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# Citrus Breeding and Genetics in China

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## ABSTRACT

Citrus is the second important fruit crop in China; the long history of citriculture and richness of germplasm has benefited genetics and breeding research in this country. Today, there are 1000 accessions or so that have been preserved *ex situ* in the National Citrus Germplasm Repository and 101 calli of citrus accessions *in vitro* are maintained in the laboratory of Huazhong Agriculture University. Selections of seedless and early- or late-ripening varieties are two important breeding goals at present in China. Using bud sport and seedling selection, at least 40 strains of seedless or with less seeds and early- or late-ripening were obtained in the past 20 years. Furthermore, China made some progress in germplasm innovation by biotechnology. Until now, about 40 interspecific and intergeneric somatic hybrid combinations were obtained; 244 triploids and 10 tetraploids gained by cross hybridization, and 1000 plantlets gained from culture of seeds and aborted ovules beneath the chimeric part of fruit with elite traits were preserved in the greenhouse or field, which highlights hope for elite cultivar selection in the future.

**Keywords:** citrus germplasm, citrus genomics, citrus industry, germplasm innovation

**Abbreviations: AFLP**, Amplified fragment length polymorphism; **CAPs**, Cleaved Amplified Polymorphic Sequence; **cpDNA**, chloroplast DNA; **cpSSR**, chloroplast Simple Sequence Repeat; **EST**, Expressed Sequence Tag; **FAO**, Food and Agriculture Organization; **HAU**, Huazhong Agricultural University; **ISSR**, Inter-Simple Sequence Repeat; **MSAP**, Methylation Sensitive Amplification Polymorphism; **MT**, million tons; **mtDNA**, mitochondria DNA; **NCGR**, National Citrus Germplasm Repository; **RAPD**, Random amplified polymorphic DNA; **RFLP**, Restriction Fragment Length Polymorphism; **SSR**, Simple Sequence Repeats

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## INTRODUCTION

Citrus is one of the world's important fruit crops which is widely grown in most areas with suitable climates between latitude 35°N~35°S. In China, citrus is the second economically cultivated fruit crop and is widely grown along the Yangtze River valley and the southern region of this river including Fujian, Zhejiang, Jiangxi, Hunan, Hubei, Guangdong, Guangxi, Chongqing and Sichuan province or municipality.

China has a long history of citriculture with more than 4,000 years of history. The book "*Chu Lu*" (translated into English by Hagerty, "*Monograph on the Oranges of Wên-chou, Chekiang*", 1923), written by Han Yen-Chih in 1178, was the first authentic monograph in the world. It mainly described 27 superior varieties in Wenzhou, Zhejiang province (China), together with many technical descriptions of seedling, grafting, cultivation, pest control, storage and processing. Some of the recorded varieties in the book can still be found in this area today.

The citrus industry in the past more than half century has developed very quickly in China. During the 1950s-1960s, national citrus annual production was 0.20-0.30 million tons (MT), which occupied only 1.5% of the total world production (Ye 2000). Later, during 1970-1980, citrus production increased slowly, and the total output doubled in just 10 years. After the 1980s, the citrus industry developed sharply under rural reform, featured by relaxing control over the market. Compared with 1985, the growing acreage and production in 2005 increased nearly 2- and 7.5-fold, respectively amounting to 1.63 million hectares and 14.96 MT (http://www.agri.gov.cn/sjzl/). Since 2004, citrus production in China exceeded America and ranked as No. 2 in the world (http://www.fao.org/ES/ESC/en/20953/20990/highlight_ 28187en.html).

In China, the citrus industry has the following main characteristics. Firstly, the most growing varieties are loose-skin mandarin, mainly including satsuma mandarins (*Citrus*

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*unshiu*) and Ponkan (*C. reticulata*), which shared two thirds of the total production. Sweet orange lies second and has a 15% share, followed by pummelo (13%), kumquat and lemon (<5%) (http://www.agri.gov.cn/sjzl/). Secondly, of the total production, 95% is for fresh market and only 5% is for processing. Thirdly, the harvest season is mainly from October to January of the following year. Fourthly, most fruits are consumed in domestic markets and only 2-4% is for export (Yu *et al.* 2006).

The government pays much attention to the citrus germplasm collection and variety selection. A set of research centers for variety improvement, including the National Center of Citrus Improvement (Chongqing), National Center of Citrus Germplasm Repository (Chongqing), National Center of Citrus Breeding (Wuhan), National Conservation Center of Virus-free Fruit Germplasm (Wuhan and Chongqing), were established recently, which will play a great role in research on citrus genetics and variety improvement. The research status and achievements on citrus breeding and genetics in the past 50 years in China have been simply reviewed in this paper.

