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Direct shoot regeneration via organogenesis in chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How)

Wei Han[1,2], Ee Liu[3], Dasen Xie[1], Qingwu Peng[1] and Xiaoming He[1]*

[1]Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, Guangdong Province 510640, China.
[2] Yingdong College of Life Sciences, Shaoguan University, Shaoguan, Guangdong Province 512005, China.
[3]College of Life Sciences, South China Agricultural University, Guangzhou, Guangdong Province 510642, China.

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A plant regeneration system was established from cotyledon explants of chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How). To obtain optimal conditions of adventitious shoot induction, the cotyledon explants were excised from seedlings of different genotypes as well as seed germination conditions, and then cultured on media containing different concentrations of 6-benzylaminopurine (6-BA)/1-naphthaleneactic acid (NAA). Among the eight genotypes, the highest rate of shoot regeneration was obtained from the cotyledons of inbred line A39. The adaxial portion of cotyledons of seedlings cultured for 3 days in darkness and 1 day in light was the appropriate explants for adventitious shoot organogenesis. The highest frequency of adventitious shoot organogenesis (52.2%) and mean number of shoots per explant (4.2) were achieved on Murashige and Skoog (MS) medium supplemented with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA. Adventitious shoots were observed to regenerate directly from cotyledons rather than from calli. A medium supplemented with $AgNO_3$ was not beneficial for shoot induction. Adventitious shoots were elongated in MS medium supplemented with 3 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA. Elongated shoots were rooted in ½ MS medium with 0.5 mg l^{-1} indole-3-acetic acid (IAA). Regenerated plantlets were transferred to a greenhouse for about 1 month, and subsequently transplanted into an open field.

Key words: *Benincasa hispida*, chieh-qua, adventitious shoot, genotypes, plant regeneration.

INTRODUCTION

Chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How), also known as hairy melon, fuzzy gourd, hairy gourd and moa qua (Cantwell et al., 1996), is a member of the Cucurbitaceae family. This family consists of many plants cultivated for their edible fruits. Other well-known members of this gourd family include cucumber, watermelon and squash.

Chieh-qua is an Asiatic crop widely farmed throughout southern China and Southeast Asia with a cultivation history of more than 300 years. There is approximately 15 000 ha of land annually cultivated for Chieh-qua in the Guangdong province of China alone.

In recent years, diseases such as *Fusarium* wilt and *Phytophthora* blight have greatly reduced the cultivation yield of chieh-qua. Breeding for disease resistance has been one of the primary objectives of chieh-qua improvement. In addition to conventional breeding methods, plant biotechnology techniques are expected to contribute to the improvement of disease resistance by means of *in vitro* selection and genetic transformation. An efficient plant regeneration system is a prerequisite for using somaclonal variation techniques and gene transfer technology.

Plant regeneration are affected by factors such as plant

*Corresponding author. Email: xiaominghe626@hotmail.com. Tel: +86-20-87560714. Fax: +86-20- 38469565.

genotype, explant type, seedling age, culture medium, concentration and a combination of plant growth regulators (PGRs). Up to now, some efficient plant regeneration protocol has been established in some crops of Cucurbitaceae (Chaturvedi and Bhatnagar, 2001; Soniya and Das 2002; Kintzios et al., 2002; Curuk et al., 2002; Lee et al., 2003; Ananthakrishnan et al., 2003; Sultana et al., 2004; Akasaka-Kennedy et al., 2004; Thomas and Sreejesh, 2004; Kathiravan et al., 2006; Selvaraj et al., 2007; Vasudevan et al., 2007a; Suratman et al., 2010). However, the regeneration of Cucurbitaceae crops is not accomplished like other model plants. Our preliminary study provided a regeneration protocol of cotyledon explants of chieh-qua (He et al., 2007). Nevertheless, the frequency of shoot organogenesis was too low (26.6%) to meet the requirement of bio-technique operation.

