Chapter 9
Poncirus

Jaya R. Soneji and Madhugiri Nageswara Rao

9.1 Basic Botany of the Genus

Poncirus trifoliata (trifoliate orange or Japanese bitter orange) belongs to the subtribe Citrinae, tribe Citreaea, subfamily Aurantioideae of the family Rutaceae and is a close relative of citrus. It is thought to be a native of China and Korea. It was brought to North America as an ornamental plant. It is widely found in all citrus-growing regions of the world, as it is commonly used as a rootstock for citrus and it improves frost hardiness and fruit quality. It is usually propagated from seeds. It is a small, fast-growing, deciduous, perennial tree, which grows upto a height of 3–10 ft (2–3 m) and has a green bark with brown streaks. The twigs are covered with sharp spines. It is a very frost hardy tree and can withstand temperatures upto −5°F (−21°C) and lower. The leaves are trifoliate rarely having four or five leaflets, alternate with leaflets being glabrous, elliptic, oblong to obovate, sessile, and 2–6 cm long. The rachis is broadly winged. Flowers are simple, fragrant, sessile, white, borne on axillary inflorescence on previous year's growth, and have five sepals and petals, eight to ten stamens, which are entirely free, and a six-celled pubescent ovary. Though the flower buds are formed in early summer, they pass the winter in a dormant condition and are protected by bud scale. Fruits are small, pubescent, round to pear-shaped, yellow when ripe, and sour to bitter in taste with numerous seeds. Unlike citrus, the fruits of *Poncirus* are inedible in USA. However, in China, the dried mature fruits are used medicinally. The peel is candied and is used as a spice while the juice is used as flavoring syrup. It is used as a source of pectin and grown as an ornamental in the European countries. Due to its spines, it is also grown as a hedge plant in Brazil.

Poncirus species are mostly diploid with $2x = 2n = 18$ chromosomes. Chromomycin A 3 (CMA) and 4,6-diamidino-2-phenylindole (DAPI) have been used for chromosome banding for characterization of chromosomes of *P. trifoliata* (Moraes et al. 2008). This has allowed the separation of the nine chromosome pairs into three groups (4B + 8D + 6F) of which only one F chromosome pair could be distinguished (Befu et al. 2000; Brasileiro-Vidal et al. 2007). In situ hybridization demonstrated that two B chromosome pairs were different from those previously found in citrus as they displayed a 45S rDNA (ribosomal DNA) site colocalized with a CMA + proximal band and a 5S rDNA site adjacent to this band while only one pair of the four D chromosome pairs showed adjacent 5S and 45S rDNA sites, with a 45S rDNA site colocalized with a CMA + band (Brasileiro-Vidal et al. 2007). Bacterial artificial chromosome (BAC) in situ hybridization has also been attempted in *P. trifoliata* and allowed the identification of seven chromosome pairs while the other two were recognized by the presence of 45S rDNA associated with the CMA + band in the first one, and lack of any single copy signal and the presence of a terminal CMA + band in the second one (Moraes et al. 2008). All chromosome pairs were homomorphic, indicating a high level of chromosomal homozygosity. Thus, a combination of chromosome morphology, fluorochrome banding, and fluorescent in situ hybridization (FISH) with rDNA probes distinguished the *Poncirus* chromosomes successfully (Roose et al. 1998).

J.R. Soneji (✉)
Citrus Research & Education Center, University of Florida, IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850, USA
e-mail: jayasoneji@yahoo.co.in

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits*,
DOI 10.1007/978-3-642-20447-0_9,

9.2 Conservation Initiatives

Though *Poncirus* is widely used as a rootstock for citrus, very little information is available on the extent and distribution of its genetic diversity. It has traditionally been conserved in very small scale in clonal orchards belonging to botanical gardens or scientific institutions, where citrus is primarily conserved. It has been conserved along with citrus in large ex situ collections in Argentina, Australia, Brazil, France, Morocco, New Zealand, South Africa, Spain, Turkey, and USA (Krueger and Navarro 2007). No information is available on in situ conservation of *Poncirus*. Shoot tips from juvenile plants of *P. trifoliata* have been cryopreserved using encapsulation–dehydration method (Gonzalez-Arnao et al. 1988). Attempts have been made to cryopreserve seeds and embryonic axes of *P. trifoliata* in vitro. The seeds were found to be sensitive to desiccation, whereas the excised embryonic axes could be easily desiccated and successfully preserved in liquid nitrogen (Radhamani and Chandel 1992). Shoot tips excised from axillary buds of Troyer citrange (*P. trifoliata* × *C. sinensis*) have been cryopreserved using encapsulation–dehydration and encapsulation–vitrification methods (Wang et al. 2000).

