

**STUDY ON *IN VITRO* MICROPORPAGATION OF
TARAXACUM OFFICINALE (L.) WEBER EX F.H. WIGG PLANT
BY TISSUE CULTURE**

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Abstract

Taraxacum officinale (L.) Weber ex F.H. Wigg plant is an herbal medicinal plant, which suffers from wide limitations when cultivated in the soil, including environmental limitations as well as the low rate of seed germination. In this study, it involved using ovary culture, callus culture and sub-culturing of *Taraxacum officinale* (L.) Weber ex F.H. Wigg plant, locally collected from Chonbuk National University Campus, South Korea-Iksan Country. That basal cultured on Murashige and Skoog (MS) medium, supplemented with different combinations and concentrations of growth regulators (2, 4-D, BAP, NAA and IAA) with registering the obtained response and periods taken until a response to establish a rapid and efficient protocol for *in vitro* micropropagation of the plantlets.

Key words: prepare of media test, ovary culture test, callus culture test, sub-culturing test

Introduction

Since ancient times, mankind has been dependent on plants for food, flavours, medicinal and many other uses (Sidhu, 2010). Micropropagation is the process of vegetative growth and multiplication from plant tissues or seeds (Fowler, 1993). Tissue culture is based on concept of totipotency; the ability of plant cells and tissues to develop into whole new plant (Fowler, 1993).

Taraxacum officinale (L.) Weber ex F.H. Wigg plant is the largest family of Asteraceae. It is an herbaceous perennial herb commonly called dandelion, found especially in lawns and along roadsides, and it is used as a medicinal herb and in food preparations. In addition, the plant consumed for its nutritional properties since leaves have a value as a salad crop, while the roots are used in the production of a coffee substitute (Bajaj, 1994). *T. officinale* is a medicinal plant species having several active compounds useful for choleric, diuretic, anti-inflammatory (Jeon *et al.*, 2008), antioxidative, and anti-carcinogenic activities such as breast and uterus cancer (Bae *et al.*, 2005; Schutz *et al.*, 2006).

Previous research on *T. officinale*

reported that different parts of plants originated from different field areas gave different antioxidant activity (Ermayanti *et al.*, 2009). But, the cultivation and propagation of dandelion are difficult due to low rates of germination and specific environmental requirements (Lee *et al.*, 2009; Schippmann *et al.*, 2002). The speed of seeds germination and the percentage of seeds that germinate vary during seasons and largely depending on temperature and moisture (Martinkova *et al.*, 2014).

Therefore, this research was aimed to investigate plant regeneration from ovary of *T. officinale* as explants grown on selected media using different concentrations and combinations of growth regulators.



Fig. (1) Habit of Dandelion plant

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Materials and Methods

Collection and sterilizing of *Taraxacum officinale* (L.) Weber ex F.H. Wigg (Dandelion ovary)

To get the Dandelion specimens (ovary) were collected from Chonbuk National University Campus, Iksan City, South Korea Country, during June 2019. After collection, these specimens (ovary) were sterilized with 15% Sodium Hypochlorite (NaOCl) for 10 or 15 minutes; then washed 4 times with sterilized distilled for 5 minutes each. After that, they were placed on sterilized filter papers to eliminate moisture and be ready for planting. These were shown in figure 2.



Fig. (2) Collected and Sterilized the Dandelion ovary specimens

Prepare of culture medium used as MS basal medium and Hormones (Murashige & Skoog, 1962)

I. First step (MS basal medium for ovary culture)

1 liter of basal MS medium was supplemented with 1 to 4 stock solution 10 ml/L, 5 to 6 stock solution 5 ml/L, agar powder 8 g/L, sucrose 30 g/L, distilled water 912 ml/L. pH medium was adjusted at 5.8, and then the medium was autoclaved at 120°C, 1 atm for 15 min.

II. Second step (MS basal medium and 2,4-D & BAP hormones for callus culture)

1 liter of basal MS medium was supplemented with 1 to 4 stock solution 10 ml/L, 5 to 6 stock solution 5 ml/L, agar powder 8 g/L, sucrose 30 g/L, 2,4-D and BAP hormone 1.5 ml/L, distilled water 910.5 ml/L. pH medium was adjusted at 5.8, and then the medium was autoclaved at 120°C, 1 atm for 15 min.