Accordingly, the objective of the present study was to establish an efficient and stable regeneration system via shoot organogenesis from cotyledon explants of chieh-qua. Special attention was given to the composition of culture medium, genotypic and seedling stage. We also focused on clarifying the effectiveness of $AgNO_3$ in shoot induction from cotyledon explants in chieh-qua.

MATERIALS AND METHODS

The seeds of chieh-qua were provided by the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences. The mature seeds of eight chieh-qua inbred lines, namely, A39, A19, A10, A02, A14, A12, A06 and B05, were used.

Seed sterilization and explants isolation

After the removal of seed coats, the seeds were surface sterilized in 75% (v/v) ethyl alcohol for 30 s followed by 0.1% mercuric chloride ($HgCl_2$) from 7 to 8 min, and then rinsed five times with sterile distilled water. The sterilized seeds were placed in Petri dishes with filter paper and water for germination (Figure 1A). The dishes were autoclaved at 121°C for 20 min before use. The seeds were germinated in darkness for 2, 3, 4 or 5 days at 28 ± 2°C, respectively and then transferred to light (30 µmol m^{-2} s^{-1}) for 1 day (Table 3). Cotyledons from seedlings of different ages were excised as explants.

Media for adventitious shoot induction

Adaxial portions of cotyledons were implanted in Petri dishes with Murashige and Skoog (MS) medium containing different concentrations and combinations of 1-naphthaleneactic acid (NAA; 0, 0.2, 0.4 and 0.6 mg l^{-1}) and 6-benzylaminopurine (6-BA; 4, 6 and 8 mg l^{-1}). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. 0.7% agar and 3.0% sucrose was added to each medium. Cultures were maintained at 25 ± 1°C and 12 h photoperiod with a light intensity of 30 µmol $m^{-2}s^{-1}$. The effect of various concentrations of $AgNO_3$ (0, 0.05, 0.1, 0.5 and 1 mg l^{-1}) in media on the induction of adventitious shoot containing 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA was investigated. $AgNO_3$ was sterilized by filtration and added to the medium after autoclaving. After 4 weeks, the frequencies of callus induction and shoot organogenesis were recorded.

Media for adventitious shoot elongation and rooting

After 4 weeks of growth in shoot induction media, the adventitious shoots were cultured in an elongation medium containing 3 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA.

Shoots longer than 2 cm were cut and transferred to ½ MS medium or ½ MS medium with 0.5 mg l^{-1} indole-3-acetic acid (IAA), 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} indole-3-butyric acid (IBA) for rooting. The IAA was sterilized by filtration and added to the medium after autoclaving.

Acclimatization

After 3 weeks of growth in rooting medium, plants with well-developed roots were carefully collected and washed with tap water to remove agar clinging to the roots. The rooted plantlets were transferred to plastic cups containing garden soil and commercial compost (1:1), and then placed in a greenhouse. A high humidity was maintained in the greenhouse until new leaves emerged. After about 1 month, the regenerated plants were planted in the field.

Statistical analysis

A completely randomized design was used in all the experiments. Each treatment consisted of 30 explants (six explants per dish), and each experiment was repeated thrice. Data analyses of the variance and mean values were carried out using Duncan's multiple range tests. Significant differences were determined at the 5% level (Gomez and Gomez, 1984).

RESULTS

Effect of plant growth regulators (PGRs) on shoot induction

The cotyledon explants were cultured on MS medium with different concentrations and combinations of NAA (0 mg l^{-1} to 0.6 mg l^{-1}) and 6-BA (6 to 8 mg l^{-1}) (Table 1 and Figure 1C). The explants were considerably enlarged during the first 3 or 4 days of culture. White friable and non-organogenesis callus occurred at the cut surface of the proximal half of explants after 7 days of culture initiation in all samples, except the explants cultured on the medium without NAA. After 15 days of culturing, small protuberances were observed on the most proximal part of the cotyledon. After about 3 to 4 weeks, adventitious shoots were observed on the cuts of the proximal half of the explants cultured in combinations of 6-BA (4 to 8 mg l^{-1}) and NAA (0.2 to 0.4 mg l^{-1}). The highest frequency of adventitious shoot regeneration (52.2%) was achieved on the medium containing 6-BA (6 mg l^{-1}) and NAA (0.2 mg l^{-1}), but the frequency sharply decreased to 33.3% when the NAA concentration was increased to 0.4 mg l^{-1} (Table 1 and Figure 1D). The shoot regeneration frequencies on media containing 6-BA (4 and 8 mg l^{-1}) and NAA (0.2 and 0.4 mg l^{-1}) had no significant difference as compared to the medium containing 6-BA (6 mg l^{-1}) and NAA (0.6 mg l^{-1}). A shoot regeneration frequency of 3.3% was obtained from the medium containing 6-BA (4 and 8 mg l^{-1}) and NAA (0.6 mg l^{-1}). However, no adventitious shoot was