9.3 Molecular Mapping in *Poncirus*

Poncirus has been extensively used as a parent in intergeneric crosses to facilitate optimum polymorphism to construct a number of molecular genetic linkage maps (Cai et al. 1994; Gmitter et al. 1996; Tozlu et al. 1999a, b; Ling et al. 2000); however, the hybrids obtained have inedible fruits making these mapping populations of limited use for fruit traits (Gmitter et al. 2007). A population obtained from an intergeneric backcross of *C. grandis* cv. "Thong Dee" and *P. trifoliata* cv. "Pomeroy," using the former as the recurrent (female) parent, has been used for performing genetic linkage analysis using restriction fragment length polymorphism (RFLP) and isozyme (Durham et al. 1992) markers. Another study used isozymes and RFLP for the construction of a genetic map based on the segregation of 8 isozyme, 1 protein, and 37 RFLP loci in 60 progeny of a cross of two intergeneric hybrids, "Sacaton" citrumelo (*C. paradisi* × *P. trifoliata*) and "Troyer" citrange (*C. sinensis* × *P. trifoliata*), often used as rootstocks (Jarrell et al. 1992). Linkage maps have also been constructed using various molecular markers from intergeneric crosses such as *C. grandis* × (*C. grandis* × *P. trifoliata*), *C. sunki* × *P. trifoliata* cv. Rubidoux, *C. grandis* cv. "Thong Dee," and *P. trifoliata* cv. "Pomeroy" (Cai et al. 1994; Cristofani et al. 1999; Sankar and Moore 2001; Table 9.1). Five genetic linkage maps have been constructed for the parents of progenies of *C. aurantium* × *P. trifoliata* var. Flying Dragon, *C. volkameriana* × *P. trifoliata* var. *Rubidoux* and a self-pollination of *P. trifoliata* var. Flying Dragon using simple sequence repeats (SSRs) for genome comparison (Ruiz and Asins 2003). Recently, an F_1 intergeneric population of *C. sinensis* × *P. trifoliata* was used to construct genetic maps in which 11 linkage groups with 113 markers in *C. sinensis*, nine with 45 markers in *P. trifoliata*, and 13 with 123 markers in the cross-pollinator consensus of both, were constructed (Chen et al. 2008).

Resistance to citrus tristeza virus (CTV) was found to be dominant by performing enzyme-linked immunosorbent assay (ELISA) on several *Poncirus*-derived populations. Using bulked segregant analysis (BSA) approach (Michelmore et al. 1991) and RAPD markers, a map was developed and the *Ctv* gene was identified from *Poncirus* (Gmitter et al. 1996; Fang et al. 1998). Two BAC contigs with integrated fine maps were constructed that resulted in the full-length sequencing of the locus spanning several hundreds of kilobases and identification of the candidate genes (Deng et al. 1997, 2001; Yang et al. 2001, 2003). Prolonged CTV challenge led to the suggestion that more than one gene may be involved in CTV resistance (Mestre et al. 1997). One CTV-resistant gene was later mapped in a different location within linkage group 4 of *Poncirus* from a population of citradias (derived from the cross between sour orange and *Poncirus*) suggesting a deviation from the single gene hypothesis, which could be quantitative trait loci (QTLs) (Bernet and Asins 2003; Asins et al. 2004). It was found that a major QTL, designated *Tyr1*, controls resistance to citrus nematode (Ling et al. 2000) and was adjacent to the *Ctv* region (Ling et al. 1999). In the *C. volkameriana* and *P. trifoliata* progeny, 11 putative QTLs have been detected in *P. trifoliata* that control the number of fruits per tree (Garcia et al. 2000). A *C. grandis* × *P. trifoliata* F_1 pseudo-testcross population was used to map QTLs associated with

