Evidence of Auxin Induced Dissimilar Pigments in *Momordica charantia* L. Callus

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บทคัดย่อ

ศึกษาผลของ 2,4-D และ NAA ต่อการเกิดสารสีระหว่างการชักนำแคลลัสของมะระขี้นก *(Momordica charantia* L.*)* จากเมล็ดที่มีเยื่อหุ้มเมล็ดและเยื่อหุ้มเมล็ดที่ระยะแตกต่างกันคือยังไม่เจริญเต็มวัย เขียวเต็มวัยและสุก นำเมล็ดที่มีเยื่อหุ้มเมล็ดและเยื่อหุ้มเมล็ดมาวางบนอาหารกึ่งแข็งสูตร Murashige and Skoog (MS) ซึ่งเติม 2,4-D หรือ NAA 0, 1, 2 และ 4 มิลลิกรัมต่อลิตร แล้วนำไปเก็บภายใต้สภาวะที่มีแสงหรือที่มืดที่ 25°ซ เป็นเวลา 6 สัปดาห์ พบว่าอาหารสูตร MS ที่มี 2,4-D สามารถชักนำให้เกิดแคลลัสสีเหลืองและสีแดง ในขณะที่แคลลัสสีแดงไม่สามารถชักนำให้เกิดบนอาหารสูตร MS ที่มี NAA ได้ อัตราร้อยละของการเกิดแคลลัสสีเหลืองสูงสุดพบเมื่อเพาะเลี้ยงเมล็ดที่มีเยื่อหุ้มเมล็ดในระยะยังไม่เจริญเต็มวัยบนอาหารสูตร MS ที่มี NAA 4 มิลลิกรัมต่อลิตร ภายใต้สภาวะที่มืดเป็นเวลา 4 สัปดาห์ ในขณะที่อัตราร้อยละของการเกิดแคลลัสสีแดงสูงสุดพบเมื่อเพาะเลี้ยงเยื่อหุ้มเมล็ดในระยะยังไม่เจริญเต็มวัยบนอาหารสูตร MS ที่มี 2,4-D 2 มิลลิกรัมต่อลิตร ภายใต้สภาวะที่มืด เป็นเวลา 2 สัปดาห์

คำสำคัญ: การชักนำแคลลัส การมีสารสี แคลลัสสีเหลือง แคลลัสสีแดง มะระขี้นก

Abstract

The effects of 2,4-D and NAA on pigmentation during callus induction from seed with aril and aril of *Momordica charantia* L. at various stages of fruit: immature, mature-green and ripe were studied. Seed with aril and aril were placed on Murashige and Skoog (MS) semi-solid medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D or NAA. All cultures were kept under light or dark conditions at 25°C for 6 weeks. The results showed that MS medium containing 2,4-D could induce the formation of yellow and red callus whereas the red callus was unable to be initiated on MS medium comprised NAA. The highest percentage of yellow callus formation was obtained after culturing seed with aril at immature stage on MS medium consisted of 4 mg/l NAA under dark condition for 4 weeks, while

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Received 24 January 2011 ; Accepted 29 March 2011

the maximum percentage of red callus production was found after culturing immature aril on MS medium fortified with 2 mg/l 2,4-D under dark condition for 2 weeks.

Keywords: Callus Induction, Pigmentation, Yellow Callus, Red Callus, *Momordica charantia* L.

1. Introduction

Momordica charantia L. is a flowering climber that belongs to the Cucurbitaceae family. It is one of the renowned vegetable for human consumption in all regions of Thailand. All parts of this plant such as immature fruit, young shoot and leaves contain bitter substances. Before consume those parts, they are generally boiled, steamed or stir fried to slightly reduce the bitter taste. At ripening stage of *M. charantia* fruit, pericarp was rich in β-carotene whereas lycopene dominated in aril [1], [2]. Apart from cuisine, their phytochemicals also have biological and pharmacological activities, e.g. anticancer, anti-HIV, anti-inflammatory and antioxidant [3]. Its fruit juice helps decreasing blood glucose (antidiabetic) and reducing adiposity [4], [5]. Moreover, the extract of *M. charantia* fruit has good larvicidal activity against mosquitoes and significant antimutagenic activity as well [6], [7].