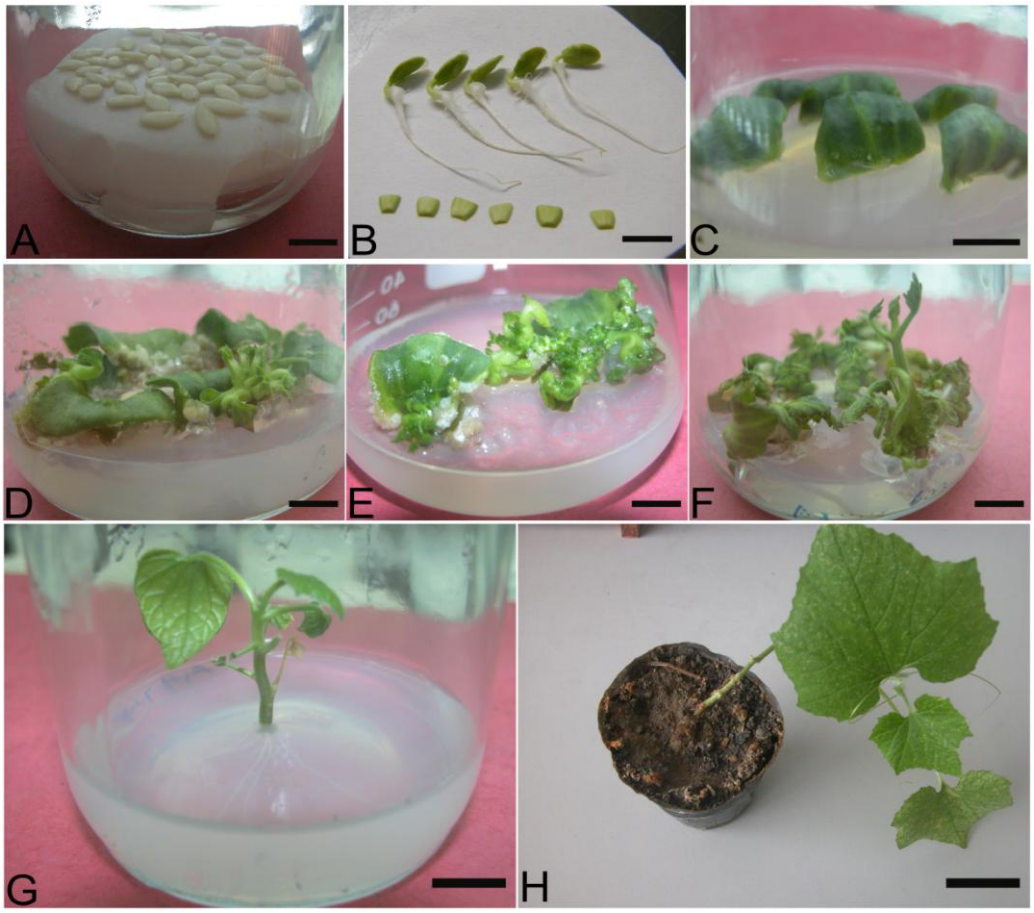

Figure 1. Shoot induction and plant regeneration in chieh-qua. (A) Seeds were germinated on a dish medium with filter paper and water, bar: 1 cm; (B) The adaxial portion of cotyledons of seedlings cultured for 3 days in darkness and 1 day in light was appropriate explants for adventitious shoot regeneration, bar: 1 cm; (C) Cotyledons were cultured on MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA, bar: 1 cm; (D) Adventitious shoot regeneration from the proximal portion of cotyledons cultured for 3 weeks on MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA, bar: 1 cm; (E) Shoot proliferation occurred when adventitious shoots were subcultured in MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA, bar: 1 cm; (F) Adventitious shoots were elongated in MS medium supplemented with 3 mg l^{-1} BA and 0.2 mg l^{-1} NAA, bar: 2 cm; (G) Elongated shoots were rooted in ½ MS medium with 0.5 mg l^{-1} IAA, bar: 3 cm; (H) Rooted plants were acclimatized in a greenhouse, bar: 3 cm.

found on the medium containing 6-BA alone or the combination of 6-BA (4, 6 and 8 mg l^{-1}) and IAA (0.1, 0.3 and 0.5 mg l^{-1}) (data not shown).

Effect of genotype on shoot organogenesis

The cotyledon explants of eight genotypes were tested on the medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA (Table 2). There was no significant difference among the callus formations of all eight genotypes tested; however, the adventitious shoot regeneration frequency and number of shoot per explants varied. The shoot regeneration frequency ranged from 0 to 52.2%. The cotyledon explants of inbred line A39 exhibited the highest rate of shoot differentiation (52.2%) and number of shoots (4.2) on the medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA. The shoot regeneration frequency of inbred lines A02 and A19 had no significant difference. Adventitious shoots were not found in inbred lines A12, A14 and B05.

Effect of seedling age and seed germinating condition on shoot induction

