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Chieh-qua Biotechnology: Progress and Prospects

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ABSTRACT

Chieh-qua (*Benincasa hispida* Cogn. var. Chieh-qua How) is a member of the family Cucurbitaceae. It is a native and important vegetable in China, and widely cultivated throughout south China and Southeast Asia for its immature fruits. The improvement of yield, resistance to disease and stress are the main aims in production and breeding of Chieh-qua. Biotechnology has provided promising approaches for cultivar improvement of this crop. This mini-review introduces the recent researches of *in vitro* culture and molecular marker application in Chieh-qua, including shoot tip and cotyledon culture, mutant selection *in vitro*, application of RAPDs for the testing of seed purity and identification of cultivars, development of molecular markers linked to genes associated with gynoecy. The prospects of biotechnology in heredity and breeding in Chieh-qua are discussed.

Keywords: *Benincasa hispida*, *in vitro* culture, molecular marker, somaclonal variation
Abbreviations: BA, benzyladenine; **2,4-D**, 2,4-dichlorophenoxy acetic acid; **FA**, fusaric acid; **IAA**, indole-3-acetic acid; **ITS**, internal transcribed spacer; **NAA**, α-naphthalene acetic acid; **ZYMV**, *Zucchini yellow mosaic virus*

CONTENTS

INTRODUCTION

Chieh-qua (*Benincasa Hispida* Cogn.var. Chieh-qua How) is also called fuzzy melon, hairy melon or moqua (Cantwell *et al*. 1996). It is a member of the family Cucurbitaceae, with a vigorous annual vine that is usually allowed to grow upright on trellis. The immature fruits are cylindrical and roundish with many bristle-like trichomes on the epidermis, and have skin color of various shades of green, depending on variety. The flesh of the fruit is commonly white with a delicious flavor (**Fig. 1**). The diversity and antiquity of cultivars in China suggest that this crop may be indigenous to southern China (Yang and Walters 1992). Being a native and important vegetable, Chieh-qua is now widely cultivated throughout southern China and Southeast Asia for the immature fruits with about 14,000 ha cultivated every year only in southern China. The other variety of *Benincasa hispida*, wax gourd, ash gourd or winter melon, is produced for its mature fruits.

However, continuous production has caused a series of problems such as diseases. Being more and more serious, Chieh-qua diseases (i.e. fusarium wilt, phytophthora blight and viruses) have led to thousands of tons of fruit to be lost in fields every year. For this reason, improvement of resistance to disease and stress, as well as yield, should be the main aims in production and breeding of Chieh-qua.

Attempts to utilize heterosis in Chieh-qua have made done since the 1980s, in which gynoecy was selected (Wei *et al*. 1996; Chen *et al*. 1999; Peng *et al*. 2002). Based on the research of gynoecy, hybrids with higher yield and resistance were released instead of conventional cultivars (Chen *et al*. 2001; Lin *et al*. 2006; Peng *et al*. 2006). In addition, studies on the genetic basis of main agricultural traits and methods for identification of resistance to Fusarium wilt were carried out (Xie *et al.* 2001; Li *et al*. 2003; Xie *et al.* 2003). Because of the development of new cultivars and efficient techniques, great progress was achieved in Chieh-qua production (Xie *et al*. 2004). However, it is well known that the germplasm of Chieh-qua is limited. It is difficult to find resistant materials especially those with resistance to fusarium wilt, phytophthora blight or viruses. So, screening and creating of new material have been urgent tasks for improvement of Chieh-qua.

Genetic improvement through biotechnology offers potential routes for improving yield and resistance by screening useful genes effectively, introducing recombinant genes or generating somaclonal variants with improved resistance to biotic or abiotic stresses (Compton *et al*. 2004).

This mini-review introduces the recently studies of *in vitro* culture and molecular markers on Chieh-qua, including shoot tip culture, cotyledon culture, mutant selection *in vitro*, application of RAPD on the testing of seed purity and identification of cultivars, and development of molecular markers linked to genes associated with gynoecy. The prospects of biotechnology in heredity and breeding in Chieh-qua are discussed.

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Invited Mini-Review

Fig. 1 Chieh-qua fruit growing on the plant. Cultivar: Xiaguan No. 1; Age of fruit: 10 days after pollinated.

Fig. 2 Clustered buds of Chieh-qua *in vitro*.

Fig. 3 Rooted plantlets transferred to plastic cups with garden soil.