Table 9.1 *Poncirus* linkage maps

Mapped parent	Type of cross	Population size	Markers used	Total markers	Map length (cM)	Linkage groups	Reference
C. grandis cv. Chandler × *P. trifoliata* cv. Webber-Fawcett	Intergeneric F_1	35	Isozymes	3	–	2	Torres et al. (1985)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	65	Isozymes, RFLP	52	533	11	Durham et al. (1992)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_1	60	Isozymes, RFLP	38	351	10	Jarrell et al.(1992)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	60	Isozymes, RAPD, RFLP	189	1,192	9	Cai et al. (1994)
C. reshni × *P. trifoliata*	Intergeneric F_1	52	RAPD	97	1,503	12	Luro et al. (1996)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_1	57	ISSR, RFLP	48	410	12	Kijas et al. (1997)
P. trifoliata	F_1	80	RAPD	62	866	8	Cristofani et al. (1999)
C. volkameriana × *P. trifoliata* cv. Rubidoux	Intergeneric F_1	80	CAPS, Isozymes, RAPD, RFLP, SSRs	38	–	3	Garcia et al. (1999)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	65	AFLP, RAPD	337	1,031	14	Ling et al. (1999)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_2	57	ISSR, RAPD, RFLP	153	701	16	Roose et al. (2000)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	60	Isozymes, ISSR, RAPD, RFLP	310	874	9	Sankar and Moore (2001)
P. trifoliata	Self F_1	80	IRAP, RAPD, SSR	48	10	342	Ruiz and Asins (2003)
P. trifoliata	F_1	97	EST-SSRs	45	426	8	Chen et al. (2008)

AFLP amplified fragment length polymorphism, *CAPS* cleaved amplified polymorphic sequence, *EST-SSR* expressed sequence tag-simple sequence repeat, *IRAP* interretrotransposon amplified polymorphism, *ISSR* intersimple sequence repeat, *RAPD* randomly amplified polymorphic DNA, *RFLP* restriction fragment length polymorphism, *SSR* simple sequence repeat freezing tolerance (Weber et al. 2003). The dominant trifoliate leaf character of *Poncirus* has also proven to be advantageous in developing mapping populations, as it allows the direct identification of zygotic hybrids from true nucellar seedlings. F_1 progeny of *C. sunki* × *P. trifoliata* were evaluated for the detection of QTLs linked to citrus *Phythophthora* gummosis resistance. Two QTLs linked to gummosis resistance were detected in linkage groups 1 and 5 of the *P. trifoliata* map and one in linkage group 2 of the *C. sunki* map (Siviero et al. 2006). QTL analysis of morphological traits in an intergeneric BC_1 progeny of *C. grandis* × *P. trifoliata* under saline and non-saline environments has also been attempted (Tozlu et al. 1999b).

9.4 Role in Crop Improvement Through Traditional and Advanced Tools

9.4.1 Traditional Breeding Efforts

Poncirus is commonly used as a rootstock for most citrus species and is also the most valuable genetic resource for the genetic improvement of citrus (Gmitter et al. 2007). It produces fertile hybrids with citrus and is an important source of useful genes for citrus rootstocks (Roose et al. 1998). It is resistant or tolerant to CTV, Phytophthora root rot, citrus nematode, cold accumulation, and other environmental stresses and has been explored for use in citrus scion and rootstock genetic improvement programs via conventional breeding and molecular approaches (Cai et al. 1994; Gmitter et al. 1996; Tozlu et al. 1999a, b; Ling et al. 2000). Sexual hybridization using *P. trifoliata* as one of the parents has been used to produce genetically improved combinations of rootstocks for use in citrus propagation. Carrizo and Troyer citranges (*C. sinensis* × *P. trifoliata*) and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) rootstocks were selected from intergeneric hybrid progeny and were found to have *Phythophthora*, virus, and nematode tolerance inherited from *P. trifoliata*. "US-852," a hybrid obtained from sexual hybridization of *C. reticulata* × *P. trifoliata*, exhibited outstanding effects on sweet orange fruit yield, producing fruit with high soluble solids on medium size trees (Bowman et al. 1999). Four new rootstocks, two (Forner Alcaide 5 and Forner Alcaide 13) obtained by sexual hybridization between Cleopatra mandarin × *P. trifoliata*, one (Forner Alcaide 418) of Troyer citrange × *P. trifoliata*, and one (Forner Alcaide 517) of King mandarin × *P. trifoliata*, resistant or tolerant to CTV and salinity have been released (Nicotra 2001; Forner et al. 2003). "X639", a hybrid between "Cleopatra" mandarin × *P. trifoliata*, though susceptible to nematodes and root pathogens, proved to be an excellent rootstock for lemons and mandarins (Miller et al. 2003).

