IAA	100	83.3	f,e	c,o	brownish	green	4	1
1.0 TDZ + 0.1 IAA	100	100	f,o	c,o	brownish	green	4	1
1.5 TDZ + 0.1 IAA	100	100	f	c,o	brownish	green	4	4
2.0 TDZ + 0.1 IAA	100	66.7	f,e	f,c	brownish	green	3	3
2.5 TDZ + 0.1 IAA	100	100	c,o	c,o	brownish	green	3	3

* **Callus Type** : f, friable; c, compact; o, organogenic; e, embryogenic

** **Callus Score**: 0, no callus formed; 1, quarter of explant formed callus; 2, half of explant formed callus; 3, entire explant formed callus; 4, callus size is twice of the initial explant

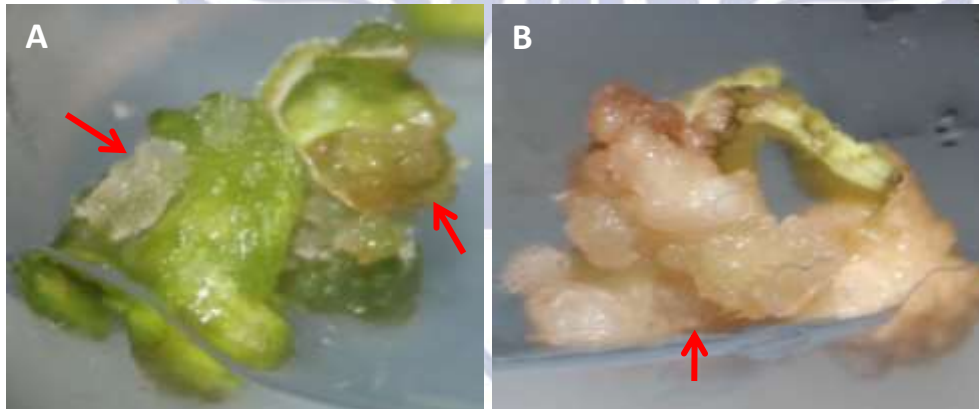


Figure 1. Friable callus. A, light condition (MS + 1.0 ppm BAP + 0.1 ppm IAA); B, dark condition (MS + 2.5 ppm BAP + 0.1 ppm IAA)

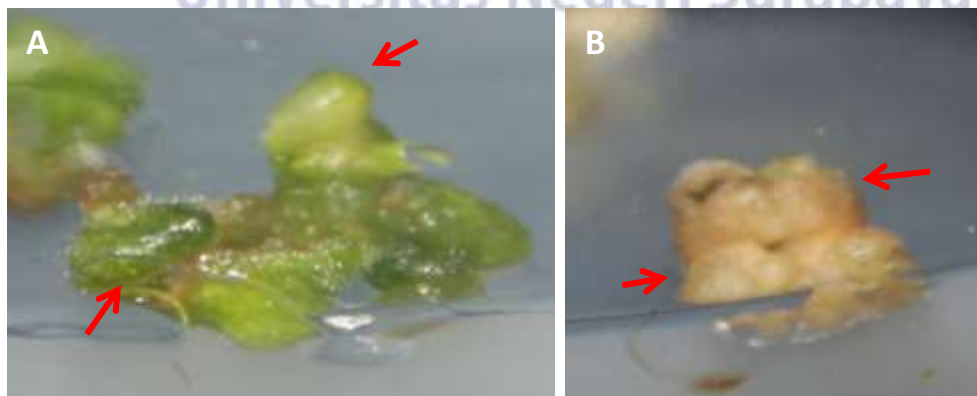


Figure 2. Compact callus. A, light condition (MS + 1.0 ppm TDZ + 0.1 ppm IAA); B, dark condition (MS + 2.5 ppm TDZ + 0.1 ppm IAA)

