IN VITRO CALLUS INDUCTION OF WHITE PUMPKIN (CUCURBITA MOSCHATA DECNE) CV CASPERITA (F1 HYBRID) THROUGH DIFFERENT EXPLANTS

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Abstract - White pumpkin (*Cucurbita moschata* Decne.) cv. Casperita (F1 Hybrid) is a popular pumpkin in many countries, however, it produces unfertile seeds. The current study determined the appropriated concentrations of 2,4-D (2,4-Dichlorophenoxy acetic acid) and BAP (6-Benzylaminopurine) on callus induction using various explants as hypocotyl, epicotyl, cotyledons, petiole, and leaf. Steriled explants were cultured on MS media with various concentrations of 2,4-D (0 and 2 mg/L) and BAP (0, 0.1, 0.3 and 0.5 mg/L) for 20 days. Results found that MS media containing all 2,4-D concentrations induced callus in all explants. MS medium containing 2 mg/L 2,4-D in combination with 0.1 mg/L BAP provided high callus percentage (100%) from hypocotyls, epicotyls and petiole. When comparison of callus formation from various explants, it was found that hypocotyl showed the highest performance on callus formation in term of fresh weight, dry weight, and callus size. This discovery can be applied to white pumpkin micropropagation improvement in the future.

Keywords - Callus induction, BAP, 2, 4-D, White Pumpkin

I. INTRODUCTION

Pumpkin species is a member of the genus *Cucurbita* within the economically important Cucurbitaceae family which has various representatives, including pumpkins (*Cucurbita moschata* and *Cucurbita pepo*) and squash (*Cucurbita maxima*), species of great economic and food value [1] Winter squash and pumpkin are usually grown for their fruits i.e. immature for summer squash, and mature for the winter squash and pumpkin [2]. Casperita cultivar is one of small white pumpkin which considerate as intermediate resistance to powdery mildew and mosaic disease [3-4]. The white fruit has a classic pumpkin shape with deep smooth ribs and a strong, green handle. This attractive pumpkin also has sweet, white flesh that eats similar to acorn squash, plus they make great fall decorations. The application of tissue culture technique for mass propagation of cucurbitaceous vegetable crops has been well demonstrated [5-9], especially callus culture. This technique has been used for many applications such as mass propagation, enzyme activity [10], genetic transformation [11], gynogenic haploid [12], polyploid induction, virus-free plant production and long term preservation [13]. Therefore, an investigation was under taken to develop a suitable protocol for efficient callus induction of Casperita cultivar F1 hybrid and it describes the comparative study of callus formation from different explants through tissue culture system.

II. MATERIALS AND METHODS

2.1 Plant material and sterilization: Mature seeds of the Casperita cultivar were obtained from the PP seed shop, Mahasarakham province, Thailand. Casperita seeds were soaked in water for 15 min prior to removing seed coat. The dehusked seeds were sterilized with 25 % (v/v) Sodium hypochlorite (Clorox) supplemented with 2-3 drops of Tween 20 for 25 mins with shaking, and then rinsed three times with sterile distilled water. **Seed germination:** Sterilized seeds were cultured on MS (Murashige and Skoog, 1962) medium without plant growth regulator. **Callus induction:** Different explants of 8 cm casperita plantlets were used as initiated explants for callus induction; hypocotyl, epicotyl, cotyledon, petiole and leaf. Approximately 1 cm of hypocotyl, epicotyl, petiole and 1 x1 cm of cotyledon and leaf were cultured on MS media supplemented with 3 % (w/v) sucrose and various concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D; 0 and 2 mg/l) and 6-Benzylaminopurine, benzyl adenine (BAP; 0, 0.1, 0.3 and 0.5 mg/l). The pH of the media was adjusted at 5.70-5.80 and solidified by adding 0.8 % (w/v) agar powder. After that, cultures were incubated at 25 ± 2 ºC under light condition (16/8 h light/dark). Callus induction percentage, callus feature, length, width, fresh and dry weight of callus were determined for 21 days after culture initiation. Dry weight was evaluated after incubation at 70 ºC for 3 days. Each treatment consisted of five replicates, and five explants were cultured in each replicate.

2.2 Statistical analysis: The experiments were Factorial in completely randomized design (CRD) with five replications. Data were tested by the one-way Analysis of Variance (ANOVA) and difference between means was separated by the Duncan's Multiple Range test ($p = 0.05$). The result was expressed as the mean ± standard error of mean (SE).