## GERMPLASM COLLECTION AND PRESERVATION

China is the most important place of origin for citrus. The long history and diversified climates enable China to harbor the most citrus varieties. Southern China is one of the centers of diversity for *Citrus* and related genera such as *Fortunella*. During the 1950s-1960s and 1970s-1980s, a national survey and collection of indigenous citrus genetic resources were carried out under the government support. A number of local elite cultivars, such as '*Nanfenmiju*', '*Jincheng*', '*Shatian*' pummelo, '*Bendizaoju*' and '*Dahongtiancheng*' were uncovered, as were some wild citrus species, such as *C. honghensis*, *C. mangshanensis*, *C. daoxianensis*, and *Poncirus polyandra*.

Beginning in the early 1980's, a National Citrus Germplasm Repository (NCGR) was established at Beibei, Chongqing, and regional citrus germplasm repositories in Huangyan, Zhejiang province; Guiling, Guangxi province; Changsa, Hunan province; Guangzhou, Guangdong province; Jiangjin, Sichuan province. As recorded in 1996, the NCGR conserved 1041 accessions. As of 2000, the number of preserved accessions decreased to 944, including 9 genera, 24 species and 14 varieties of *Citrus* and related genera, of which indigenous and introductions from abroad accounted for 65.5% and 34.5%, respectively (Chen 2000). Recently, since the germplasm collection was strengthened, and the government carried out a project to introduce abroad elite citrus varieties during the end of the 1990s, the numbers of repository accessions increased to 1046 before the end of 2004 (www.ziyuanpu.net.cn/intro.aspx?puname=CRICAAS), of which the indigenous and foreign accessions accounted for 56.9% and 43.1%, respectively (**Table 1**). In addition, Huazhong Agricultural University (HAU, Wuhan, China) also pays much attention to citrus germplasm. Until the end of 2006, they had over 280 accessions conserved *ex situ* in the National Center of Citrus Breeding.

Though much work has been done on germplasm collection, a wide range of genetic diversity is still present *in situ*, and conservation *in vitro* just began in the early of 1990s when HAU began studying *in vitro* conservation and had constructed a citrus callus bank of 101 accessions before 2007. At the same time, China began to study cryopreservation *in vitro* of citrus germplasm. Wang and Deng (2001, 2004, 2004b) established a system of cryopreservation *in vitro* by vitrification, and showed that the regeneration rate of trifoliate orange and citrus somatic hybrid shoot tips after cryopreservation reached over 90%.

## GERMPLASM IDENTIFICATION AND EVALUATION

Germplasm identification and evaluation is the basis for reasonable utilization. As one of countries with abundant citrus genetic diversity in the world, China has identified or evaluated over 700 citrus genetic resources by morphology, isozyme analysis and molecular markers since the 1980s, and explored 45 superior cultivars or varieties (www.ziyuanpu.net.cn/intro.aspx?puname=CRICAAS).

Since RAPD was established (Shi *et al*. 1998), RFLP, SSR, AFLP, CAPs, ISSR and MSAP were subsequently established in China. In addition, Cheng *et al*. (2003) explored citrus chloroplast simple sequence repeat (cpSSR). Using such molecular analysis tools, many studies on the phylogenetics of citrus have been carried out including genetic polymorphism (Pang 2002; Liu *et al*. 2005; Fu *et al*. 2006), pedigree relationship (Fan *et al*. 2002; Pang *et al*. 2003, 2006), identification of bud sport varieties (Liao *et al*. 2006; Zeng *et al*. 2006), and identification of somatic hybrids (Guo *et al*. 2002; Cheng *et al*. 2003; Fu *et al*. 2004; Xu *et al*. 2005). Since 2000, HAU established a RAPD fingerprint of over 250 accessions (Deng, *et al*. 2000) and AFLP, SSR fingerprints of 29 *Poncirus* genetic resources (Pang 2002). Li *et al*. (2006) evaluated systemically wild loose-skin orange resources and considered China as one of the native centers of loose-skin orange. In total, about one third of the germplasm accessions have been molecularly evaluated in the past years, which enable us to clarify the relatedness of many germplasm accessions. For example, the cpSSR analysis cleared the relationship of Satsuma mandarin with '*Bendiguangju*'; the latter had been considered as the mother of Satsuma mandarin in past decades. However, cpSSR marker analysis verified that these two varieties had a different cytoplasmic background; Satsuma had the cytoplasm of tangerine, and '*Bendiguangju*' mandarin contained the cytoplasm of sweet orange. They are very similar in leaf and fruit morphology, and might be sister-varieties from same parents, but resulted from reciprocal crosses (Li *et al*. 2006).

