Shoots formation from gynosinesis *Cucumis sativus* l.

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ABSTRACT

Haploid plants achieve through androgenesis or gynogenesis. In the gynogenesis method, the ovary or ovule are used as explants induct haploid plants. The female flower one day before the flowering of *Cucumis sativus* L. are collected. Cold pretreatment of ovaries at 4°C up to 24 hours and culture under dark conditions. Significantly enhanced callus induction response is compared with cultures under 4-week cultured on CBM medium supplemented with various concentrations of TDZ 0.01 - 0.04 mg/L. After 4 weeks, ovaries are transferred to medium with kinetin 0.05 - 0.20 mg/L. Then, ovaries were transferred to medium supplemented with BA: IAA 3:1. Finally, green ovaries were transferred to BA 1.5 mg/L and GA_3 1.5 mg/L. The results showed that ovary induction has best affected on CBM with TDZ 0.03 mg/L with 11 callus/sample. Ovaries developed on kinetin 0.1 mg/L with 7.4 callus/sample. Ovaries become green and had leaves and roots formation on BA: IAA (3 mg/L: 1 mg/L). 11 plantlets were harvested from ovary culture after 12-week culture on CBM supplemented with BA 1.5 mg/L and GA_3 1.5 mg/L.

1. Introduction

Cucumber is a popular and important crop for many countries in over the world, especially tropical countries including Viet Nam. Cucumber is a highly nutritious and economically important plant. In modern agriculture, F1 hybrids are the first choice for commercial production and are being improved to achieve the best productivity. However, it takes too much time to create parental lines needed for hybrid by traditional pollination method, in the of cucumber, for 6-8 years (Gémes-Juhász, Balogh, Ferenczy, & Kristóf, 2002). By haploids *in vitro* culture can shorten the time to create pure parent lines only in 1-2 generations (Le, Nguyen, Luu, Le, & Do, 2005).

In Cucurbitaceae, haploids were created by many different methods: haploids production through *in vitro* gynogenesis in summer squash *Cucurbita pepo* L. (Shalaby, 2007), production of *in vitro* haploid plants from *in situ* induced haploid embryos in winter squash *Cucurbita maxima* Duchesne ex Lam. (Kurtar & Balkaya, 2010). Many experiments were researched in ovary cultures such as the effect of optimal stage of the female gametophyte and heat treatment *in vitro* gynogenesis induction in cucumber *Cucumis sativus* L. (Gémes-Juhász

et al., 2002), thidiazuron (TDZ) and silver nitrate (AgNO3) enhanced gynogenesis of unfertilized ovule cultures of *Cucumis sativus* L.

Several studies were conducted using ovary culture methods such as the study of the effect of female gametocyte development and pre-treatment on cucumber-growing (Gémes-Juhász et al., 2002), investigating the effects of TDZ and AgNO3 concentrations on unfermented cucumber (Li, Si, Cheng, Li, & Liu, 2013). Ovary culture has been considered to be most successful in the haploids production in many species (Alan et al.,2003; Hansen, Gertz, Joersbo, & Andersen, 1995) but were affected by many factors: genotype, stage of ovule development, temperature pretreatment, culture medium, embryo transformation, light conditions, phytohormones (Gémes-Juhász et al., 2002; Jin-Feng, Li, Ahmed, & Kere, 2010; Shalaby, 2007).

Based on the benefits of haploid production from cucumber culture and the need for cucumber breeding, we conducted the research "Shoots formation from gynosinesis *Cucumis sativus* L." to find the suitable medium for induction and shoots regeneration of cucumber.

2. Materials and methods

2.1. Material

Female flowers one day before the flowering of *Cucumis sativus* L. were collected from a greenhouse in Southern Seed Research Center in Ho Chi Minh City.