The effect of seedling age and seed germinating condition on adventitious shoot induction was examined. Callus formation was observed in all cultures. However, the shoot regeneration frequencies and number of shoots per explant were influenced by the age of the seedling that provided the cotyledon explants. The cotyledons of 4-day-old seedlings produced a higher frequency of adventitious

Table 1. Effect of plant growth regulators on shoot induction from cotyledon explants of chieh-qua inbred line.

PGRs (mg l^{-1})		Callus formation (%)	Shoot organogenesis (%)
6-BA	NAA		
4	0	0	0^{e}
6	0	0	0^{e}
8	0	0	0^{e}
4	0.2	100	22.2 ± 3.9^{c}
4	0.4	100	25.6 ± 1.9^{c}
4	0.6	100	3.3 ± 0.0^{d}
6	0.2	100	52.2 ± 5.1^{a}
6	0.4	100	33.3 ± 3.3^{b}
6	0.6	100	21.1 ± 5.1^{c}
8	0.2	100	21.1 ± 5.1^{c}
8	0.4	100	20.0 ± 3.3^{c}
8	0.6	100	3.3 ± 0.0^{d}

Proximal cotyledon explants from 4-day-old seedlings of inbred line A39 cultured on MS medium were evaluated after 4 weeks of culture. Means followed by different letters are significantly different at the 5% level. PGRs, Plant growth regulators; 6-BA, 6 benzyl amino purine; NAA, naphthalene acetic acid.

Table 2. Effect of genotype on shoot organogenesis from cotyledon explants of chieh-qua.

Genotype	Callus formation (%)	Shoot organogenesis (%)	No. of shoots per explant
A39	100	52.2 ± 5.1^{a}	4.2 ± 0.2^{a}
A06	100	15.6 ± 3.9^{b}	3.7 ± 0.3^{b}
A10	100	7.8 ± 1.9^{c}	2.3 ± 0.3^{cd}
A02	100	4.4 ± 1.9^{cd}	2.5 ± 0.5^{c}
A19	100	3.3 ± 0.0^{de}	2.0 ± 0.0^{d}
A12	100	0^{e}	0^{e}
A14	100	0^{e}	0^{e}
B05	100	0^{e}	0^{e}

Proximal cotyledon explants from 4-day-old seedlings cultured on MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA was evaluated after 4 weeks of culture. Means followed by different letters are significantly different at the 5% level.

shoot regeneration than those of 6-day-old seedlings (Table 3). The seed germinating condition also influenced shoot induction. The highest percentage of shoot regeneration (52.2%) was obtained from 4-day-old seedlings that had been incubated for 3 days in darkness and 1 day in light. The highest number of shoots per explant (5.2) obtained from 6-day-old seedlings incubated for 5 days in darkness and 1 day in light was not significantly different from that of 4-day-old seedlings incubated for 3 days in darkness and 1 day in light (Figure 1B).