## RESEARCH ON CITRUS GENETICS

Research on citrus genetics faces many serious impediments due to citrus being highly genetic heterozygosity, its longer juvenility, nucellar embryo interference, self-sterility or incompatibility of partial species, and because most citrus physiological and morphological traits are controlled by QTLs. During the 1980s-1990s, Chen *et al*. (1990, 1993) studied the heredity of citrus' main traits. As for the color heredity of citrus pulp, *orange or red* is a dominant allele while *yellow* recessive; *Poncirus* CTV resistance is controlled by a single allele, i.e. segregation of CTV resistance in the off-spring from the cross conforms to Mendel's Law of Segregation; citrus pollen fertility of male sterility is controlled by multiple minor genes and abides by consecutive and quantitative heredity. Chen *et al*. (1994) studied fruit shape heredity by 21 cross combinations and 1049 offspring, and found that the fruit shape of $F_1$ was made up of consecutive variation.

Recently, citrus molecular genetics and biotechnology work have been accelerated in China. The hereditary characteristic of nuclear and cytoplasmic somatic hybrids by cell fusion was widely studied in the citrus group of the National Key Lab of Crop Genetic Improvement of HAU

**Table 1** The contents of citrus germplasm in NCGR until the end of 2004.

| Categories | Abroad introduction (43.1%) | Indigenous accessions (56.9%) | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | Local cultivars | Breeding cultivars | Wild species | Related genera | Genetic materials | Mutants | Varieties | |
| Numbers | 451 | 296 | 37 | 116 | 8 | 105 | 17 | 16 | 1046 |

From www.ziyuanpu.net.cn/intro.aspx?puname=CRICAAS

(Chen *et al*. 2004; Hao *et al*. 2004a, 2004b; Liu *et al*. 2004a, 2004b; Guo *et al*. 2006). Their results showed that the tetraploid somatic hybrids possessed additive nuclear components from both fusion parents, whereas the diploid somatic hybrids only owned leaf parents' nuclear material; furthermore, chloroplast DNA (cpDNA) in most of the somatic hybrids showed unilateral or uniparental segregation with a few exceptions of co-existence of both fusion partners; however mitochondria DNA (mtDNA) in the somatic hybrids was mainly derived from the embryogenic parents and recombination or loss of mtDNA were found in some fusion combinations. Zhang *et al*. (2003, 2006) evaluated genetic variation of citrus callus bank *in vitro* and showed that chromosome doubling is a common factor observed in most citrus calli except that of '*Ruby*', '*Weizhang*' and '*Kinnow*'. Moreover, there existed a significant difference in the extent of variation among genotypes by Duncan Analysis, and calli of some genotypes still maintained their embryogenic capacity though they were conserved for a long time (over 15 years).

## BREEDING GOALS AND ACCOMPLISHMENTS

The goal of improving fresh fruit in the world has been to create, in the past 30 years, seedlessness, easy-peeling, enriched flavor and aroma, and extension of the ripening periods (Deng 2005). In China, one of the breeding goals is to acquire seedless varieties. Using bud sport and seedling selection, at least 150 strains of seedless or less seeds were obtained until now, such as '*Qianyang*', a seedless red sweet orange (Chen *et al*. 1992), seedless '*Shatian*' pummelo (Li *et al*. 1994), seedless '*Shatangju*' (Ye *et al*. 2006), seedless 'Ponkan' and '*Xuegan*' sweet orange (Chen 1997).

The second important breeding goal in China is to select early-, or late-ripening varieties as an important strategy to extend the harvest seasons. By bud-sport selection, some superior and promising varieties were released, e.g. '*Yanxi wanlu*' Ponkan, a late-ripening bud sport of 'Ponkan' selec-ted in Fujian, China, postponed the harvest time for 2 months from late November to Feburary (Zhong and Chen 1994). '*Mingliu Tianju*', selected from '*Chun Tianju*' tangerine in Guangdong province, is a late-ripening mutant suitable for planting in regions without frost disaster and ripens during late December and early March (Zeng *et al*. 2006). Recently, cooperating with local technical institutes, HAU selected '*Fengjiewancheng*' and '*Zaohong*' navel orange in Three Gorge orchard. The ripening period of '*Fengjiewancheng*' was at least 1 month later than its original cultivars (Liu *et al*. 2006), and '*Zaohong*' ripens in October, one month earlier than the parental line (Zhang *et al*. 2007). Moreover, HAU carried out a bud sport selection project in the main producing areas of navel orange and Ponkan orchards since 2002 and got a dozen promising strains with potential to extend the ripening period of navel orange and Ponkan.