9.4.2 Ploidy Manipulation

Anther culture has been used to recover haploid plantlets from *P. trifoliata* (Hidaka et al. 1979). Deng et al. (1992a) were able to obtain only heterozygous plantlets from anther culture of *P. trifoliata*. In *P. trifoliata,* pollen culture has also been attempted; however, plantlets were not obtained (Germana et al. 1996). The pollen developmental stages, genotype used as well as the tissue culture parameters affect the success of anther culture. The effect of different developmental stages of *P. trifoliata* pollen grains on the formation of embryoids, pseudobulbils, and calli has been studied (Hidaka et al. 1979). For embryoid production, the early uninucleate stage was the most suitable while anthers at other developmental stages from pollen mother cell to bicellular stage produced only calli (Germana 2007). Hidaka (1984) studied the effects of sucrose concentration (1, 3, 5, 7, and 9%) on embryoid and callus formation and found that 3% sucrose was ideal for embryoid formation in *P. trifoliata*. Medium supplemented with 0.2 mg/l of both indole-3-acetic acid (IAA) and kinetin (Kn) was found to be efficient for embryoid formation while the addition of 2,4-dicholorphenoxy acetic acid (2,4-D) induced callus formation in *P. trifoliata*. Deng et al. (1992b) found that the addition of α-naphthaleneacetic acid (NAA) and activated charcoal in the medium induced embryoid formation in *P. trifoliata.*

Hybrid embryo rescue has also been exploited for the genetic improvement of *Poncirus*. *P. trifoliata* is cold hardy and resistant to root-rot, CTV, and citrus-browning nematode. However, it is susceptible to citrus exocortis viriod (CVC). Controlled crosses, followed by triploid hybrid embryo rescue, were carried out between Red tangerine (which is resistant to

CVC) × *P. trifoliata* as well as Satsuma mandarin (which is citrus canker tolerant) × *P. trifoliata* to introduce these new characters into *P. trifoliata* (Tan et al. 2007). To produce triploid intergeneric hybrids, gametosomatic fusion between *P. trifoliata* tetrads and somatic protoplasts of *C. sinensis* has been reported (Deng et al. 1992b).

Somatic hybridization allows the production of somatic hybrids that incorporate genomes of the two parents without recombination, thus avoiding the problem of the high heterozygosity (Navarro et al. 2004). Production of tetraploid somatic hybrids that combine complementary diploid rootstock germplasm via protoplast fusion has become a practical strategy with the overall objective of packaging necessary disease and pest resistance into horticulturally desirable, widely adapted rootstock such as *Poncirus*. In *Poncirus*, the first somatic hybrid was obtained between *C. sinensis* and *P. trifoliata* (Ohgawara et al. 1985). These results allowed the establishment of rootstock breeding programs in several countries. A number of somatic hybrids with *Poncirus* as one of the parents have been generated (Table 9.2) and are at different stages of field trial (Grosser et al. 2000). Somatic hybrid rootstocks are showing good potential to reduce tree size, as needed, for more efficient high-density plantings with good yields of high quality (Grosser 2003). Seed trees of most of these somatic hybrid rootstocks are also producing adequate nucellar seeds for standard propagation (Grosser et al. 2000).

9.4.3 Genetic Engineering

Genetic transformation of *Poncirus* and its hybrids has been achieved (Table 9.3). The first efficient protocol for transformation of seedling explants of *P. trifoliata* was established by Kaneyoshi et al. (1994), which was subsequently used by many groups (Kobayashi et al. 1996; Kaneyoshi and Kobayashi 1999; Wong et al. 2001; Iwanami et al. 2004; Endo et al. 2005). A similar protocol was used to transform Carrizo citrange (*C. sinensis* × *P. trifoliata*) that did not respond as well as *P. trifoliata* (Pena et al. 1995a). Hence, several factors affecting transformation and regeneration were critically studied (Cervera et al. 1998a). Cocultivation of epicotyl or internodal stem segments with *Agrobacterium tumefaciens* has been the most commonly used systems to efficiently produce transgenic plants of *P. trifoliata* (Kaneyoshi et al. 1994) and citrange (*C. sinensis* × *P. trifoliata*, Pena et al. 1995b; Gutierrez et al. 1997; Cervera et al. 1998b). To enhance both regeneration and transformation frequency, Yu et al. (2002) proposed cutting longitudinally the epicotyl segments of Carrizo citrange in two halves. Thin layers of about 1–2 mm cut transversally from etiolated epicotyls were found to be highly organogenic in *P. trifoliata,* Swingle citrumelo and Carrizo citrange transformation (Le et al. 1999; Molinari et al. 2004a, b).