Effect of $AgNO_3$ on shoot organogenesis

The medium supplemented with $AgNO_3$ (0.05 to 1 mg l^{-1}) was used to assess the effect of Ag^{+} on shoot organogenesis (Table 4). Cotyledons cultured on shoot regeneration medium became swollen, and adventitious shoots emerged from the cuts of the proximal half of the explants. However, the shoots appeared abnormal and unhealthy. Hyperhydricity was also observed. The $AgNO_3$ concentration highly influenced the frequency of shoot regeneration. The frequencies of callus formation and shoot induction significantly decreased on medium supplemented with $AgNO_3$, but the number of shoots formed per explant had no significant difference from the control.

Shoot proliferation

Shoot proliferation occurred when cotyledon explants with emerging buds were subjected to two successive transfers at an interval of 15-days each in the MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA (Figure 1E).

Elongation

The proliferated multiple shoots were separated into small

Table 3. Effect of cotyledon age and seed germination condition on shoot organogenesis from cotyledon explants of chieh-qua inbred line.

Germination time and condition	Callus formation (%)	Shoot organogenesis (%)	No. of shoots per explant
Darkness, 3 days	100	20.0 ± 5.8d	4.0 ± 0.9^{ab}
Darkness, 2 days; light, 1 day	100	38.9 ± 1.9b	4.3 ± 0.1^{ab}
Darkness, 4 days	100	37.8 ± 5.1b	3.7 ± 0.2^{b}
Darkness, 3 days; light, 1 day	100	52.2 ± 5.1a	4.2 ± 0.2^{ab}
Darkness, 5 days	100	14.4 ± 1.9de	3.7 ± 0.7^{b}
Darkness, 4 days; light, 1 day	100	29.7 ± 3.4c	3.3 ± 0.3^{b}
Darkness 6 days	100	13.3 ± 3.0de	3.6 ± 0.6^{b}
Darkness, 5 days; light, 1 day	100	7.8 ± 1.9e	5.2 ± 1.0^{a}

Proximal cotyledon explants from 4-day-old seedlings of inbred line A39 cultured on MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA were evaluated after 4 weeks of culture. Means followed by different letters are significantly different at the 5% level.

Table 4. Effect of $AgNO_3$ concentrations on shoot organogenesis from cotyledon explants of chieh-qua inbred line.

$AgNO_3$ (mg l^{-1})	Callus formation (%)	Shoot organogenesis (%)	Number of shoots per explant
0.0	100	52.2 ± 5.1^{a}	4.2 ± 0.2^{a}
0.05	100	13.5 ± 2.1^{d}	3.2 ± 0.2^{ab}
0.1	100	15.5 ± 0.7^{d}	2.8 ± 0.2^{b}
0.5	60	42.0 ± 2.8^{b}	4.1 ± 0.7^{ab}
1.0	16	22.0 ± 2.8^{c}	4.1 ± 0.5^{ab}

Proximal cotyledon explants from 4-day-old seedlings of inbred line A39 cultured on MS medium with 6 mg l^{-1} 6-BA and 0.2 mg l^{-1} NAA were evaluated after 4 weeks of culture. Means followed by different letters are significantly different at the 5% level.

Table 5. Effect of the medium elements on root induction in chieh-qua inbred line A39.