The breeding goal of citrus rootstock in the world is to: 1) make scions more vigorous, dwarf-type, with high and stable production and good quality; 2) disease-resistant, especially against *Citrus tristeza* virus, Citrus nematodes and root rot; 3) tolerance to environmental stress, such as cold, salt, drought and high humidity. However, few studies on rootstock breeding have been carried out in China. China is one of the main native regions of most species in *Citrus* and related genera with plentiful genetic resource with resistant characteristics. Hence, citrus planting regions could select directly suitable rootstock from local resources and exhibits regional speciality in the application of rootstocks.

## BREEDING TECHNIQUES AND CULTIVARS

### Bud sport and seedling selection

Many countries began cross breeding during the 1920s-1930s. In China, cross breeding began in the 1940s. However, since the impediments mentioned above, cross breeding was in a very embarrassed status. Almost all the cultivars selected in the past 20 years came from bud sport or chance seedling selection with few from cross breeding (**Table 2**).

**Table 2** Important cultivars selected during the past 20 years in China.

| Types | Variety name | Released | Breeding region | Breeding method |
|---|---|---|---|---|
| *Citrus reticulata* | Ponggan Dong-13# | 1990 | Guangdong | Bud sport |
| | Longshen Pongan | 1993 | Guangxi | Bud sport |
| | Jinshui Ponggan | 1993 | Hubei | Bud sport |
| | Zaoshu Nanfengmiju | 1994 | Jiangxi | Bud sport |
| | Yanxi Wanlu | 1995 | Fujian | Bud sport |
| | Ganpon 1# | 1997 | Jiangxi | Bud sport |
| | Qianyang seedless Pongan | 1998 | Hunan | Bud sport |
| | Huagan 2# | 2005 | Hubei | Seedling |
| | Shiyuechu | 2005 | Guangdong | Seedling |
| | Mingliu Tianchu | 2006 | Guangdong | Bud sport |
| *C. sinensis* | Hongjiangcheng | 1986 | Guangdong | Seedling |
| | Daguo Bingtangcheng | 1987 | Hunan | Bud sport |
| | Beibei 447# | 1988 | Shichuang | Bud sport |
| | Tongshui72-1 Jincheng | 1988 | Shichuang | Bud sport |
| | Seedless Dahongcheng | 1992 | Hunan | Bud sport |
| | Navel orange 4# | 1996 | Shichuang | Bud sport |
| | Navel orange 7802# | 1996 | Hunan | Bud sport |
| | Huahong Navel orange | 1996 | Hubei | Bud sport |
| | Zhongyu 7# | 1997 | Chongqing | Radiation mutation |
| | Seedless xuegan | 1997 | Fujian | Radiation mutation |
| | Fengjiewancheng | 2005 | Chongqing | Bud sport |
| | Xinjing No.101 | 2005 | Hubei | Bud sport |
| | Zaohong Navel orange | 2006 | Hubei | Grafting Chimera |
| *C. unshiu* | Guoqing 1# | 1987 | Hubei | Bud sport |
| | Guoqing 4# | 1987 | Hubei | Bud sport |
| | Zaojin | 1990 | Shichuang | Bud sport |
| | Longyuanzao | 1990 | Hunan | Bud sport |
| | Wugangtezao | 1990 | Hunan | Bud sport |
| | Egan 2# | 2004 | Hubei | Bud sport |
| *C. grandisi* | Tongxianyou | 1986 | Sichuan | Seedling |
| | Seedless shatian pummelo | 1994 | Guangxi | Laser mutation |
| | Longhuizaoshu | 1999 | Jiangxi | Bud sport |
| | Feicuiyou | 2002 | Zhejiang | Seedling |
| | Taoxizaoyou | 2003 | Jiangxi | Seedling |
| | Chuhongyou | 2004 | Zhejiang | Seedling |
| | Hongroumiyou | 2004 | Fujian | Bud sport |
| Hybrid | Kaixuangan | 1989 | Zhejiang | Cross |
| | Hongyugan | 1989 | Zhejiang | Cross |
| | Wanmi 1# | 1991 | Zhejiang | Cross |
| | Jinshayou | 2001 | Jiangxi | Cross |

### Polyploidy breeding

To create triploid seedless fruit has always been a research hotspot in the world since 1960s for it can meet consumer's preference. China began polyploid breeding in 1970s. In 1974, a tetraploid trifoliate orange, '*GuanYun No.1*' was discovered at Guanyun prefecture of Jiangsu province (Chen and Song 1989). In 1985, Chen and Ou reported a seedless tetroploid '*Shiyueju*' tangerine created by treating the shoot of diploid '*Shiyueju*' with colchicine. Subsequently, Li and Zhang (1988) got seedless tetroploid *Fortunella japonica* by the same treatment. Hong *et al*. (2005) obtained tetraploid 'Ponkan' by culturing the embryos of mature seeds. Though