III. RESULTS AND DISCUSSION

Almost treatments which treated with 2 mg/l 2,4-D showed callus formation derived from different explants. The highest callus induction percentage was recorded in hypocotyl, epicotyl and petiole after cultured on MS medium supplemented with 2 mg/l 2,4-D in combination with 0.1 mg/l BAP while in cotyledon culture was MS medium supplemented with 2 mg/l 2,4-D and 0.3 mg/l BAP. In contrast, the suitable medium for callus induction from leaf was MS medium added 2 mg/l 2,4-D without BAP. Callus features derived from hypocotyl, epicotyl and petiole exhibited compact callus with yellow and white color while loose, brown and white calli were observed in cotyledon and leave derived calli (Figure 1-2). Considering callus size, we found that callus sizes approximately in width x length derived from hypocotyl, epicotyl, cotyledon, leave and petiole were 0.8 x 1.0, 0.8 x 0.9, 1.2 x 1.8, 0.8 x 1.5 and 0.8 x 1.1 cm, respectively. Our results were similar to Thiruvengadam et al. (2006) [14] who reported the development of melon (*Momordica charantia* L.) culture using various concentrations of 2,4-D and BAP.

Plant growth regulators (PGRs) are the most important factor to induce callus. Therefore, efficiency of callus induction depends on the ratio between auxins and cytokinins in medium cultivation. Callus can be effectively induced by culturing on media supplemented with relatively high level of both auxin and cytokinin found in some plants [15-16]. Auxin commonly used in callus induction is 2,4-D, while BAP is the most common of cytokinin used in callus induction process. Our results indicated that 2 mg/l 2,4-D was the most effective on callus induction of all explants culture. Therefore, 2,4-D is a necessary PGRs to induce calli from Pumpkin cv. Casperita (F1 Hybrid) explants, because 2,4-D is the most effective auxin to suppress organogenesis and trigger proliferation of cells in scutellum and mesocotyl to form callus [17]. There are many researches confirmed that 2,4-D was adequate for callus induction of pumpkin and squash [7, 18, 19]. Moreover, 2,4- D may play the role for only cell expansion which cannot divide in some case but when using 2,4-D in combination with BAP, the cell starts to growth in common with division process [20]. However, some researchers reported that MS medium with 1 mg/l 2,4-D and 2 mg/l kinetin was the optimum condition for callus induction of *Barringtonia racemosa* L. [21]. Therefore, type and concentration of PGRs are required for callus induction depend on plant species.

Hypocotyl and epicotyl were suitable explants for callus induction in this study that in accordant with Pal et al. (2007) [19] who reported that hypocotyl was high callus induction potential than cotyledon, petiole and leaf in summer squash (*Cucurbita pepo* L.) culture. However, regeneration from callus to plantlets of the Casperita cultivar is also required for mass propagation.

IV. CONCLUSIONS

This current study reports a successful and high frequency callus induction protocol of pumpkin cv. Casperita (F1 Hybrid) from different explants through *in vitro* culture system. The study demonstrated that 2 mg/l 2,4-D with 0.1 mg/l BAP under light condition provided the most effective callus induction of pumpkin cv. Casperita (F1 Hybrid). The novel discovery found in this study is the fact that hypocotyl and epicotyl are suitable explants that appropriated for callus induction in pumpkin cv. Casperita (F1 Hybrid). This discovery can be applied for micropropagation of other pumpkin and squash cultivars. In addition, the finding of this study will be useful for the first step of pumpkin improvement or rapid *in vitro* propagation through any innovative biotechnological approaches which provide a simple and reliable protocol for *in vitro* generating high frequency callus and further cultivar genetic transformation experiment toward high yielding improved through plant tissue culture and genetic transformation techniques.