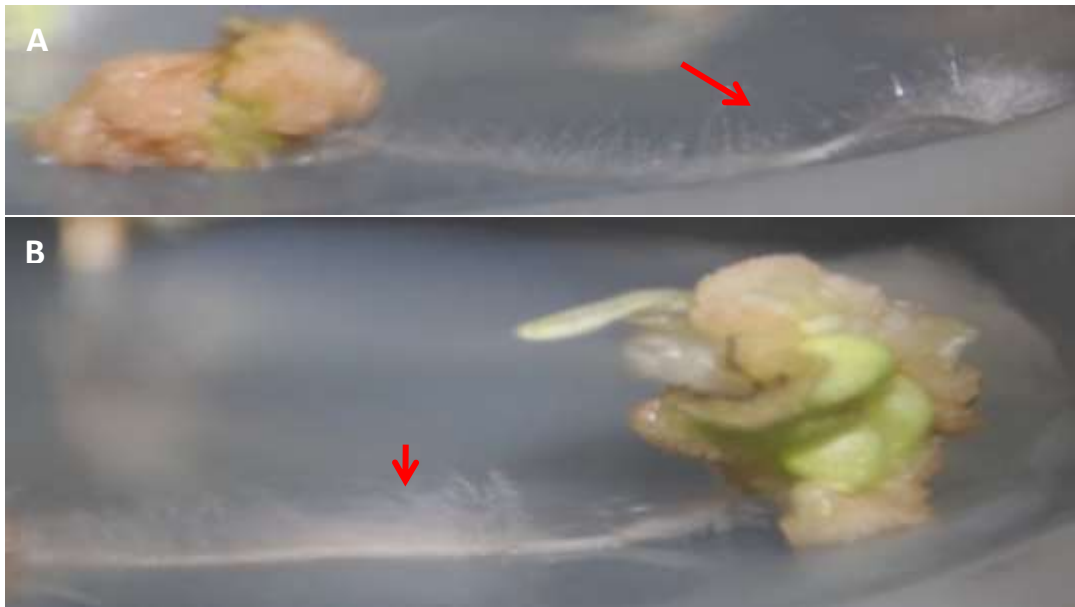


Figure 3. Rooty callus from leaf explants incubated in dark condition. A, on MS + 0.5 ppm BAP + 0.1 ppm IAA; B, on on MS + 0.5 ppm TDZ + 0.1 ppm IAA