SHOOT TIP MICROPROPAGATION

Shoot tip culture has been widely applied in the rapid propagation of elite cultivars and virus elimination. Micropropagation protocols have been developed in some members of the cucurbit family, such as the micropropagation protocol of triploid cultivars of watermelon by which about 1.2 million plants could be obtained in just over 6 months from an initial 100 shoot tip explants (Compton *et al*. 2004). Micropropagation of Chieh-qua has been carried out using shoot tips of tube-seedlings. Shoot tips 0.5 cm long produced 3-5 clustered buds (**Fig. 2**) every 3-4 weeks when cultured on MS solid medium containing 17.76 μM benzyladenine (BA) and 1.14 μM indole-3-acetic acid (IAA) (He *et al*. 2002a). However, the propagation coefficient of Chieh-qua shoot-tips is lower than that of wax gourd (the other variety of *Benincasa hispida*). The highest propagation coefficient (25.6) of wax gourd was obtained on MS medium supplemented with 4 mg/L BA and 1 mg/L α-naphthalene acetic acid (NAA) (Lin and Xu 1999a). Lin and Xu (1999b) also found that the propagation was effected by pH of the medium for pro-culture, as well as concentration of growth regulators in medium. Shoot tips from seedlings pre-cultured 15-20 d on medium at a pH of 7.2 was optimal for micropropagation of wax gourd.

Clustered buds of Chieh-qua elongated on medium with reduced BA and increased IAA (MS+ BA 4.44 μM +IAA 2.85 μM). Elongated shoots (more than 1 cm long) rooted easily on MS medium with 2.85 μM IAA. Cluster buds without being elongated in culture could not produce roots normally. Rooted plantlets were transferred to plastic cups with garden soil (**Fig. 3**) and placed in a greenhouse under high humidity (about 90%). The humidity of the glasshouse was gradually reduced as the plants grew. Using this method, more than 60% of plantlets survived acclimatization in the greenhouse (He *et al*. 2002b).

ADVENTITIOUS SHOOT ORGANOGENESIS

In the Cucurbitaceae, tissues can be regenerated both via organogenesis (i.e. adventitious bud formation), and somatic embryogenesis (Dong *et al*. 1991; Compton *et al*. 1993; Nakagawa *et al*. 2001). However, to date regeneration from cotyledons of *B. hispida* has only been reported via organogenesis (He *et al*. 2002a; Thomas and Sreejesh 2002; He *et al*. 2006a). The cotyledons of *in vitro*-germinated seedlings were the preferred explants for shoot organogenesis. To obtain seedlings, uncoated seeds of Chieh-qua were germinated on ½-strength of both macro- and micronutrients of MS medium supplemented with 3% sucrose and 0.7% agar (He *et al*. 2002a). In wax gourd, however medium supplemented with 4 μM BA was found to be optimal for embryo germination (Thomas and Sreejesh 2002).

Basal sections of Chieh-qua cotyledon had organogenic competence. Adventitious shoots formed on the cut edge of basal sections of cotyledons rather than on top (He *et al*. 2002a). Similar results were obtained in cucumber and watermelon, in which the proximal end of cotyledons had the greatest regeneration rate (Compton 2000).

The composition and concentration of growth regulators in the medium affected directly shoot organogenesis in Chieh-qua. Adventitious shoots were introduced directly from cotyledons of Chieh-qua when cultured on MS medium supplied with BA as the only plant growth regulator. However, the regeneration rate was improved by adding IAA. Optimum shoot regeneration was achieved in MS medium supplemented with 26.64 μM BA and 0.57 μM IAA, by which 2.85 shoots per cotyledon were obtained (He *et al*. 2006a). At higher concentrations of BA (over 8 mg/L), fewer buds were induced, part of which were abnormal and wilted in subculture. Similar results were reported with the combination of BA and IAA in other members of the Cucurbitaceae (Compton 1999, 2000). Furthermore, Thomas and Sreejesh (2002) described a protocol for the production of complete plantlets through multiple shoots from the cotyledon-de-

rived calli of ash gourd (*B. hispida*). In the protocol MS medium fortified with 4 μM 2,4-D was used for callus initiation from the cotyledons, and MS medium fortified with 4 μM BA and 0.2 μM NAA was recommended as the optimum medium for shoot regeneration.