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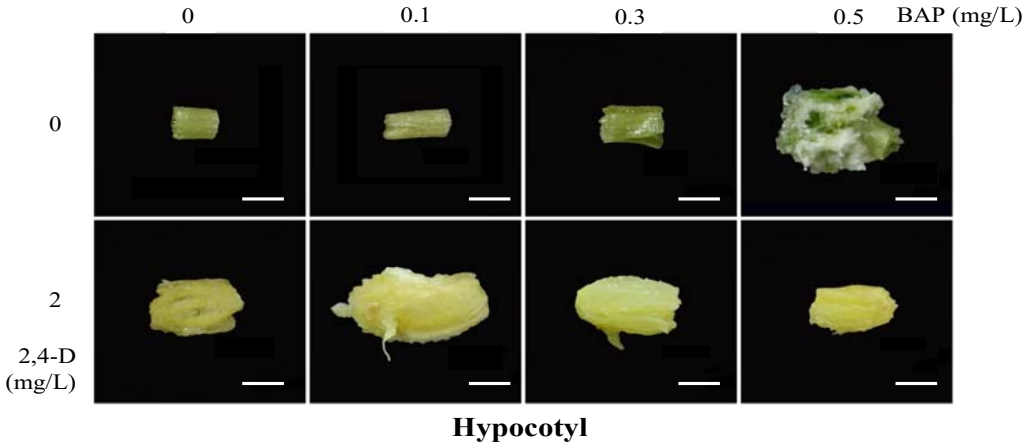
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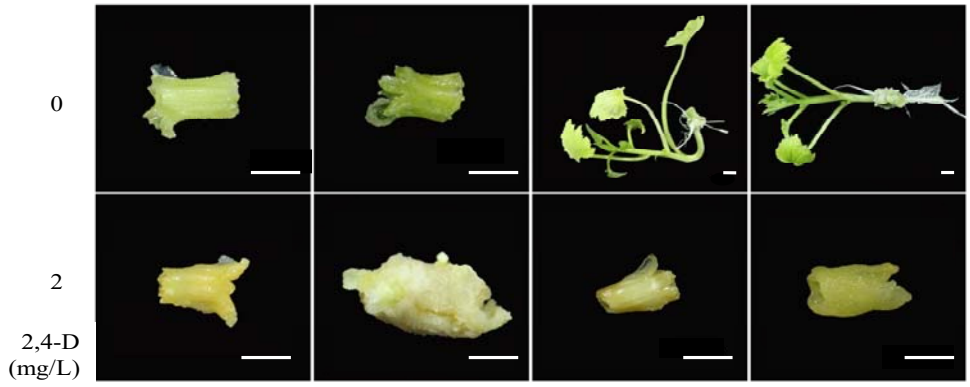
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Hypocotyl



Epicotyl

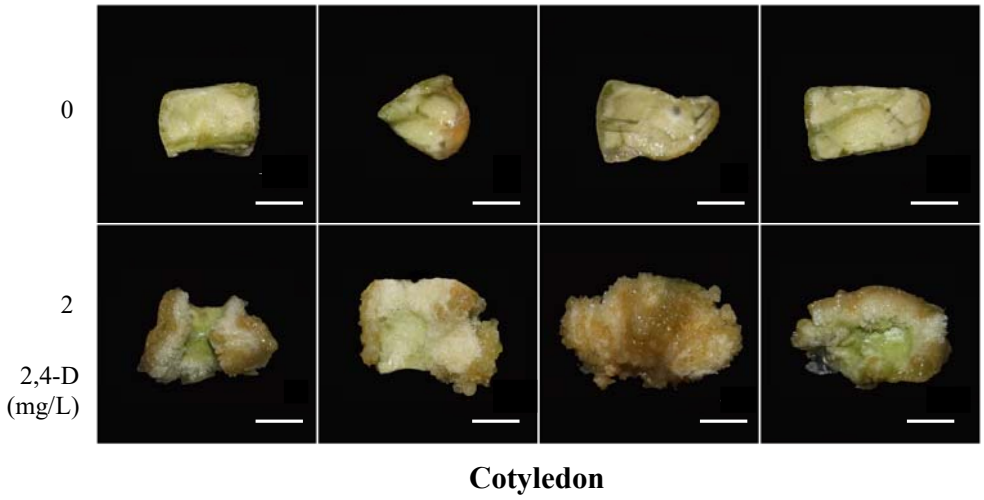


Fig.1. Callus features derived from hypocotyl, epicotyl and cotyledon cultured on MS media supplemented with various concentrations of 2,4-D and BAP for 21 days (scale = 0.5 cm)