**Fig. 1 Chinese Citrus germplasm.** (**A**) The relative of *Citrus*, *Murraya paniculata*. (**B**) The key rootstock of citrus in China, *Poncirus trifoliata*. (**C**) Wild Kumquat, *Fortunella hindsii*. (**D**) Cultivated kumquat *F. crassifolia*, 'Rong-an Jingan'. (**E**) Red tangerine (*C. reticulata*), an old variety of citrus in China. (**F**) Nanfeng tangerine (*C. reticulata*), a kind of small citrus with long culture history in China. (**G**) Ponkan (*C. reticulata*), the most widely cultivated citrus in China. (**H**) Jincheng No.101 sweet orange (*C. sinensis*), a variety suitable for high quality juice. (**I**) Shatian pummelo (*C. grandisi*), most famous pummelo in China.

there has a hope to breed superior triploids by cross hybrid-dization of tetraploids with diploids, no positive results were reported by field cross hybridization due to lack of suitable tetraploid parent and nucellar embryo interference. Recently, HAU created about 40 allopolyploids via protoplast fusion and more than dozen combinations of triploid plants via somatic hybrids with diploid seedy cultivar with the aid of embryo rescue (Yi *et al*. 1997, 1998; Guo *et al*. 2000; Liu *et al*. 2002; Song *et al*. 2005; Guo *et al*. 2006), which highlights the hope to obtain superior triploid cultivars and polyploid rootstocks in the future.

## Biotechnology and germplasm innovation

Somatic cell fusion could overcome sexual incompatibility and long juvenility, and may play a potential role in citrus genetic improvement, including producing directly or indirectly superior varieties, improving citrus scion and rootstock, or creating allopolyploids for triploid breeding (Guo *et al*. 2000; Guo and Deng 2001).

The techniques of protoplast isolation (Deng *et al*. 1988), somatic cell fusion (Deng *et al*. 1992, 1993) and embryo rescue technology (Yi *et al*. 1997) were subsequently established in China since the late 1980s. In the past, creating new germplasm by conventional cross hybridization seemed difficult due to the obstacles mentioned above. However, these obstacles were overcome by the integration of tissue culture and embryo rescue technology, which could play a very important role in citrus germplasm innovation. To date, about 40 interspecific and intergeneric somatic hybrid combinations were obtained (Deng *et al*. 2005). Song *et al*. (2005) obtained 244 triploids and 10 tetraploids from a cross combination of allopolyploid × diploids via embryo rescue technology. By using a tissue culture system, Zhang (2006) recovered 1508 plantlets from the seeds and aborted ovules beneath the chimera part of the fruit with elite traits, and 1000 plantlets were successfully transferred to the greenhouse or field, which will be the candidates for new cultivar selection in the future.

## Others

The techniques mentioned above were mainly used in China for cultivar breeding and germplasm innovation. Except for these, Laser (Li *et al*. 1994), radiation (Chen 1997), and conventional cross hybridization (Chen, *et al*. 1991) were occasionally used to implement breeding goals in the past (**Table 2**). However, with the application of embryo rescue technology, and the requirement for construction genetic linkage maps, China began to strengthen the use of cross hybridization. Recently, HAU created 10 cross hybridization combinations and got over 1000 hybrid accessions verified by SSR analysis.

## RESEARCHES ON CITRUS GENOMICS

Research on citrus genomics in China was set up in the past for EST exploration and utilization, gene cloning, genomic sequencing and genetic map construction. The first cDNA library published in China was constructed from 'Cara Cara' navel orange pulp by Tao *et al*. (2006). Recently, different kinds of citrus cDNA libraries are being constructed in different research institutes. At the same time, EST sequencing is under way with about 700 ESTs having been obtained. In addition, citrus EST-SSR primers were exploited and utilized for marker-assisted selection (Meng *et al*. 2005; Cheng *et al*. 2006; Jiang *et al*. 2006). As for gene cloning and functional analysis, though 33 non-virus genes related to fruit development and ripening, sugar, acid and carotenoid metabolism were cloned and logged in the nucleotide database of NCBI (http://www.ncbi.nlm.nih.gov/) by China research institutes until the end of 2006, only a few gene functions were simply analyzed. Qin *et al*. (2004) cloned two citrus fructokinase genes (*Cufrk1* and *Cufrk2*) and found that they had tissue-and development-specific expression patterns. Tao *et al*. (2007) cloned a citrus phytoene synthase gene (*Psy*) and verified that its enhanced expression was related with exclusive phytoene accumulation during fruit ripening of 'Cara Cara' navel orange.