2.2. Method

After cold pretreatment at 4^{o}C up to 24 hours, the un-pollinated ovaries were rinsed in 70% alcohol for 3 minutes, followed by soaking in $Ca(OCl)_2$ 15% for 15 minutes and washed three times with sterile distilled water. After that, the ovaries have removed the skin and sliced thin. Sliced ovaries were cultured on CBM medium supplied with TDZ 0.01 - 0.04 mg/L or kinetin 0.05 - 0.20 mg/L, sucrose 3% and agar 8 g/l, under dark, 24 ± 2^{o}C, humidity 70% conditions.

After 4-week culture, callus were transferred to CBM medium supplied with BA: IAA (ratio 3:1 or 0.3: 0.1 mg/L) under 16/8h (light/dark) photoperiod, 24 ± 2^{o}C, humidity 70% conditions to induct shoots. After the next 8 weeks of culture, green shoots were transferred to medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 to growth shoots. Shoots began to form and grow in size after 4 - 6 weeks of culture. By the 8 - week cultivation, the shoots developed strongly and separately into complete plantlets. Well-developed plantlets were transferred onto free hormone basal medium (CBM) with 3% sucrose and cultured at 26^{o}C under a 16:8h (light/dark condition) for further development. The plantlets still were cultured continuously in basal medium (CBM) until reaching a height of over 3 cm. After that, plantlets were transferred to the external environment.

3. Result and disscusion

The highest number of inducted callus were 11 samples on medium supplemented 0.03 mg/L TDZ and 7.4 callus/sample on medium supplemented 0.10 mg/L kinetin. The results from Table 1 indicated at all treatments containing TDZ and kinetin showed higher callus than control. That means TDZ and kinetin had a strong impact on cucumber ovaries induction (Figure 1).

Table 1

Inductive callus on CBM medium with TDZ or kinetin after 4-week culture

TDZ concentration (mg/L)	Number of callus	Kinetin concentration (mg/L)	Number of callus
Control	2.80[e]	Control	1.80[d]
0.01	4.80[d]	0.05	2.60[c]
0.02	6.80[c]	0.10	7.40[a]
0.03	11.00[a]	0.15	4.00[b]
0.04	8.40[b]	0.20	3.00[c]