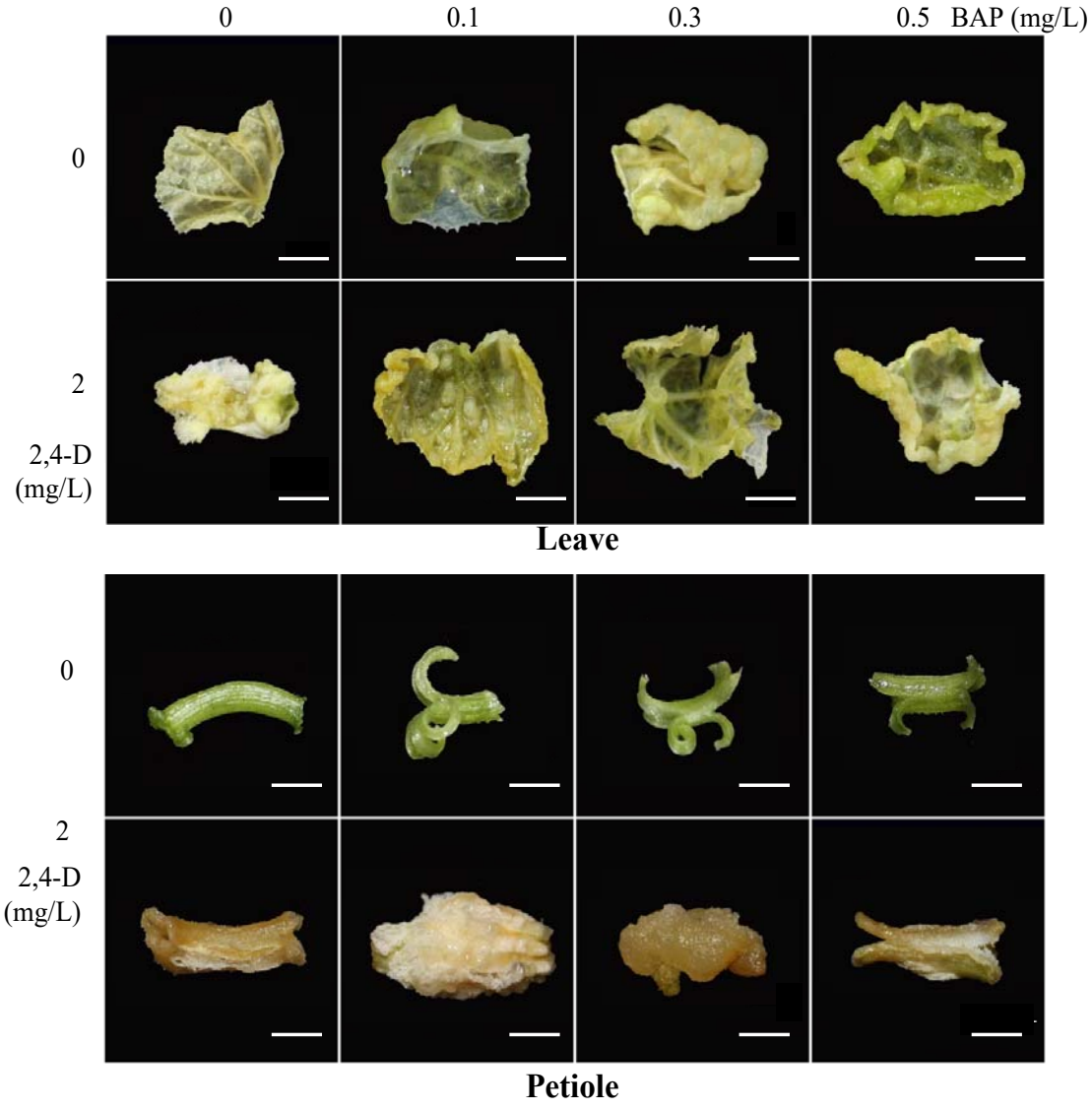


Fig.2. Callus features derived from leaf and petiole cultured on MS media supplemented with various concentrations of 2,4-D and BAP for 21 days (scale = 0.5 cm)