For the in vitro culture of *M. charantia*, there were a number of researches had been reported so far. Flowers could be developed after cultured shoot tips of *M. charantia* on Murashige and Skoog (MS) medium [8] consisted of kinetin or N^6-benzyladenine [9]. Clonal propagation of *M. charantia* via direct and indirect organogenesis was successfully obtained from many works too [10] - [12]. In the case of somatic embryogenesis, numerous globular embryos of *M. charantia* had been found in MS liquid medium comprised 1.5 mg/l 2,4-D and when plant growth regulator was removed from this medium, the later developmental phases, heart and torpedo stages, were noticed [13]. Besides, adding exogenous polyamines to the MS medium showed an increase in fresh weights and number of somatic embryos of *M. charantia* embryogenic calli [14].

In aseptic plant culture, callus, a group of unorganized plant tissues, is an important plant biotechnology tool which can be extensively used in various ways, such as, indirect somatic embryogenesis [15], generation of somaclonal variation [16], and secondary metabolite production [17]. Though callus culture of *M. charantia* had recently been applied in flavonoid production [18], effects of synthetic auxin on pigment formation in *M. charantia* callus was still concealed. Hence, the purposes of this study were to initiate callus from seed with aril and aril of *M. charantia* and investigate variations in pigment production.

2. Materials and Methods

Fresh fruits of *Momordica charantia* L. at various stages: immature, mature-green and ripe (Figure 1 to 3) were purchased from a local market in Nonthaburi province, Thailand. After cleaning with tap water, they were soaked under laboratory detergent for 15 min and rinsed again with tap water. Consequently, the fruits were immersed in 70% (v/v) ethanol for 3 min and flamed. Under aseptic conditions, each fruit was cut open and seeds with arils were taken from the fruit. Seed with aril was then placed on Murashige and Skoog (MS) semi-solid

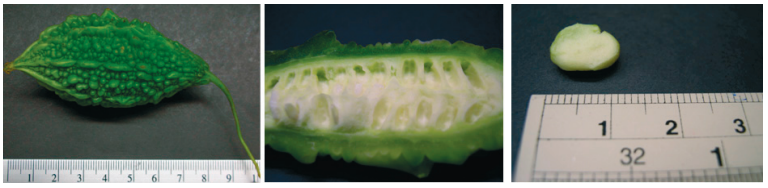

Figure 1 *M. charantia* immature fruit (left), inside the immature fruit (middle) and immature seed with aril (right).

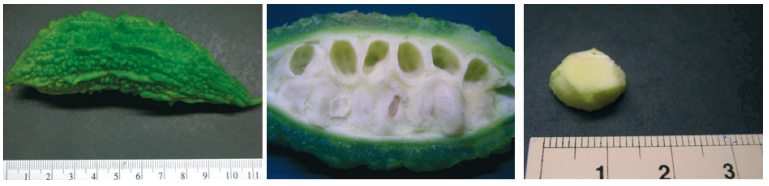

Figure 2 *M. charantia* mature-green fruit (left), inside the mature-green fruit (middle) and mature-green seed with aril (right).

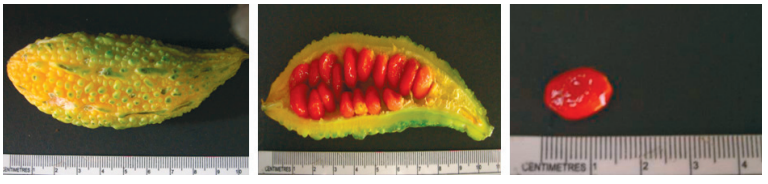

Figure 3 *M. charantia* ripe fruit (left), inside the ripe fruit (middle) and ripe seed with aril (right).

medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D or NAA. For individual aril, the aril was removed from seed and transferred to MS semi-solid medium consisted of 0, 1, 2 and 4 mg/l 2,4-D or NAA. All media in this research were adjusted to pH 5.7, gelled with 0.9% (w/v) agar, and autoclaved at 121 °C and 15 psi for 20 min. Cultures were kept in a growth room under dark condition or 16 h of illumination from cool white fluorescent lamps (24.2 µmol m^{-2} s^{-1} light intensity) and 8 h of darkness at 25±2 ˚C. The data of pigment production in callus were collected for 6 weeks. The quantity of color that callus produced was determined by the area of color on each callus in which the maximum percentage should not more than 100%.