Several authors have proposed the use of *rol* genes from the Ri plasmid as transgenes to produce dwarf *P. trifoliata* and citrange rootstocks (Gentile et al. 1998; Kaneyoshi and Kobayashi 1999). *rolC* gene from *A. rhizogenes* has been successfully incorporated into *P. trifoliata* (Kaneyoshi and Kobayashi 1999). Human epidermal growth factor (hEGF) has also been incorporated into *P. trifoliata* (Kobayashi et al. 1996). A citrus gibberellin (GA) 20-oxidase cDNA (*CcGA20ox1*) gene that controls the plant architecture and a halotolerance gene *HAL2* have been introduced into Carrizo citrange (Cervera et al. 2000a; Fagoaga et al. 2007). *Arabidopsis* genes such as *LEAFY* or *APETALA1* that alter the growth habit, reduce juvenility, and regulate vegetative and other behavior have been introduced into juvenile Carrizo citrange seedling explants (Pena et al. 2001). Regenerants of Carrizo citrange obtained under selective conditions after *Agrobacterium*-mediated transformation have been used for the characterization of these regenerants into silenced and/or chimeric plants (Dominguez et al. 2004). Gene constructs have been created for various types of CTV-derived genes and have been introduced into Carrizo citrange in efforts to induce resistance to the CTV virus (Gutierrez et al. 1997). A citrus blight-associated gene has also been introduced into Carrizo citrange (Kayim et al. 2004). Coat protein gene from citrus mosaic virus (CiMV) has been incorporated into *P. trifoliata* (Iwanami et al. 2004). Another gene *FLOWERING LOCUS T* that reduces the time of flowering has been incorporated into *P. trifoliata* and the transformed regenerants flowered in less than 8 months with four out of six transgenic lines developed normal fruits with intact seeds (Endo et al. 2005). In Spain, 16 transgenic plants of Carrizo citrange, with two plants each from eight independent transgenic lines, have been released under an experimentally controlled field for further evaluation (Pena et al. 2008).

Table 9.2 Somatic hybrids regenerated using *Poncirus* as one of the parents

Embryogenic parent	Leaf parent	Somatic hybrids regenerated	Reference
Trovita sweet orange	*Poncirus trifoliata*	–	Ohgawara et al. (1985)
Trovita sweet orange	Troyer citrange	–	Kobayashi and Ohgawara (1988)
Trovita sweet orange	*P. trifoliata*	–	Kobayashi and Ohgawara (1988)
Navel orange	Troyer citrange	>5	Ohgawara et al. (1991)
Valencia sweet orange	Carrizo citrange	7	Louzada et al. (1992)
Milam lemon	*C. depressa* × *P. trifoliata*	23	Tusa et al. (1992)
Cleopatra mandarin	Argentine trifoliate orange	6	Grosser et al. (1994)
Succari sweet orange	Argentine trifoliate orange	>200	Grosser et al. (1994)
Sour orange	Flying Dragon trifoliate orange	210	Grosser et al. (1994)
Shirayanagi navel orange	*P. trifoliata*	12	Kaneko et al. (1995)
Red Marsh grapefruit	Argentine trifoliate orange	33	Grosser et al. (1996)
Red Marsh grapefruit	Flying Dragon trifoliate orange	46	Grosser et al. (1996)
Willow leaf mandarin	Pomeroy trifoliate orange	24	Ollitrault et al. (1996)
P. trifoliata	*Fortunella hindsii*	17	Miranda et al. (1997)
Milam lemon hybrid	Swingle citrumelo	>100	Grosser et al. (1998)
Milam lemon hybrid	Carrizo citrange	15	Grosser et al. (1998)
Cleopatra mandarin	Carrizo citrange	>100	Grosser et al. (1998)
Sour orange	Carrizo citrange	>100	Grosser et al. (1998)
Page tangelo	*P. trifoliata*	>150	Deng et al. (2000)
Red tangerine	*P. trifoliata*	>30	Deng et al. (2000)
Red tangerine	*Citrange*	10	Deng et al. (2000)
Hamlin sweet orange	Flying Dragon trifoliate orange	>800	Grosser et al. (2000)
Valencia sweet orange	Carrizo citrange	>75	Grosser et al. (2000)
Cleopatra mandarin	Flying Dragon trifoliate orange	>300	Grosser et al. (2000)
Cleopatra mandarin	Swingle citrumelo	>300	Grosser et al. (2000)
Murcott tangor	Cohen citrange (pentaploid)	3	Grosser et al. (2000)
Nova tangelo	Cohen citrange (pentaploid)	>30	Grosser et al. (2000)
Cleopatra mandarin	Cohen citrange (pentaploid)	>30	Grosser et al. (2000)
Sour orange	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Washington Navel sweet orange	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Changsa mandarin	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Duncan grapefruit	*P. trifoliata* 50-7	>50	Grosser et al. (2000)
Sour orange	Benton citrange	>50	Grosser et al. (2000)
C. sunki	Carrizo citrange	–	Ollitrault et al. (2000)
C. aurantifolia	Carrizo citrange	–	Ollitrault et al. (2000)
Amblycarpa mandarin	Benton citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Carrizo citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	C-35 citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Flying Dragon trifoliate orange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Rubidoux trifoliate orange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Somatic hybrid of sour orange + Flying Dragon trifoliate orange	<50	Medina-Urrutia et al. (2004)