III. Third step

(MS basal medium and BAP+NAA & IAA+NAA hormones for sub-culturing)

1 liter of basal MS medium was supplemented with 1 to 4 stock solution 10 ml/L, 5 to 6 stock solution 5 ml/L, agar powder 8 g/L, sucrose 30 g/L, hormones 1.5 ml/L, distilled water 910.5 ml/L. pH medium was adjusted at 5.8, and then, the medium was autoclaved at 120°C, 1 atm for 15 min.

These preparation and experiment tests were shown in figures 3 to 17.



Fig. (3) Take the stock 1 - 6 solution



Fig. (4) Agar 8 g/l

Fig. (5) Sucrose 30 g/l

Fig. (6) Distilled water



Fig. (7) 2,4-D&BAP Fig. (8) BAP&NAA Fig. (9) IAA&NAA Fig. (10) Fix pH 5.8



Fig. (11) Preparation of materials for autoclave at 120°C, 1 atm for 15 min

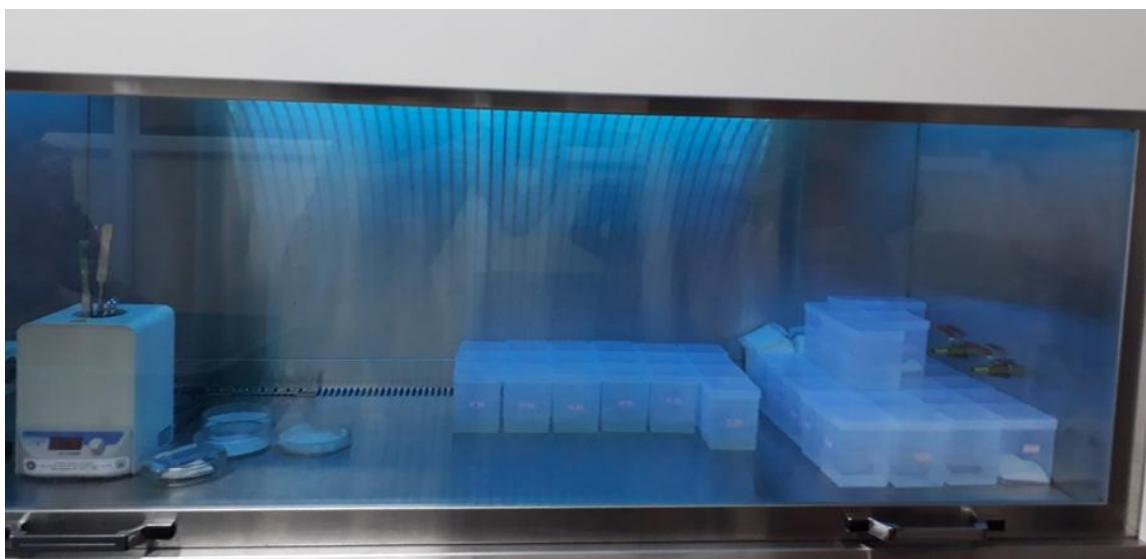


Fig. (12) Stored the sterilized materials in the antiseptic chamber for a day to solidify the media