3. Results and Discussion

Tissue culture of *Momordica charantia* L. was done with various explants. However, the culture of seed with aril or aril of this plant had never been described. It is quite interesting that aril, a fleshy seed cover or coating, of *M. charantia* has the bright-red color when fruit ripen (Figure 3). This red pigment was identified as lycopene which contained 96% of the carotenoids of the ripe seeds [1]. As a consequence, our experiment attempted to culture the seed with aril or aril of the ripe fruit on MS medium containing various concentrations of 2,4-D or NAA with the anticipation of red callus production. Unfortunately, ripe seed with aril and ripe aril were unable to form any calli on these

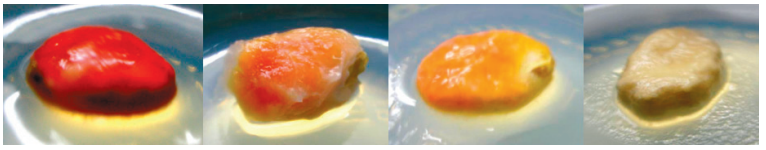

Figure 4 Development of ripe seed with aril of *M. charantia* on MS medium containing 2,4-D or NAA after culturing 0, 2, 4 and 6 weeks (left to right, respectively).

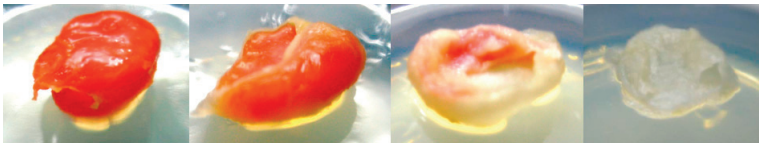

Figure 5 Development of ripe aril of *M. charantia* on MS medium containing 2,4-D or NAA after culturing 0, 2, 4 and 6 weeks (left to right, respectively).

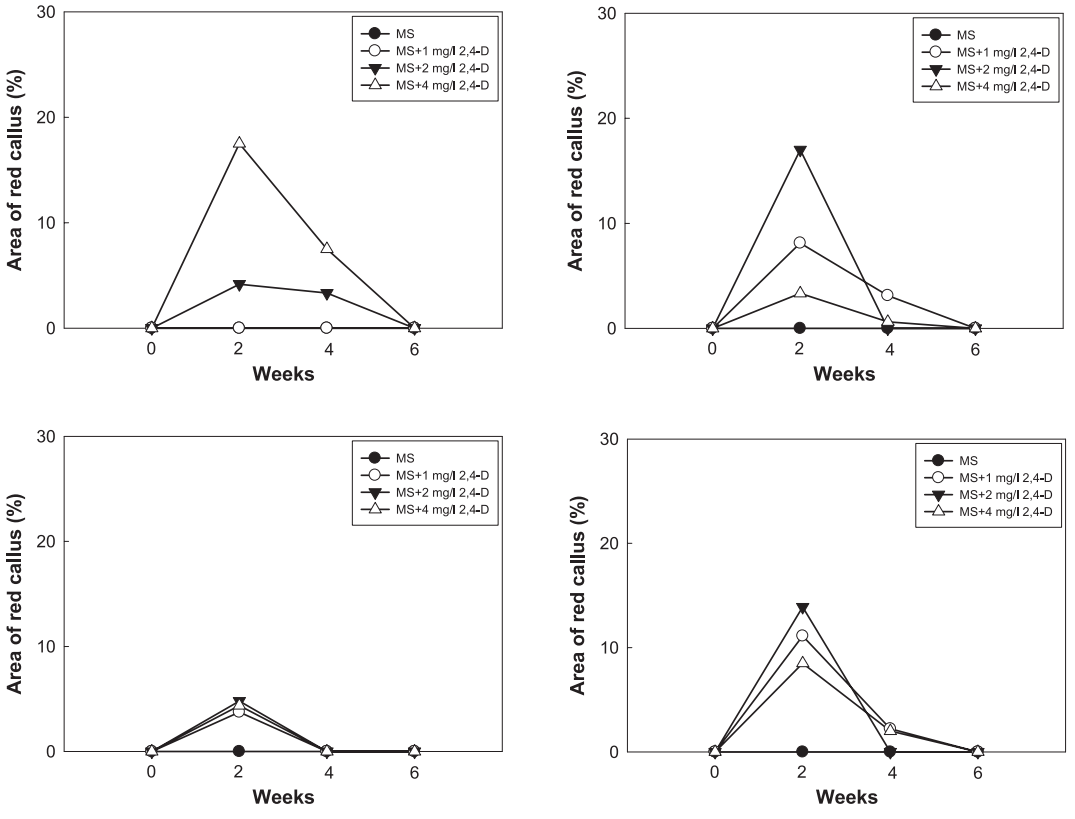

Figure 6 Percentage of red callus of *M. charantia* from immature (upper) and mature-green (lower) seed with aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D under light (left) and dark (right) conditions.