The organogenic competence of cotyledons was genotype-dependent (He *et al*. 2006a). An attempt was made to use hypocotyls and leaves of Chieh-qua as explantss, but we failed to induce adventitious shoot (He *et al*. 2002b).

Adventitious shoots regenerate from cotyledons of Chieh-qua is more poorly than other crops of Cucurbitaceae. More efforts should be applied to improve the regeneration competence of Chieh-qua.

SELECTION OF SOMACLONAL VARIATION WITH RESISTANCE

In vitro selection is a promising technique to improve resistance to disease, particularly for pathogens known to produce toxins. Compared with classical crossing methods, it is an immediate and inexpensive way of generating or selecting plant variants with tolerance to either the pathogen or its toxin from susceptible varieties. Mutants with resistance have been obtained in several crops (Pathania and Misra 2003; El-Hadrami *et al*. 2004). Somaclonal variants of cotton (*Gossypium hirsutum* L.) regenerated from embryogenic callus grown in medium with selection pressure exhibited resistance to Fusarium wilt caused by *Fusarium oxysporum* (Zhang *et al*. 1998). Similar work was attempted in Chieh-qua. A protocol of *in vitro* selection was reported using fusaric acid (FA, a toxin produced by *F. oxysporum*) as the selection agent. Cotyledon-derived clustered buds were inoculated on regeneration medium with FA. More than 60% of explants browned after about 15 days' culture. The survivors were cultured on fresh regeneration medium without FA for about 3 weeks, and selected on regeneration medium with FA again. The regenerated plantlets were found to be pathogen-resistant (He *et al*. 2006b).

APPLICATION OF MOLECULAR MARKERS

Molecular markers are valuable tools in the evaluation of genetic diversity and resistance genes. Assessing genetic diversity was carried out in one Chieh-qua cultivar and two Chinese wax gourd cultivars using RAPD genetic markers with twenty 10-mer random primers. Of the twenty primers used, one could not be amplified, 7 amplified monomorphic DNA patterns, and 12 gave reproducible polymorphic DNA amplification patterns. Only 3 primers gave unique banding patterns for each of the 3 cultivars. The three cultivars were very similar when comparison using RAPD patterns (Meng *et al*. 1996). In addition, 9 inbred lines of wax gourd were analysed using 130 RAPD primers, of which 26 were informative and amplified 163 DNA marker bands. A total of 47 polymorphic bands were obtained with a mean of 1.8 per primer which, in combination, discriminated all the inbreds from each other. Pair-wise genetic distance measurements ranged from 0.056 to 0.179, suggesting a narrow genetic base for the inbreds. The results indicated significant positive correlations of genetic distance with hybrid performance and heterosis (Sureja *et al*. 2006).

Gynoecy plays an important role in Chieh-qua heterosis breeding. Identification of the markers linked to this character will facilitate selection of gynoecious Chieh-qua lines in a breeding program. A 1150 bp gynoecy-specific fragment was amplified in the primer combination of TGA GCGGACA. This marker was verified using individual DNA of the F_2 population and the band could only be amplified in the gynoecious plants (Chen *et al*. 2002).

Seed purity testing using RAPD was also attempted in Chieh-qua. Eight random primers were used in RAPD of male/female parents and F_1. Three of these random primers had been successfully amplified; two of them were specific in the female parent. The other had specific amplified fragments that lay between those of the male/female parents and F_1 showing polymorphism of the amplified fragment (Li *et al*. 1999).

MOLECULAR BIOLOGY IN PATHOGENY ATTACKING CHIEH-QUA

Pathogenicity, vegetative compatibility grouping and AFLP analysis were correlated and could effectively distinguish isolates of *F. oxysporum* from cucurbitaceous (Vakalounakis *et al*. 1999). Differences in pathogens of brought wilt disease in wax gourd and Chieh-qua were studied. *F. oxysporum* f. sp. Chieh-qua is distinct from *F. oxysporum* f. sp. *benincasae* in cultivar susceptibility and can be differentiated by pathogenicity tests with appropriate hosts (Xie *et al*. 2006). Genetic relationships between these two specialized forms and races of *F. oxysporum* f. sp. *benincasae* were determined by the DNA sequences of ribosomal DNA internal transcribed spacer (ITS) region. The length of ITS1 and ITS2 sequence of *F. oxysporum* f. sp. Chieh-qua and *benincasae* was 558 bp and 545 bp, respectively. They were 94.3% homologous.