Source: The researcher's data analysis

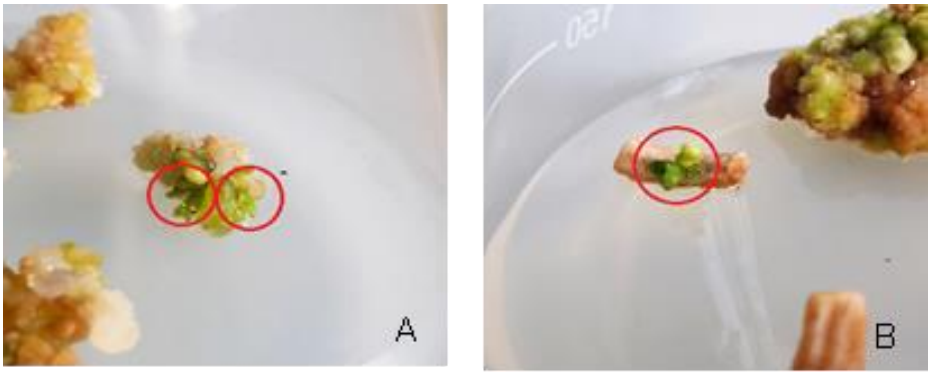

Figure 1. Green callus on medium supplemented

A: TDZ 0.03 mg/L B: kinetin 0.1 mg/L

TDZ is one of some plant growth hormones that have the role of complex auxin and cytokinin. It can induct ovaries of a female flower of cucumber forming callus. Cucumber ovaries that were cultured on medium supplemented 0.03 mg/L TDZ showed the highest inductive rate of 44.32% on cultivar 502 x 605 (Mopbeli, Peyvast, Hamidoghli, & Olfati, 2013). The highest embryogenic rates were 12.14 % in IL69 with 0.03 mg/L TDZ and IL57 with 11.11% with TDZ 0.07 mg/L were reported (Li et al., 2013). In this treatment, with number of inductive callus is 11 samples when used medium supplemented 0.03 mg/L TDZ. It is easy to see that in the case of cucumber TDZ has a much stronger impact than kinetin. This may indicate that each plant will adapt to different phytohormones and different concentrations. Results from Table 2 showed that the number of green callus obtained in medium supplemented BA: IAA (3:1 mg/L) was highest at 9.4 callus. In the remaining treatments, some of the callus also formed roots, albeit in very small proportions. The experiment combined auxin and cytokinin with the expectation that auxin will support cytokinin stimulates the development of shoots or embryos. However, until 8-week after cultivation, the callus still did not produce shoots or embryos but started appearing roots. The endogenous concentration of auxin in plants was too high plus auxin was added from the medium to stimulate root formation (Figure 2).

Table 2

Number of green callus on CBM medium supplemented BA: IAA at different ratio after 8-week culture

Concentration (mg/L)	Number of green callus (≥ 1 mm)
Control (0:0)	1.20^{c}
BA:IAA (3:1)	9.40^{a}
BA:IAA (0.03:0.01)	2.60^{b}

Source: The researcher's data analysis

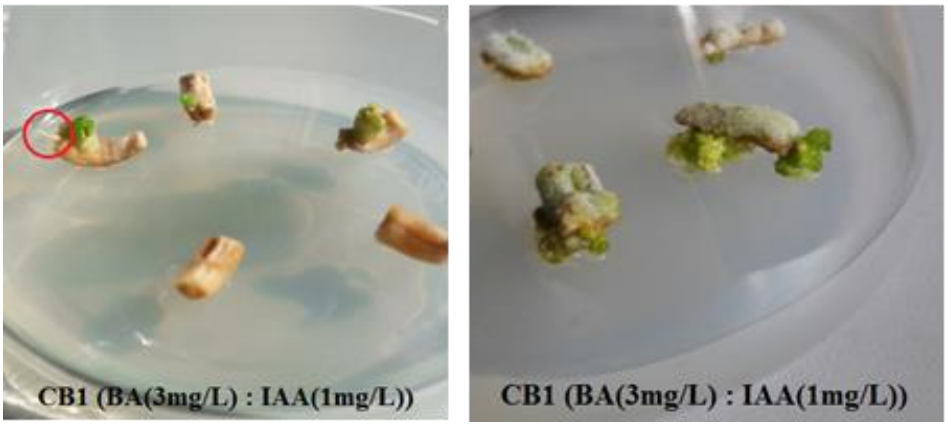

Figure 2. Callus on medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 after 2 weeks of culture

According to Bui (2000), auxin combined with cytokinin promotes shoot growth and initiates the creation of apical meristems from the parenchyma. However, in high concentrations of auxin inhibits the development of newly formed shoot buds or axillary buds, the shoots will now be pushed into the latent state. Cytokinin supports auxin in growth but also has antagonism between auxin (root formation) and cytokinin (shoot formation). It can be said that budding or root formation is very dependent on the rate of auxin/cytokinin. If this ratio is high, it will help to create roots and creating shoots when this ratio is low (Bui, 2000). Therefore, it is useful to use phytohormone with appropriate concentrations.

When green callus was transferred from medium supplemented BA: IAA (3:1 mg/L) to medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3. Similar shoots had formed (Figure 2) after only 2 weeks of culture. After a few weeks of culture, the leaves were gradually formed (Figure 3).

With adaptable concentrate, formation shoots from the callus of ovaries were inducted by BA and IAA. Auxin is a substance that stimulates root formation but when combined with cytokinin helps to promote the creation of buds. However, high levels of auxin concentration, will hinder the development and pushed the shoots into the latent state. Therefore, when green callus was transferred through medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3, auxin activated the latent shoots, helping them to form the initial leaf. Growth leaves were developed from shoots under a mixture of BA and GA_3 medium. Because GA_3 makes cells of

the primary leaf to be longer and BA inducts cells mitosis.