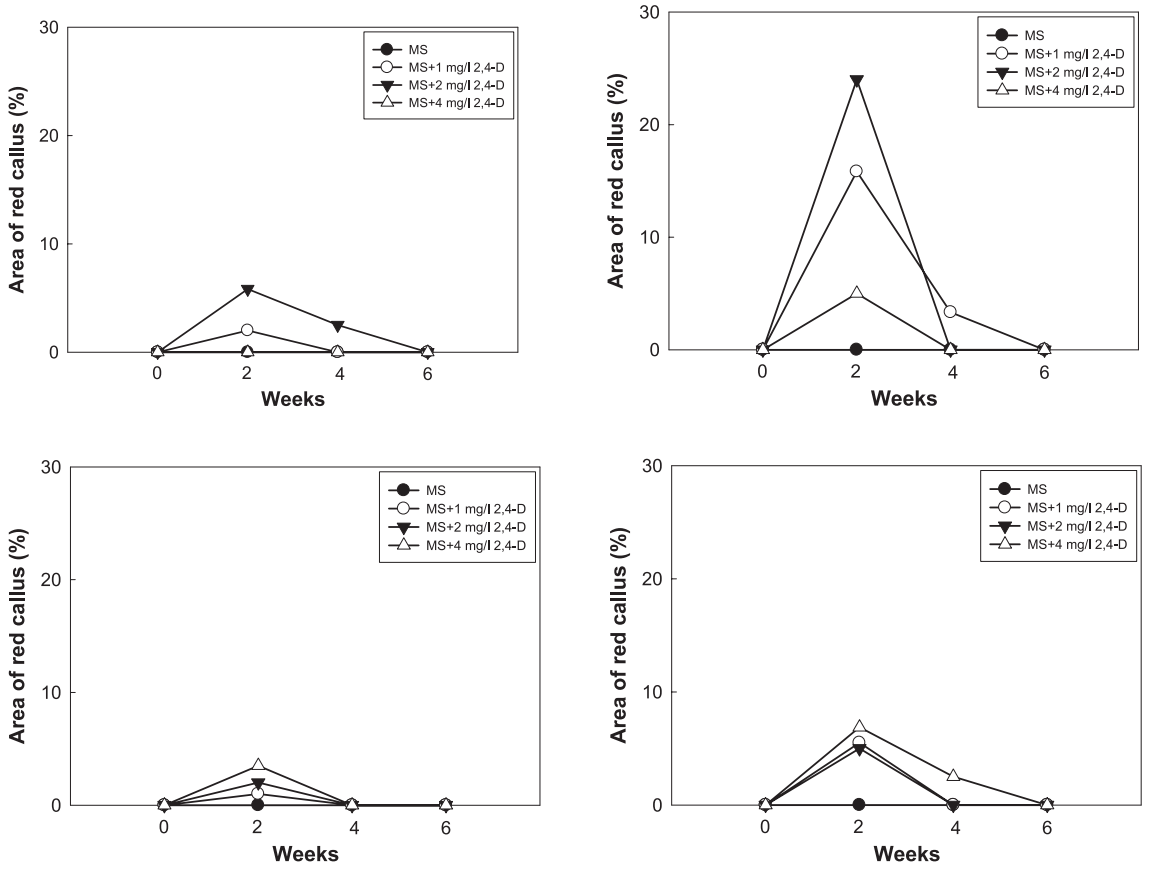

Figure 7 Percentage of red callus of *M. charantia* from immature (upper) and mature-green (lower) aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D under light (left) and dark (right) conditions.

media. The color of seed pulp or seed membrane changed from red into colorless at last (Figure 4 and 5). This result indicated that tissue of ripe seed with aril and ripe aril may develop beyond the dedifferentiation process and these mature cells could not reverse into meristematic stage and form the undifferentiated callus tissue. Conversely, seed with aril or aril of the immature and mature-green *M. charantia* fruits on MS medium supplemented with different amounts of 2,4-D or NAA were capable of developing the pigmented calli.