Viral diseases have become a devastating disease of Chieh-qua in GuangDong province since 2000. *Zucchini yellow mosaic virus* (ZYMV) is an important pathogeny. A Guangzhou isolate of ZYMV infecting Chieh-qua was identified by indicator tests and partial sequence amplification using 2 primers (ZCP5F (5′-GCCAAGCTTTCGGGCACTC AGCAAACTG-3′) and ZCP3R (5′-GGTCTCGAGCTGCA TTGTGTTCACACCCA-3′). The coat protein (CP) gene of this virus was amplified by RT-PCR, and ligated into the expression vector PET-22b(+). The recombinant plasmid PET-ZCP was transformed into *E. coli* BL21 (DE3) and then induced to express by IPTG. The CP gene was highly expressed by SDS-PAGE and western blot analysis. The molecular weight of the recombinant protein was about 330 kD. Antiserum with high specificity was produced after a rabbit was immunized with purified recombinant protein, and the titer was determined to be 1/4096 by antigen coating plate-ELISA (Song *et al*. 2005).

CONCLUDING REMARKS

It has been proved that biotechnology has provided promising approaches for cultivar improvement of Chieh-qua. Efficient methods for plant regeneration *in vitro* have paved the way for variant selection *in vitro* and genetic transformation. Somaclonal variation has potential application for developing disease resistant genotypes. Identification of markers linked to useful characters facilitated selection of prospective lines in breeding programs. However, further studies about culture of different tissues and markers linked to important characters should be carried out in order to improve the breeding efficiency of Chieh-qua. In addition, different ways to create new material of Chieh-qua ought to be explored. It is expected that biotechnology will play an important role in Chieh-qua breeding.

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REFERENCES

*** In Chinese**

Cantwell M, Nie X, Zong R, Yamaguchi M (1996) Asian vegetables: Selected fruit and leafy types. In: Janick J (Ed) *Progress in New Crops*, ASHS Press, Arlington, VA, pp 488-495

Chen QH, Peng QW, **Zhuo QY, Huang T, He XZ, Lin YE, Li TY** (1999) Breeding and application of gynoecy in chieh-qua. *Guangdong Agricultural Sciences* **4**, 16-17*

Chen QH, Xie DS, He XM, Peng QW (2003) DNA marker links to gynoecious genes in chieh-qua. *Guangdong Agricultural Sciences* **5**, 19-21*

Compton ME, Gray DJ (1993) Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid and tetraploid watermelon. *Journal of the*

American Society of Horticultural Science **118**, 151-157

Compton ME (2000) Interaction between explant size and cultivar impacts shoot organogenic competence of watermelon cotyledons. *HortScience* **35**, 749-750

Compton ME, Gray DJ, Gaba VP (2004) Use of tissue culture and biotechnology for the genetic improvement of watermelon. *Plant Cell, Tissue and Organ Culture* **77**, 231-243

Dong JZ, Jai SR (1991) High frequency plant regeneration from cotyledon of water melon (*Citrullus vulgaris* Schrad.). *Plant Cell Reports* **9**, 559-562

El-Hadrami A, El-Idrissi-Tourane A, El-Hassni M, Daayf F, El-Hadrami I (2005) Toxin-based *in-vitro* selection and its potential application to date palm for resistance to the bayoud Fusarium wilt. *Comptes Rendus Biologies* **328**, 732-744

Ganesan M, Jayabalan N (2006) Isolation of disease-tolerant cotton (*Gossypium hirsutum* L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate. *Plant Cell, Tissue and Organ Culture* **87**, 273-284

He XM, Xie DS, Peng QW (2002a) Effects of culture factors on plant regeneration of chieh-qua. In: Lei J (Ed) *Advances in Horticulture*, Guangzhou Publishers, Guangzhou, China, pp 457-462*

He XM, Xie DS, Peng QW (2002b) Tissue culture and rapid propagation of *Benincasa hispida* var. chiehqua. *Plant Physiology Communications* **38**, 593*

He XM, Xie DS, Peng QW, Mu LX (2006a) Plant regeneration from cotyledon of chieh-qua (*Benincasa hispida* Cogn. var. *Chieh-qua* How.). In: *Abstracts of 27th IHC*, Seoul, Korea, p 349

He XM, Xie DS, Peng QW, Mu LX (2006b) Study on selection *in vitro* of the mutants with fusarium acid resistance in chieh-qua (*Benincasa hispida* Cogn. var. *Chieh-qua* How.). *Guangdong Agricultural Sciences* **1**, 49-51*