Figure 3. Plantlets on medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 *after 4 weeks of culture.*

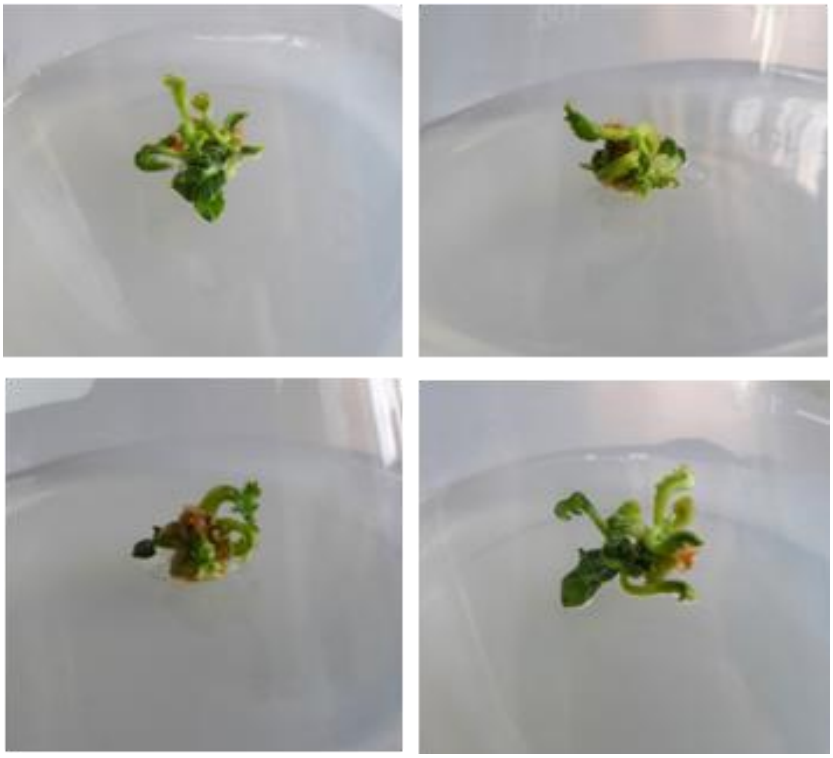

Figure 4. Plantlets on medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 after 8 weeks of culture

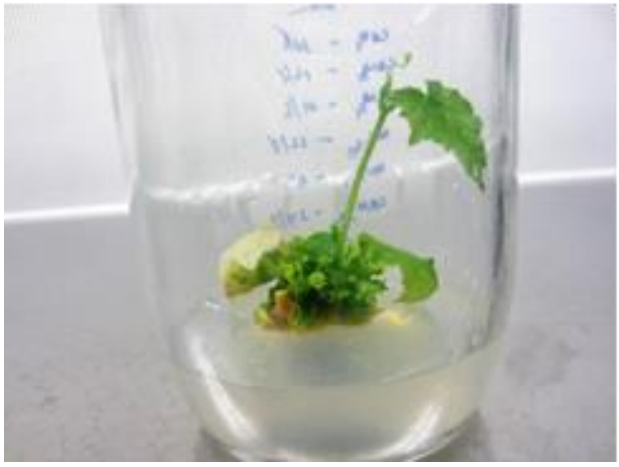

Figure 5. Plantlets on medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 after 12 weeks of culture

The plantlets still were cultured continuously in basal medium (CBM) until reaching a height of over 3 cm. After that, transfer plantlets to the external environment.

Figure 6. Plantlets on medium supplemented with 1.5 mg/L BA and 1.5 mg/L GA_3 after 8 weeks of culture

4. Conclusion

The results showed that ovary induction had best affected on CBM with TDZ 0.03 mg/L with 11 callus/sample. Ovaries developed on kinetin 0.1 mg/L with 7.4 callus/sample. Ovaries became green and had leaves and roots formation on BA: IAA 3 mg/L: 1 mg/L. 11 plantlets

were harvested from ovary culture on CBM medium supplemented BA 1.5 mg/L and GA_3 1.5 mg/L after 12-week culture in this study.

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