In the present research, callus induction of *M. charantia* showed some variations in pigment production since the red callus was not found on MS medium containing NAA whether from seed with aril or aril explants. This finding revealed that types of plant growth regulators seem to have an effect on pigmentation of *M. charantia* callus. However NAA, in this case, did not promote red callus production. Nevertheless, MS media comprised 2,4-D showed different responses on the production of red pigment during callus initiation between seed with aril and

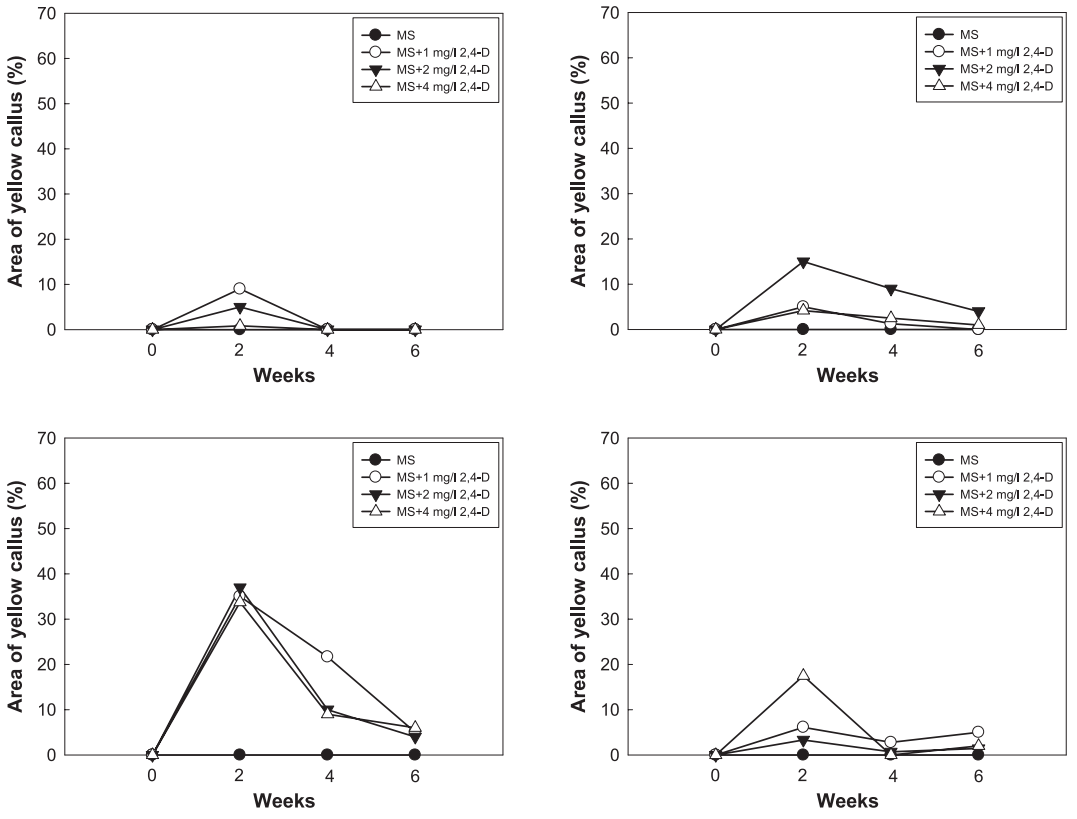

Figure 8 Percentage of yellow callus of *M. charantia* from immature (upper) and mature-green (lower) seed with aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D under light (left) and dark (right) conditions.

aril. The immature seed with aril produced red callus better than mature-green seed with aril under light condition while the immature aril gave the highest percentage (24%) of red pigment-producing callus over other treatments on the MS medium adding 2 mg/l 2,4-D in dark condition for 2 weeks (Figure 6 and 7).

While red callus was unable to obtain from MS media consisted of NAA, yellow callus could be initiate on MS media fortified with both 2,4-D and NAA. Overall, MS media comprised NAA were obviously competent to induce yellow callus better than 2,4-D (Figure 8 to 11). The maximum yellow callus production from MS media consisted of 2,4-D was on the second week whereas MS media containing NAA mostly had the highest peak on 4 weeks. Although 2,4-D appeared to enhance the yellow pigment formation of *M. charantia* callus more rapidly than NAA, the latter synthetic hormone could initiate higher amount of yellow callus on seed with aril and aril than the former. In addition, all concentrations of NAA were efficient to promote yellow callus formation but high concentration (4 mg/l) seemed to have more potential than the