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## REFERENCES

* In Chinese

**Chen C-L, Guo W-W, Yi H-L, Deng X-X** (2004) Cytogenetic analysis of two interspecific citrus allotetraploid somatic hybrids and their diploid fusion parents. *Plant Breeding* **123**, 332-337

**Chen D-C, Ou Y-R** (1985) An promising mutated polyploids-tetroploid 'Shiyueju'. *China Citrus* **3**, 2-3*

**Chen G-M, Huang S-J, Chen C-M, Chen S-P, Cheng J-Y** (1997) Selection of seedless 'xuegan'. *Fujian Fruits* **2**, 46*

**Chen K-L, Chen L-G, Zhong G-Y, Hong Q-B** (1994) The heredity of fruit shape in citrus. *Journal of Southwest Agricultural University* **16**, 120-123*

**Chen L-G, Chen K-L, Li J, Hu Y-Q** (1993) Studies on the rind color heredity of citrus. *Acta Horticulturae Sinica* **20**, 22l-222*

**Chen L-G, Hu Y-Q, Chen K-L, Zhou J-X, Zhou Y-B** (1991) A new late-ripening Satsuma mandarin - 'Wanmi 1#'. *Journal of Fruit Science* **18**, 18l-184*

**Chen L-G** (1990) Genetic and breeding of citrus traits. *China Citrus* **4**, 17-18*

**Chen Q-M, Wei W-N, Yang X-G** (1992) Selection of seedless 'Qianyang dahong' sweet orange. *China Citrus* **21**, 3-4*

**Chen Q-Y, Song N-J** (1989) A tetraploid trifoliate orange 'Guanyun NO.1'. *Acta Horticulturae Sinica* **16**, 78-80*

**Chen Z-S** (2000) The status of citrus germplasm conservation, evaluation and utilization in China. *South China Fruits* **29**, 14-15*

**Cheng Y-J, Meng H-J, Guo W-W, Deng X-X** (2006) Universal chloroplast primer pairs for simple sequence repeat analysis in diverse genera of fruit crops. *Journal of Horticultural Science and Biotechnology* **8**, 132-138

**Cheng Y-J, Guo W-W, Deng X-X** (2003) cpSSR: a new tool to analyze chroloplast genome of citrus somatic hybrids. *Acta Botanica Sinica* **45**, 906-909

**Deng X-X, Grosser JW, Gmitter FG Jr.** (1992) Interspecific somatic hybrid of rough lemon and 'Hamlin' sweet orange via protoplast fusion. *Acta Genetica Sinica* **19**, 140-144

**Deng X-X, Hu C-G, Huo H-Q, Guo W-W, Yi H-L** (2000) A preliminary study on citrus germplasm conservation and evaluation by RAPD analysis. *Acta Horticulturae* **535**, 99-105

**Deng X-X, Xiao X-Y, Deng Z-A** (1993) Interspecific somatic hybrid of ichang papeda with valencia orange. *Chinese Journal of Biotechnology* **9**, 128-131*

**Deng X-X, Zhang W-C, Wan S-Y** (1988) Study of citrus protoplast isolation and its plant regeneration. *Acta Horticulturae Sinica* **l5**, 99-102*

**Deng X-X** (2005) Advances in world-wide citrus breeding. *Acta Horticulturae Sinica* **32**, 1140-1146*

**Fan M-T, Gao J, Li W-X, Xu M-H, Wu X-E, Long W-H** (2002) The RAPD analysis of fifteen germplasm resources of *Citrus*. *South China Fruits* **31**, 3-6*

**Fu C-H, Chen C-L, Guo W-W, Deng X-X** (2004) GISH, AFLP and PCR-RFLP analysis of an intergeneric somatic hybrid combining Goutou sour orange and *Poncirus trifoliata*. *Plant Cell Reports* **23**, 391-396

**Fu C-H, Chen F-Y, Deng X-X** (2006) Genetic diversity of *Citrus succosa* Hort. *ex* Tanaka from seven provinces as revealed by AFLP analysis. *Yi Chuan* **28**, 268-272

**Guo W-W, Cheng Y-J, Deng X-X** (2002) Intergeneric somatic hybrids of *Citrus reticulata* with *Poncirus trifoliate* for complementary rootstock improvement and their identification by AFLP analysis. *Plant Cell Reports* **20**, 829-834

**Guo W-W, Cheng Y-J, Chen C-L, Deng X-X** (2006) Molecular analysis revealed autotetraploid, diploid and tetraploid cybrid plants regenerated from an interspecific somatic fusion in *Citrus*. *Scientia Horticulturae* **108**, 162-166