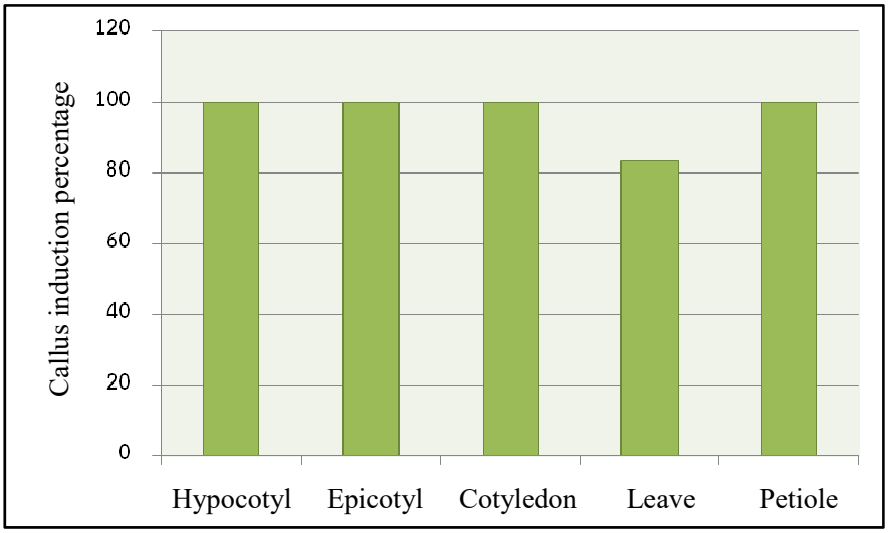
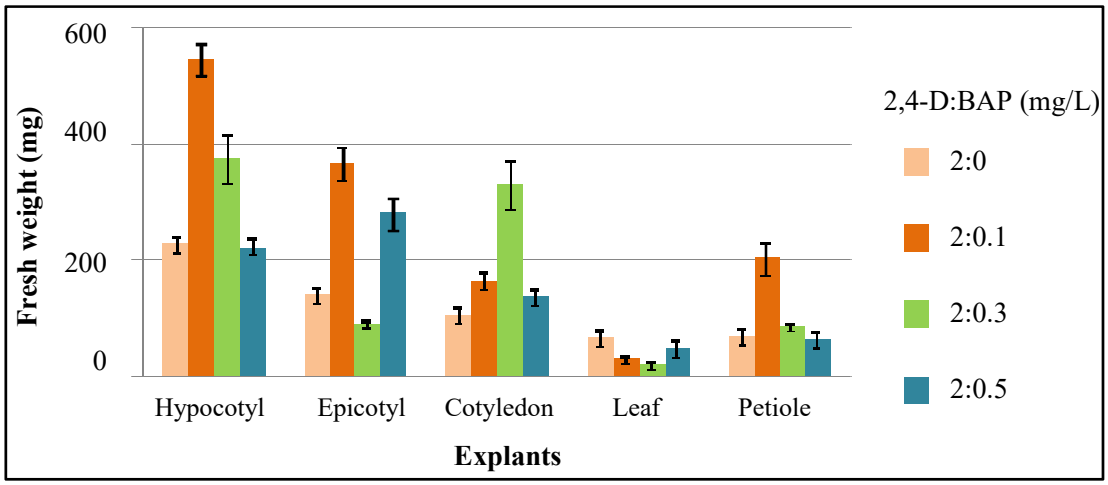


Fig.3. Callus induction percentage from different explants after cultured on the suitable formula of MS media supplemented with various concentrations of 2,4-D and BAP for 21 days.



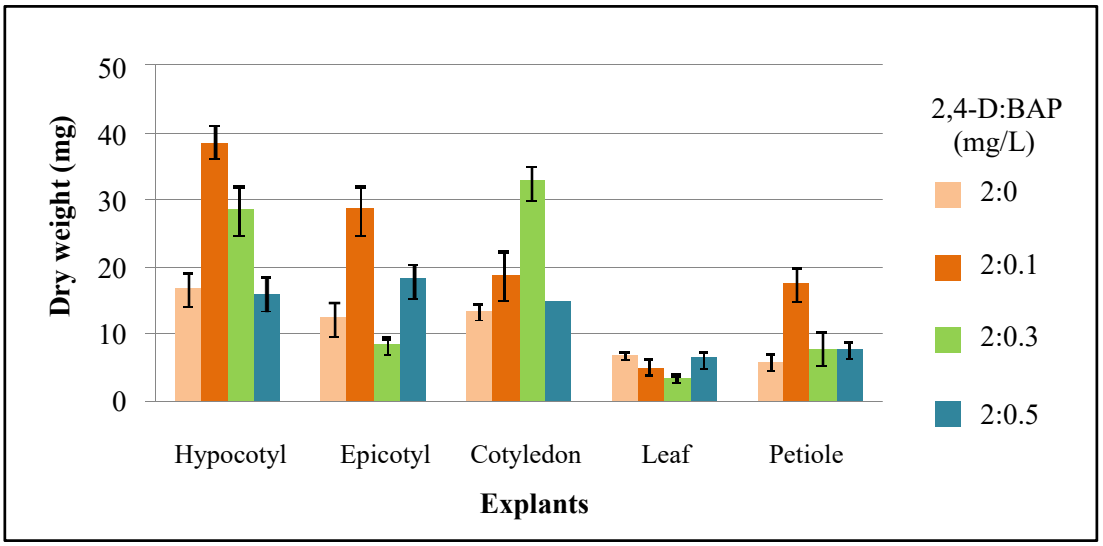


Fig.4. Fresh and dry weight of callus derived from different explants after cultured on the suitable formula of MS media supplemented with various concentrations of 2,4-D and BAP for 21 days.

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