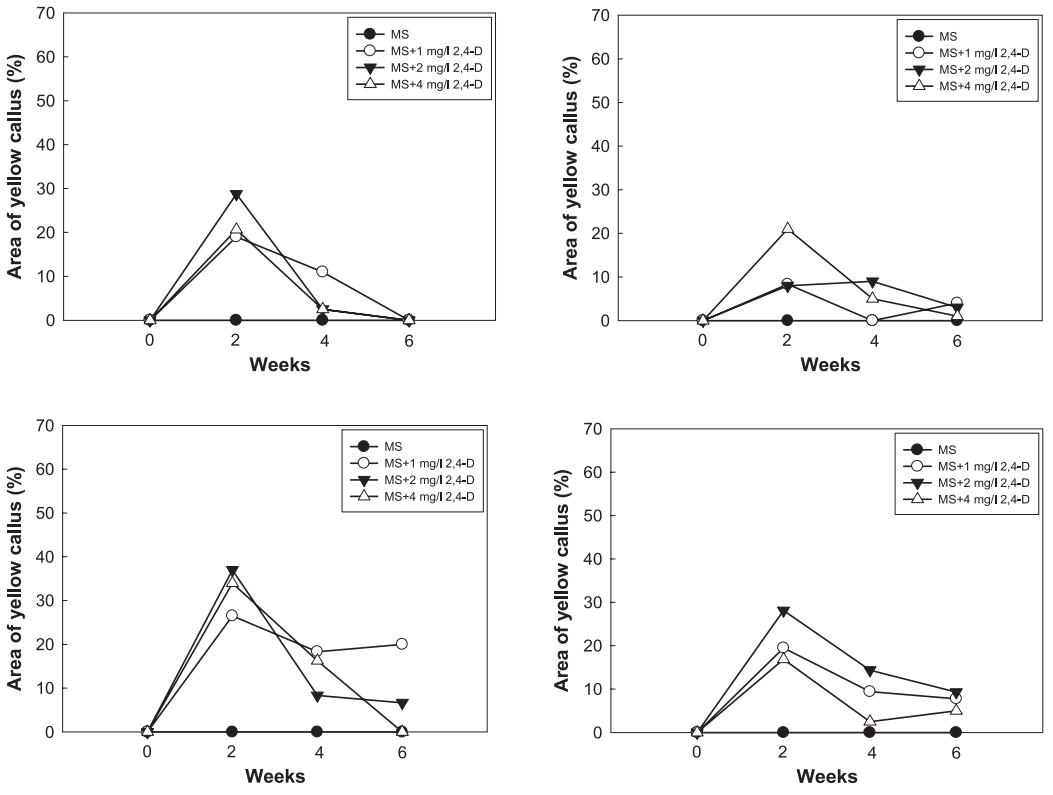

Figure 9 Percentage of yellow callus of *M. charantia* from immature (upper) and mature-green (lower) aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l 2,4-D under light (left) and dark (right) conditions.

lower ones. The greatest percentage of yellow callus formation (64.17%) was accomplished after culturing immature seed with aril on MS medium consisted of 4 mg/l NAA under dark condition for 4 weeks.

Pigment production from callus typically illustrated some alterations according to plant growth regulator and illumination. In *Lithospermum erythrorhizon,* variation in shikonin derivatives biosynthesis was controlled by auxin and light. This pigment content increased when callus had been cultured on medium containing IAA in the dark. When IAA was replaced with 2,4-D or illuminated with blue light, the colorant decreased markedly [19]. On the other hand, the colorization in *Portulaca* callus was induced by light, but disappeared in the dark. Betalain pigment synthesis was noticeable within 30 h after irradiation and showed positive correlation with illumination periods [20]. Additionally, various 2,4-D concentrations also had an effect on betalain accumulation in pigmented callus of *Portulaca* [21]. For callus of potato, different kinds of plant growth regulators demonstrated the deviation on pigment formation. For example, NAA mostly gave