**Guo W-W, Deng X-X, Yi H-L** (2000) Somatic hybrids between navel orange and grapefruit for seedless triploid breeding. *Euphytica* **116**, 281-285

**Guo W-W, Deng X-X** (2001) Wide somatic hybrids of Citrus with its related genera and their potential in genetic improvement. *Euphytica* **118**, 175-183

**Hao Y-J, You C-X, Deng X-X** (2004a) Evidences for the control of chromosome number variation by a programmed-cell-death-like pathway in citrus callus. *Euphytica* **140**, 205-212

**Hao Y-J, Wen X-P, Deng X-X** (2004b) Genetic and epigenetic evaluation of citrus calluses recovered from slow growth. *Journal of Plant Physiology* **161**, 479-484

**Hong L, Liu Y-Z, Deng X-X** (2005) Obtaining of ponkan (*Citrus reticulata* Blanco) tetraploid by culturing embryos of mature seeds. *Acta Horticulturae Sinica* **32**, 688-690*

**Jiang D, Zhong G-Y, Hong Q-B** (2006) Analysis of microsatellites in citrus unigenes. *Acta Genetica Sinica* **33**, 345-353*

**Li C-S, Zhang M-Z** (1988) Seedless *Fortunella* tetroploid induced by colchicine. *China Citrus* **17**, 39*

**Li Y-Z, Cheng Y-J, Guo W-W, Xu Q, Deng X-X** (2006) Genetic diversity in mandarin landraces and wild mandarins from China based on nuclear and chloroplast simple sequence repeat markers. *Journal of Horticultural Science and Biotechnology* **81**, 371-378

**Li Z, Li R-Y, Qing X-X, Chen M-X, Li T, Chen T-T** (1994) Study on laser-mutagenesis of shatian pumello. *Acta Laser Biology Sinica* **3**, 386-389*

**Liao Z-K, Zhang Q-M, Liu W-G, Ding W-P, Wang C-M** (2006) Identification of citrus mutants by APLP technique. *Journal of Fruit Science* **23**, 486-488*

**Liu J-H, Hu C-G, Deng X-X** (2002) Production of *Citrus* intergeneric tetraploid somatic hybrids plant from electricity-mediated protoplast fusion. *Acta Horticulturae Sinica* **29**, 372-374*

**Liu J-H, Xu X-Y, Deng X-X** (2004a) Regeneration of interspecific diploid somatic hybrid plants derived from protoplasts fusion in *Citrus*. *Journal of Agricultural Biotechnology* **12**, 237-246

**Liu J-H, Xu X-Y, Deng X-X** (2004b) Characterization of nuclear and cytoplasmic compositions of somatic hybrid plants between sweet orange and sour orange. *Acta Botanica Sinica* **46**, 1206-1211

**Liu Y, Sun Z-H, Liu D-C, Wu B, Tao J-J** (2005) Assessment of the genetic diversity of pummelo germplasms using AFLP and SSR markers. *Scientia Agriculturae Sinica* **38**, 2308-2315*

**Liu Y-Z, Tang P, Tao N-G, Xu Q, Peng S-A, Deng X-X, Xiang K-S, Huang R-H** (2006) Fruit coloration difference between Fengwan, a late-maturing mutant and its original cultivar Fengjie72-1 of navel orange (*Citrus sinensis* Osbeck). *Journal of Plant Physiology and Molecular Biology* **32**, 31-36

**Meng H-J, Cao Q-Q, Hu Z-Y, Liu G-P, Cheng Y-J, Deng X-X** (2005) Analysis of SSR in Citrus sequence from EMBL database. *Agricultural Science in China* **4**, 501-506

**Pang X-M** (2002) Studies on phylogeny of *Citrus* and its relatives and on genetic diversity of *Poncirus* using molecular markers. PhD Thesis, Huazhong Agriculture University, Wuhan, China, 103 pp*

**Pang X-M, Hu C-G, Deng X-X** (2003) Phylogenetic relationships as revealed among citrus and its relatives by SSR marker. *Acta Genetica Sinica* **30**, 81-87

**Pang X-M, Wen X-P, Hu C-G, Deng X-X** (2006) Genetic diversity of *Poncirus* accessions as revealed by amplified fragment length polymorphism (AFLP). *The Journal of Horticultural Science and Biotechnology* **81**, 269-275

**Qin Q-P, Zang S-L, Chen J-W, Xie M, Jin Y-F, Chen K-S, Asghar S** (2004) Isolation and expression analysis of fructokinase genes from citrus. *Acta Botanica Sinica* **46**, 1408-1415