Jain MS (2001) Tissue culture-derived variation in crop improvement. *Euphytica* **118**, 153-166

Krug MGZ, Stipp LCL, Rodriguez APM, Mendes BMJ (2005) *In vitro* organogenesis in watermelon cotyledons. *Pesquiza Agropecuária Brasileira* **40**, 861-865

Li CW, Li ZJ, Cheng RZ (1999) Studies on the application of RAPD on the testing of hair squash seed purity. *Seed* **3**, 14*

Li WJ, Fang FX, Li LZ, Liang RF, Kang DX (2003) Corralation and path analysis of main agronomic traits in chieh-qua. *Acta Horticulticae Sinica* **30**, 734-736*

Lin J, Xu H (1999a) Tissue culture and rapid propagation of *Benincasa hispida*. *Plant Physiology Communications* **35**, 472*

Lin J, Xu H (1999b) Research on high efficient plantlet regeneration of *Benincasa hispida* (Thunb.) Cogn. *Acta Botanica Boreali-Occidentalia Sinica* **19**, 1-4

Lin JY, Xie WP, Tan X, Zhang H, Li LF (2005) A new chieh-qua F_1 hybrid-Guanhua No. 4. *China Vegetable* **10**, 87-88*

Meng XD, Wei YY, Ma H, Zhang WH, Li JR (1996) Identification of Chinese wax gourd and chieh-qua cultivars using RAPD markers. *Acta Agriculture ShangHai* **12**, 45-49*

Nakagawa H, Saijyo T, Yamauchi N, Shigyo M, Kako S, Ito A (2001) Effects of sugars and abscisic acid on somatic embryogenesis from melon (*Cucumis melo* L.) expanded cotyledon. *Scientia Horticulturae* **90**, 85-92

Pathania NS, Misra RL (2003) *In vitro* mutagenesis studies in *Gladiolus* for induction of resistance to *Fusarium oxysporum* f.sp. *gladioli*. *Acta Horticulturae* **624**, 487-494*

Peng QW, Chen QH, Xie DS, He XZ, He XM, Lin YE (2002) Selection and application of gynoecies with sleek fruits in chieh-qua. *Guangdong Agricultural Sciences* **4**, 16-17*

Peng QW, Chen QH, He XM, Xie DS (2006) A new chieh-qua F_1 hybrid-Xiaguan No. 1. *China Vegetable* **10**, 25-26*

Song LM, Lu CH, Liang WX, Huang JG, Li HF (2005) Cloning, expression of the CP gene and antiserum preparation of *Zucchini yellow mosaic virus* infecting *Benincasa hispida* Cogn. Var. *Chieh-qua* How. *Acta Microbiologica Sinica* **45**, 132-134*

Sureja AK, Sirohi PS, Behera TK, Mohapatra T (2006) Molecular diversity and its relationship with hybrid performance and heterosis in ash gourd [*Benincasa hispida* (Thunb.) Cogn.]. *Journal of Horticultural Science and Biotechnology* **81**, 33-38

Thomas TD, Sreejesh KR (2004) Callus induction and plant regeneration from cotyledon explants of ash gourd (*Benincasa hispida* L.). *Scientia Horticulturae* **100**, 359-367

Vakalounakis DJ, Fragkiadakis GA (1999) Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. *Phytopathology* **89**, 161-168

Wei YY, Zhang QP (1996) Breeding of more female chieh-qua and preliminary utilization. *Journal of Shandong Agricultural University* **27**, 275-280

Xie DS, Peng QW, He XM, Lin YE, He XZ (2001) Genetic study of yield traits on chieh-qua. *Journal of Guangxi Agricultural and Biological Science* **20**, 246-249

Xie DS, He XM, He SJ, Peng QW (2003) Studies on the artificial inoculation techniques of wax gourd or chieh-qua blight. *Journal of Guangxi Agricultural and Biological Science* **22**, 92-95

Xie WP, Lin JY, Huang SL (2006) Research progress of production and breeding of chieh-gua. *Journal Changjiang Vegetables* **2**, 33-35*

Zhang XL, Yao MJ, Shi PC, Dong XG (1998) Resistance identification of the lines regenerated from colonies resistant to fusarium wilt in cotton (*Gossypium hirsutum* L.). *China Cotton* **25**, 8-9*