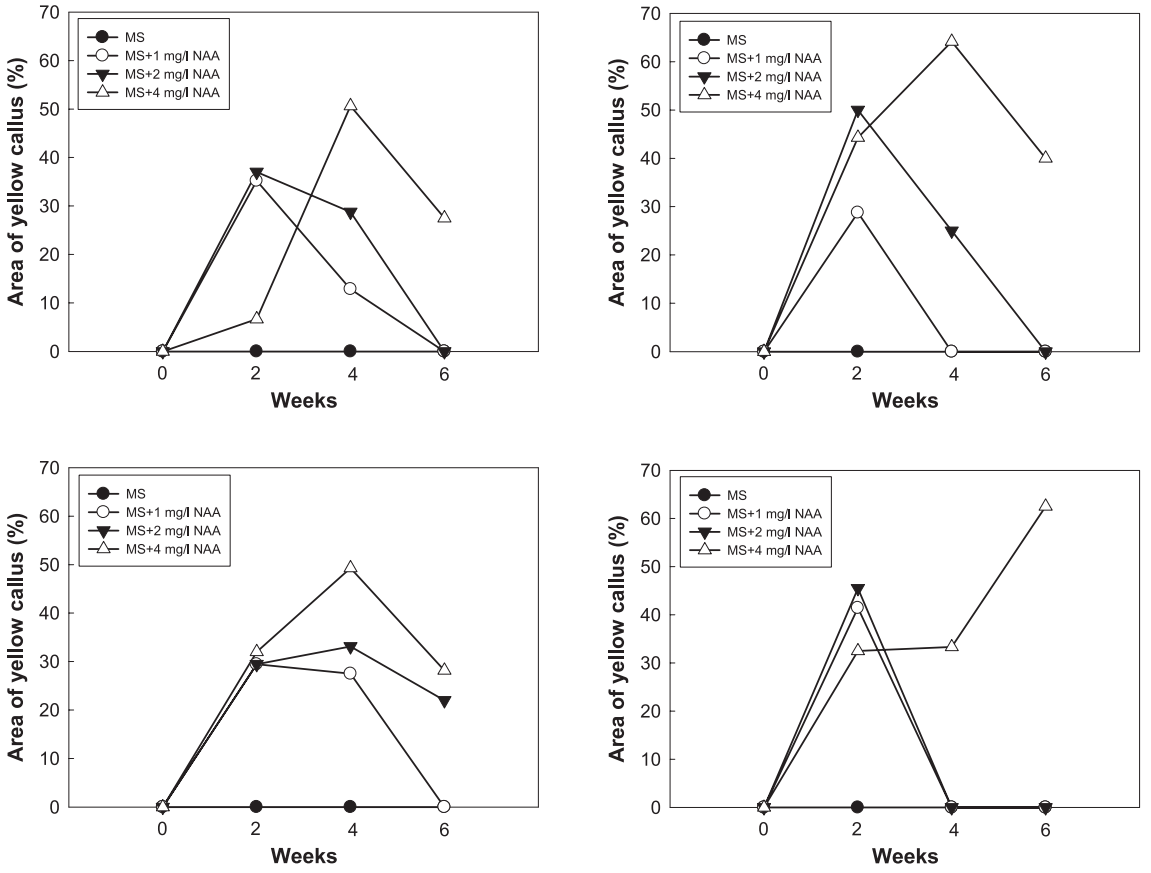

Figure 10 Percentage of yellow callus of *M. charantia* from immature (upper) and mature-green (lower) seed with aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l NAA under light (left) and dark (right) conditions.

light green callus, 2,4-D provided yellow callus and BA together with TDZ offered green callus [22]. These research outcomes mentioned above absolutely supported our finding because plant growth regulators (NAA and 2,4-D) and illuminations (light and dark conditions), in the present results, had the effect on pigmentation of *M. charantia* callus as well.

In conclusion, pigment formation in callus is an important step for large scale or industrial production of natural colorant from plant tissue culture. In this study, yellow and red callus of *M. charantia* were successfully induced on MS media consisted of NAA and 2,4-D (Figure 12). It is feasible that these pigments are carotenoids in which the yellow color is perhaps β-carotene or zeaxanthin and the red color is probably lycopene. These colorants from *M. charantia* callus should have a further examination to ascertain that they are the desirable pigments. Moreover, the low percentage of red pigmentation also needs to be improved by using other plant growth regulators alone or in combination.