**Shi Y-Z, Guo W-W, Deng X-X** (1998) Establishment of RAPD analysis techniques and identification of somatic hybrids in *Citrus*. *Acta Horticulturae Sinica* **25**, 105-110*

**Song J-K, Guo W-W, Yi H-L, Liu J-H, Chen C-L, Deng X-X** (2005) Creation of triploid citrus plants by crossing elite allotetraploid somatic hybrid pollen parents with diploid cultivars. *Acta Horticulturae Sinica* **32**, 594-598

**Tao N-G, Hu Z-Y, Liu Q, Xu J, Cheng Y-J, Guo L-L, Guo W-W, Deng X-X** (2007) Expression of phytoene synthase gene (*Psy*) is enhanced during fruit ripening of Cara Cara navel orange (*Citrus sinensis* Osbeck). *Plant Cell Reports* **26**, 837-843

**Tao N-G, Xu J, Chen Y-J, Deng X-X** (2006) Construction and characterization of a cDNA library from the pulp of Cara Cara navel orange (*Citrus sinensis* Osbeck). *Journal of Integrative Plant Biology* **48**, 315-319

**Wang Z-C, Deng X-X** (2004a) Plant regeneration from the cryopreserved somatic hybrid of *Citrus*. *Acta Horticulturae Sinica* **31**, 215-216*

**Wang Z-C, Deng X-X** (2004b) Cryopreservation of shoot-tips of citrus using vitrification: effect of reduced form of glutathione. *Cryoletters* **25**, 43-50

**Wang Z-C, Deng X-X** (2001) Cryopreservation of citrus shoot-tips by vitrification and regeneration. *Acta Horticulturae Sinica* **28**, 301-306*

**Xu X-Y, Liu J-H, Deng X-X** (2005) FCM, SSR and CAPS analysis of intergeneric somatic hybrid plants between Changsha kumquat and Dancy tangerine, *Botanical Bulletin of Academia Sinica* **46**, 93-98

**Ye Y-M** (2000) Review and prospect of citrus variety improvement. *South China Fruits* **29**, 15-17*

**Ye Z-X, Zeng T, Xu J-K, Luo Z-D, Hu G-B, Zhang Z-Q, Ji Z-L, Cheng Y-C, Cheng G-L, Cheng L-X, Ling X-Q** (2006) '*Wuzishatangju*', a new mandarin cultivar. *Journal of Fruit Science* **23**, 149-150*

**Yi H-L, Deng X-X, Shi Y-Z, Guo W-W** (1997) Studies on culture of immature triploidy embryos *in vitro*. *Acta Horticulturae Sinica* **24**, 289-291*

**Yi H-L, Deng X-X** (1998) Study of culture of citrus triploid plantlets. *Journal of Fruit Science* **l5**, 212-216*

**Yu X-J, Wang B-X, Bai S** (2006) International competitiveness of China's citrus industry and measures for its promotion. *Journal of Southwest Agricultural University (Social Science Edition)* **4**, 72-75*

**Zeng B-Q, Gan L, Xiong X-Y, Deng X-X, Deng Z-N** (2006) AFLP analysis of a superior bud mutation of 'Bingtang' orange. *Acta Agriculturae Universitis Jiangxiensis* **28**, 222-225*

**Zeng J-W, Peng C-J, Yi G-J, Du G-X, Zhang S-P, Feng C-P, Huo H-Q, Zhong Y, Zhou B-R, Huang Y-H** (2006) New late-ripening citrus variety – '*Mingliu Tianchu*'. *Acta Horticulturae Sinica* **33**, 1164*

**Zhang J-E, Liu J-H, Deng X-X** (2003) Genetic variation of citrus calli revealed by the ploidy analyser. *Acta Genetica Sinica* **30**, 169-174*

**Zhang J-E, Guo W-W, Deng X-X** (2006) Relationship between ploidy variation of citrus calli and competence for somatic embryogenesis. *Acta Genetica Sinica* **33**, 647-654

**Zhang M, Deng X-X, Qin C-P, Chen C-L, Zhang H-Y, Liu Q, Hu Z-Y, Guo L-L, Song W-H, Tan Y, Liao S-C** (2007) Characterization of a new natural periclinal navel-satsuma chimera of citrus 'Zaohong' navel orange. *Journal of the American Society of Horticultural Science* **132**, 374-380

**Zhang M** (2006) Seperation of citrus fruit sector chimeras and genetic analysis of two graft chimeras. PhD Thesis, Huazhong Agriculture University, Wuhan, China, 115 pp*

**Zhong L-S, Chen G-T** (1994) Selection of late-ripening Pongan – 'Yanxi wanlu'. *China Citrus* **23**, 14-15*