Fig. (13) Ovary culture

Fig. (14) Callus culture

Fig. (15) Sub-culture

Fig. (16) Sealed tightly

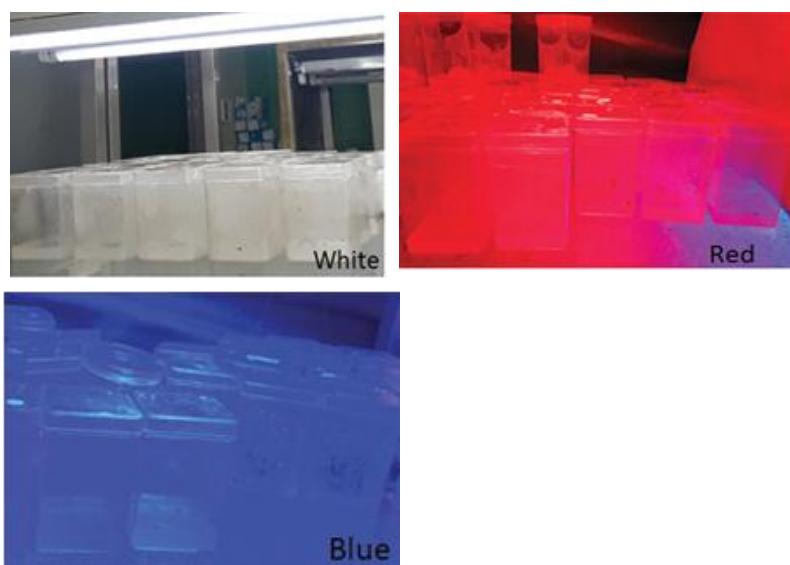


Fig. (17) Kept in a growth chamber under different flow density of white light, red light, blue light, dark with temperature (25°C) for *in vitro* micropagation of the plantlets.

These experiments of this study were conducted at Tissue culture Laboratory from Oriental Herb Science, Chungbuk National University and Botany Department, Dagon University.

Results

In these results:

- Ovary culture planting development of sample explants growth were observed in figures 18.

(MS Basal medium only)

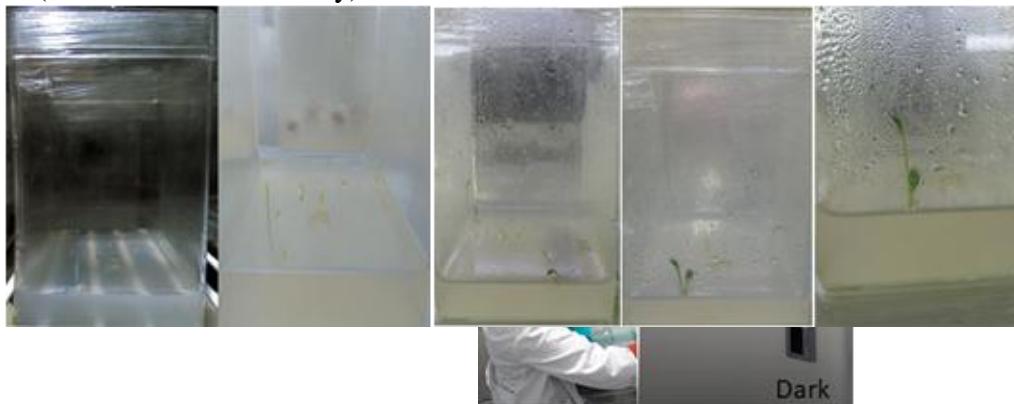


Fig. (18) After two weeks, starting the ovary culture development of new explant in the Dark

- Explants culture planting development of callus growth were observed in figures 19.

(2,4-D & BAP hormones + MS bsaal medium)