Rooting medium	Rooting response (%)	Callus formation	No. of roots per plantlet
1/2 MS	100	No callus growth	4.3^{b}
1/2 MS + 0.5 mg l^{-1} IAA	100	No callus growth	5.3^{a}
1/2 MS + 0.5 mg l^{-1} NAA	100	Good callus growth	3.0^{c}
1/2 MS + 0.5 mg l^{-1} IBA	100	Slow callus growth	4.7^{ab}

Means followed by different letters are significantly different at the 5% level.

clusters containing two to three shoots. These clusters were transferred to MS medium supplemented with 3 mg l^{-1} 6-BA and NAA 0.2 mg l^{-1}. After culturing for about 4 weeks, the shoots elongated and were available for rooting (Figure 1F).

Shoot rooting

Shoots longer than 2 cm were cut and transferred to ½ MS medium or ½ MS medium with 0.5 mg l^{-1} IAA, 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} IBA for rooting (Table 5). After about 10 days, roots were induced in all four media. The highest number of roots per plant (5.3) was achieved on ½ MS medium containing IAA. Plantlets from ½ MS medium with NAA and IBA had roots with a white callus at the shoot, which reduced their viability. ½ MS medium with 0.5 mg l^{-1} IAA was considered as the appropriate medium for chieh-qua shoot rooting (Figure 1G). The rooted plantlets were transplanted into plastic cups containing garden soil and commercial compost (1:1). The plantlets were maintained in a greenhouse for about a month (Figure 1H), and then planted in a field.

DISCUSSION

An efficient plant regeneration system via organogenesis was established for chieh-qua (*B. hispida* Cogn. var. Chieh-qua How). Cotyledon explants excised from seedlings germinated *in vitro* were used for the regeneration. The present report is probably the first detailed one on the shoot organogenesis of chieh-qua. The explant type, plant genotype, seedling age, culture medium, concentration, and a combination of PGRs were the key factors that influenced shoot organogenesis and subsequent

plant regeneration (Niederwieser and Staden, 1990; Dabauza and Peña, 2001; Ashrafuzzaman et al., 2009; Prakash and Gurumurthi, 2010). Basal MS medium has been found to be the most effective for adventitious shoot organogenesis in Cucurbitaceous cotyledon explants (Kathiravan et al., 2006; Haque et al., 2008; Zhang et al., 2011). Many reports have indicated that 6-BA alone or in combination with auxin is useful for shoot differentiation (Dong and Jia, 1991; Sarowar et al., 2003). In the present study, the combination of 6-BA/NAA was found appropriate for adventitious shoot differentiation from cotyledon explants of chieh-qua. However, the cotyledon explants of chieh-qua did not regenerate adventitious shoots in MS medium with 6-BA alone. Adventitious shoot regeneration in chieh-qua was also found to require cytokinins and auxins. This finding agreed with that reported by Sultana et al. (2004) for watermelon. Selvaraj et al. (2007) have revealed the requirement of a high auxin/low cytokinin ratio for callus formation, and a low auxin/high cytokinin ratio for shoot induction from callus. Thomas and Sreejesh (2004) have reported that the addition of 0.2 µM NAA with 4 µM 6-benzylaminopurine (BAP) was beneficial for adventitious shoot regeneration in the cotyledon-derived callus of ash gourd. When compared with cucumber (Vasudevan et al., 2004; Vasudevan et al., 2007b), watermelon (Sultana and Bari, 2003; Sultana et al., 2004), melon (Curuk et al., 2002; Muruganantham et al., 2002) and squash (Ananthakrishnan et al., 2003; Kathiravan et al., 2006), chieh-qua also needed a high concentration of 6-BA for shoot induction. Such a high concentration may influence shoot proliferation as well as elongation, and could be one of the reasons for the difficulty of regeneration of chieh-qua. Therefore, a balance between cytokinins and auxins is more important for improving the frequency of chieh-qua regeneration.

The cotyledon explants of eight genotypes were tested for adventitious shoot regeneration in the present study. Different regeneration abilities were observed among the genotypes. Hence, adventitious shoot regeneration in chieh-qua was strongly influenced by the genotype. Similar results have been reported in the tissue culture of muskmelon (Molina and Nuez, 1995) and cucumber (Mohiuddin et al., 1997). George et al. (2008) have found that direct or indirect regeneration readily occurred only in some plant species, or may even be restricted to certain varieties within species. This phenomenon may be attributed to the silencing of relevant genes in the heterochromatin. Genotypes with high regeneration abilities should be selected to establish an efficient shoot regeneration system from cotyledon explants in chieh-qua.