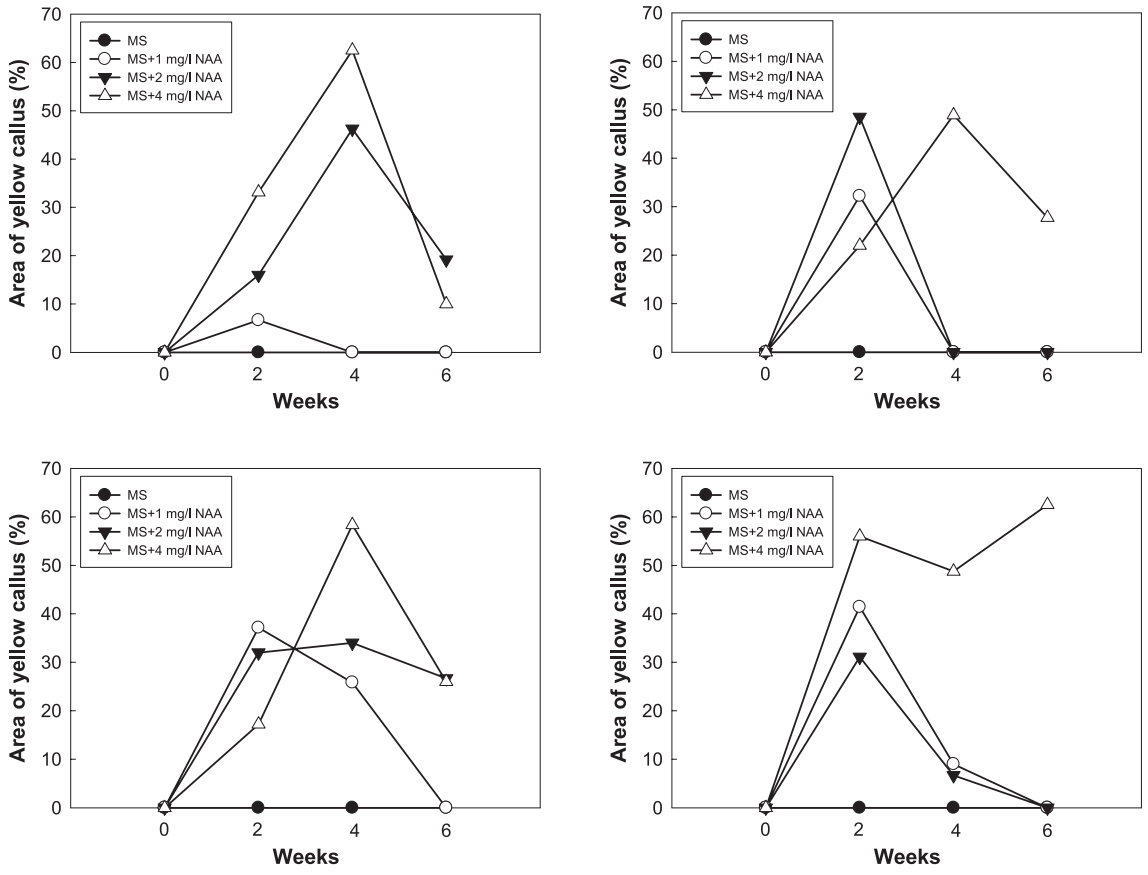

Figure 11 Percentage of yellow callus of *M. charantia* from immature (upper) and mature-green (lower) aril cultured on MS medium supplemented with 0, 1, 2 and 4 mg/l NAA under light (left) and dark (right) conditions.

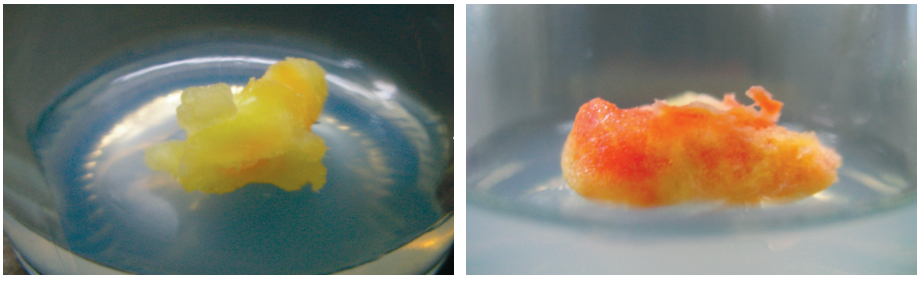

Figure 12 Yellow callus from mature-green aril *of M. charantia* culturing on MS medium supplemented with 4 mg/l NAA under dark condition for 2 weeks (left) and red callus from immature aril of *M. charantia* L. culturing on MS medium containing 2 mg/l 2,4-D under dark condition for 2 weeks (right).

4. Acknowledgement

We would like to thank Miss Butsarin Phanpoolperm, our research colleague who passed away, for her generous assistance in the media preparation, culturing the explants and data collection.

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