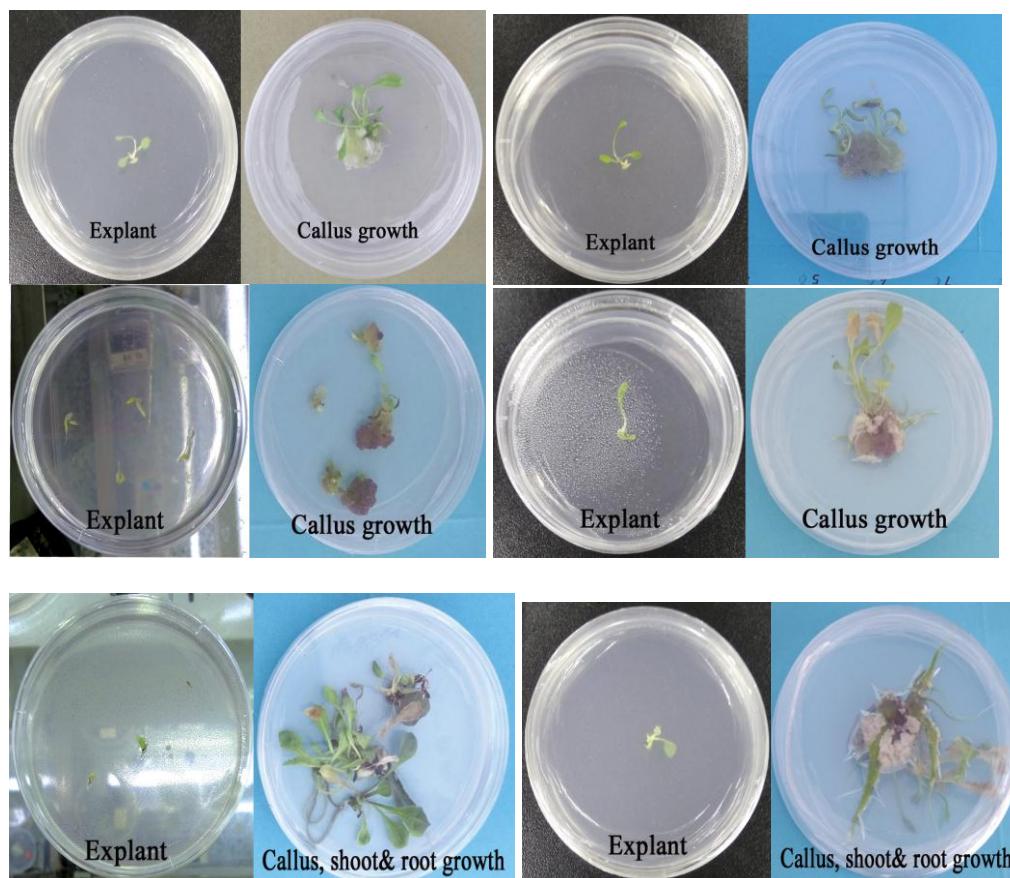


Fig. (19) After 6 to 8 weeks, growth of callus, shoot and root from new explants culture

3. Callus culture planting development of plantlets growth were observed in figures 20 to 21.
(BAP & NAA hormones + MS basal medium)



Fig. (20) Callus culture planting

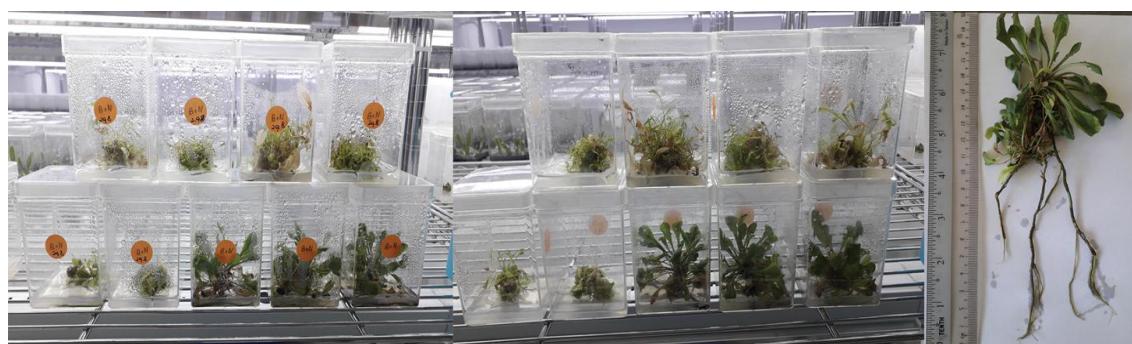


Fig. (21) After Two months, growth of plantlets from callus culture

4. Callus culture planting development of plantlets growth were observed in figures 22 to 23. (IAA & NAA hormones+ MS basal medium)