In the present study, the shoot regeneration frequency of cotyledons increased after a pretreatment combining darkness with light during seed germination. Similar results have been reported in the cotyledon culture of watermelon (Compton and Gray, 1993). In contrast, Han et al. (2004) have discovered that when compared with light pretreatment, the darkness pretreatment of seedlings decreases the frequency of the adventitious shoot regeneration of cotyledon explants in bottle gourd. Apparently, the effect of alternating darkness and light during seed germination on shoot regeneration from cotyledon varied in different species. Previous studies have shown that the frequency of shoot regeneration was influenced by the seedling age in cucurbits (Compton, 2000; Lee et al., 2003; Krug et al., 2005; Ntui et al., 2009). In the present study, the shoot regeneration frequency of cotyledons from 4-day-old seedlings was higher than those from 5 and 6-day-old seedlings. Dong and Jia (1991) have suggested that young cotyledons are physiologically very active and easily affected by environmental factors, such as exogenous hormones. Therefore, the suitable seed germination condition and seedling age for each species of Cucurbitaceous need to be determined to establish an efficient shoot regeneration system.

$AgNO_3$ inhibits the action of ethylene (Beyer, 1979), which is widely reported to be beneficial for shoot organogenesis of several crops (Burnett et al., 1994; Mohiuddin et al., 1997; Burgos and Alburquerque, 2003; Han et al., 2004; Mohiuddin et al., 2005). In the present study, we observed that the application of $AgNO_3$ negatively affected chieh-qua shoot induction. Callus induction and proliferation were also significantly decreased by high levels of $AgNO_3$. Similar results have been reported in fig leaf gourd (Kim et al., 2010). These results indicate that $AgNO_3$ may have distinct influences on shoot induction. Therefore, we proposed that the use of $AgNO_3$ should be avoided in culture media for chieh-qua shoot induction.

For the shoot induction, we found that the proximal part of the cotyledon showed a higher frequency of adventitious shoot regeneration than the distal part. In fact, no adventitious shoot was found on this part. The proximal part of the cotyledon played a key role in shoot induction. Cells in this part were sensitive to PGRs and had the potential for adventitious shoot formation. Hence, organogenesis was determined by the part of the cotyledon. A similar phenomenon has been observed in some other Cucurbitaceous species (Compton, 2000; Lee et al., 2003; Ananthakrishnan et al., 2003).

According to our results, ½ MS medium with 0.5 mg l^{-1} IAA was appropriate for chieh-qua shoot rooting. Many studies have demonstrated *in vitro* that low-salt, ½ and ¼ MS media promote shoot rooting in cucurbits (Lee et al., 2003; Han et al., 2004; Thomas and Sreejesh, 2004). Auxin is also widely used for root induction in cucurbits, including IAA (Han et al., 2004), NAA (Kathiravan et al., 2006; Kim et al., 2010) and IBA (Sarowar et al., 2003; Krug et al., 2005). All these observations revealed that the shoot rooting response may be affected by the growth medium composition. Differences in rooting response may be attributed to culture conditions or genotypes (Shakti et al., 2007). Therefore, the optimal medium for shoot rooting in an *in vitro* culture of Cucurbitaceous species need to be determined.

In summary, we established a plant regeneration system

via organogenesis from cotyledon explants of chieh-qua, which may contribute to the cell and genetic engineering of this crop. However, some problems were encountered, such as the formation of ill-defined buds and shoot-like structures either resisting elongation or producing rosettes of distorted leaves, which generally do not produce normal shoots. Similar problems have been observed in melon (Gaba et al., 1999) and pepper (Steinitz et al., 1999; Ochoa-Alejo and Ramirez-Malagon, 2001). These problems may be linked to genotypes or the medium for shoot regeneration. Future research will be focused on these areas.

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