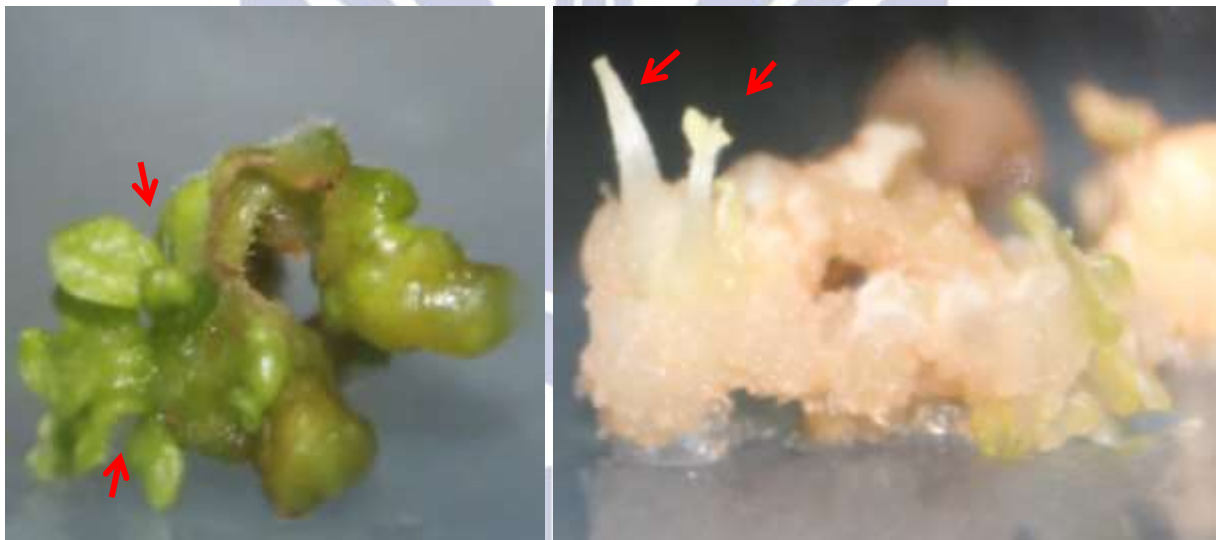


Figure 4. Shooty callus. A, light condition (MS + 2.5 ppm TDZ + 0.1 ppm IAA); B, dark condition (MS + 1.5 ppm BAP + 0.1 ppm IAA)



Figure 5. Embryonic callus from leaf explant cultured in dark condition (MS + 0.5 ppm TDZ + 0.1 ppm IAA)

DISCUSSION

All treatments incubated in dark condition successfully resulted maximum callus formation (100%). However, cultures in light condition produced lower frequency (66.7-83.3%) due to contamination. If the contamination could be avoided, there was a probability to achieve 100% callus frequency as well because the explants already showed response but no callus formed yet. So it was likely that there was no correlation between lower callus frequency and treatment in light condition.

Friable callus mostly produced in dark condition, whereas compact callus were more frequent on cultures incubated in light condition. Friable and compact callus are calluses which do not exhibit any obvious organ (Ikeuchi et al, 2013). They are differ physically, that friable callus is soft, fragile and watery, while compact callus is hard (Akaneme and Eneobong, 2008). In this research, friable and compact callus is shown on Figure 1 and 2. In dark condition, callus color was brownish, when it was green in light condition. This fact indicated that no chlorophyll produced on cultures incubated in dark condition. Moreover, callus score was higher on cultures incubated in dark than in light condition. Callus score showed the growth of callus compared to the size of initial explants. This result is inline with Jaramillo and Summers (1991) that callus induced from anther explants increased in diameter and number as the increase of dark period.

Almost all of the treatments produced organogenic callus. Organogenic callus is callus that shows distinct organ, i.e. root (rooty callus) or shoot (shooty callus) (Ikeuchi et al, 2013). Organogenic callus, in this study, was found frequently on both cultures incubated in dark and light condition. Figure 3 shows rooty callus in dark and light condition and shooty callus is as shown on Figure 4. Embryonic callus is callus which bears embryo-like structure (Ikeuchi, 2013). However, embryonic callus was only observed on cultures incubated in dark condition and only on cultures treated with TDZ (0.5 ppm and 1 ppm) in combination with 0.1 ppm IAA. Figure 5 shows embryogenic callus from explant cultured in 0.5 ppm TDZ + 0.1 ppm IAA. TDZ was reported to induce somatic embryogenesis in some species. Zhou et al (1994) reported the application of TDZ combined with naphthalene acetic acid (NAA) to induce somatic embryo from ovary explants of *Cayratia japonica*. TDZ induces somatic embryogenesis in peanut by accumulating the endogenous cytokinins (Murthy et al, 1995). Yet in tomato cv. Omdurman, TDZ was known as the inducer to produce high frequency of shoot formation (Osman et al, 2010).

The characterization of callus morphology was hardly accomplished only by naked eye. Stereo microscope was needed to make sure that a callus feature was actually an organ primordia or an embryo primordia. Furthermore, high resolution of image was also helpful to characterize the callus types. In this case, callus types could obviously observed only after the photographs were taken.

CONCLUSION

Callus formation from leaf discs of tomato cv. Tymoti by using combination of TDZ/IAA and BAP/IAA is better in dark condition than in light.

ACKNOWLEDGEMENTS

We thank to Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM), University of Surabaya (Ubaya) for funding this research.

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