Fig. (22) Callus culture planting



Fig. (23) After Two months, growth of plantlets from callus culture

5. Sub-culture planting were shown in figures 24 to 25.
(BAP & NAA hormones MS basal medium)

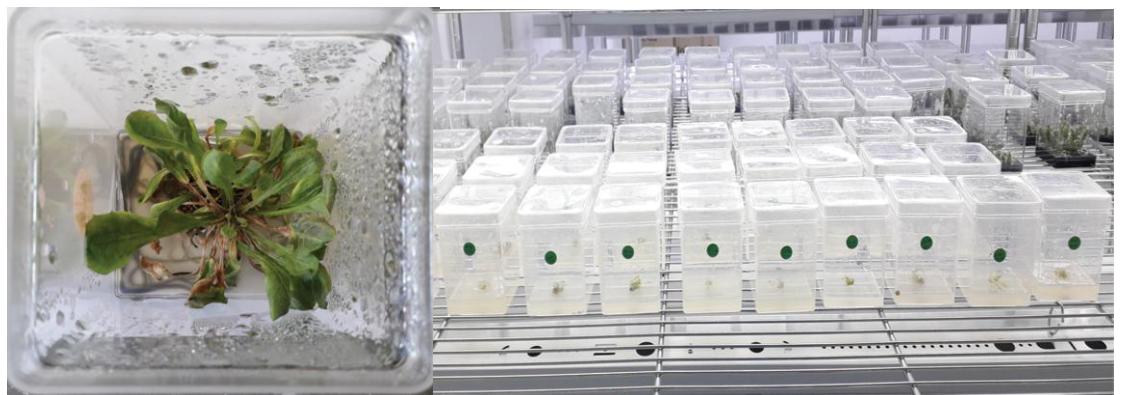


Fig. (24) Sub-culture planting

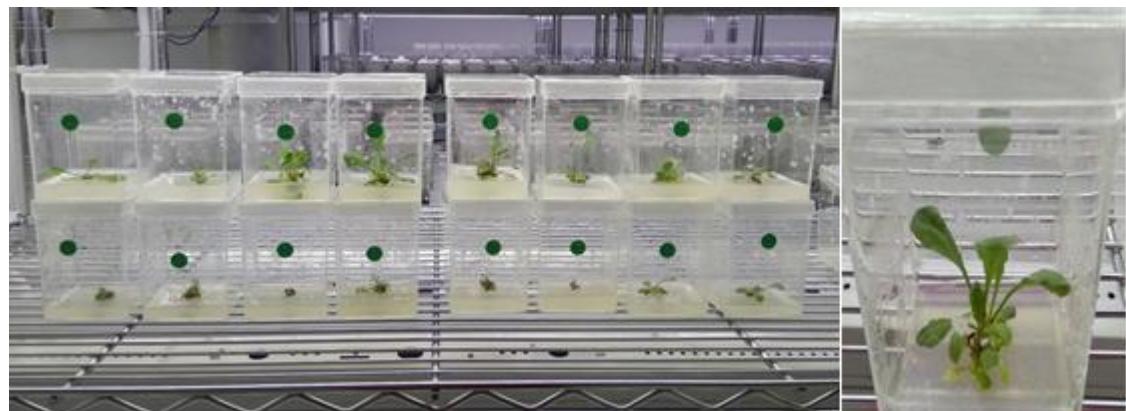


Fig. (25) After one months, growth of plantlets from sub-culture

Discussion and Conclusion

In this result, indicated that ovary of *Taraxacum officinale* developed into callus, shoots and roots regeneration on MS basal medium containing with different hormones of 2,4-D & BAP, BAP & NAA and IAA & NAA in combination. Shows the growth response of the ovary culture produced explant regeneration within a period of two to three weeks, only in MS basal medium, *in vitro* germinated. Addition of 2,4-D & BAP (1:0.5 ml/L) hormones stimulated explant culture to gave callus regeneration within a period of 6-8 weeks. Addition of BAP & NAA (1:0.5 ml/L) and IAA & NAA (1:0.5 ml/L) hormones in combination of callus culture to produced

callus, shoots and root regeneration within a period of two months, but gave different capability of explant for plantlets formation. IAA & NAA (1:0.5 mL/L) hormones produced low viability of callus, shoot and root formation. BAP & NAA (1:0.5 mL/L) hormones produced high viability of plantlets formation. Combination of BAP and NAA was suitable for regeneration of *Taraxacum officinale* plantlets.

Therefore, the best medium for *in vitro* micropropagation of *Taraxacum officinale* plantlets were MS basal medium containing 1mg/l of BAP in combination with 0.5 mg/l of NAA.

These characters were in general agreement with those mentioned by Rawa'a *et al.* (2018), Tri & Andri (2011), Chandrasekera *et al.* (2017), Ermayanti *et al.* (2009), (Rezaee and Kamali, 2014).

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