Table 9.3 Summary of *Agrobacterium*-mediated transformation of *Poncirus trifoliata* and its hybrids

Genotype used	Explant used	Gene(s) introduced	Reference
Carrizo citrange	In vitro internodal stem segments	*nptII*, *uidA*	Moore et al. (1992)
P. trifoliata	In vitro epicotyl segments	*nptII*, *uidA*	Kaneyoshi et al. (1994)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Pena et al. (1995b)
P. trifoliata	In vitro epicotyl segments	*hEGF*	Kobayashi et al. (1996)
Carrizo citrange	In vitro internodal stem segments	CTV-*CP*	Gutierrez et al. (1997)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Cervera et al. (1998c)
Troyer citrange	In vitro epicotyl segments	*nptII*, *rolA*, *rolB*, *rolC*	Gentile et al. (1998)
Carrizo citrange	In vitro epicotyl segments or greenhouse internodal stem segments	*nptII*, *gfp*	Ghorbel et al. (1999)
P. trifoliata	In vitro epicotyl segments	*rolC*	Kaneyoshi and Kobayashi (1999)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Cervera et al. (2000b)
Carrizo citrange	In vitro epicotyl segments	*HAL2*	Cervera et al. (2000a)
Troyer citrange	In vitro epicotyl segments	*nptII*, *gfp*	LaMalfa et al. (2000)
Carrizo citrange	In vitro epicotyl segments	CS-*ACS1*	Wong et al. (2001)
P. trifoliata	In vitro epicotyl segments	CS-*ACS1*	Wong et al. (2001)
Carrizo citrange	In vitro epicotyl segments	*AP1*, *LFY*	Pena et al. (2001)
Carrizo citrange	In vitro internodal stem segments	*nptII*, *uidA*	Yu et al. (2002)
Swingle citrumelo	In vitro epicotyl thin sections	*nptII*, *uidA*	Molinari et al. (2004a)
P. trifoliata	In vitro epicotyl thin sections	*nptII*, *uidA*	Molinari et al. (2004a)
Carrizo citrange	In vitro epicotyl segments	*p5cs*	Molinari et al. (2004b)
Carrizo citrange	In vitro epicotyl segments	*p12*	Kayim et al. (2004)
P. trifoliata	In vitro epicotyl segments	CiMV-*CP*	Iwanami et al. (2004)
P. trifoliata	In vitro epicotyl segments	Ci-*FT*	Endo et al. (2005)
P. trifoliata	Greenhouse internodal stem segments	CTV-*p23*	Fagoaga et al. (2005)
Carrizo citrange	In vitro epicotyl segments	*hpt, bar, gfp*	Cervera et al. (2006)
Carrizo citrange	In vitro epicotyl segments or greenhouse internodal stem segments	*ipt, R/RS* recombinase system	Ballester et al. (2007)

9.5 Genomics Resources Developed

Poncirus and its hybrids have been extensively used in EST-sequencing efforts (Talon and Gmitter Jr 2008). They have also been used to generate ESTs from several libraries under biotic (*Xylella fastidiosa*, CTV, citrus leprosis virus, *Phythophthora*, mite) and abiotic (drought) stresses, and during fruit development (Machado et al. 2007). Around 62,344 ESTs have been generated from *P. trifoliata*, and 9,791 from its hybrids with citrus (http://www.int-citrusgenome.org/usa/) using various tissues as seed, leaf, bark, greenhouse and field-grown plants, etc. An Affymetrix citrus GeneChip has been developed, which contains probe sets for detection of several pathogens and commonly used transgenes, and a representation of the region of the *P. trifoliata* genome containing *Ctv*, the CTV resistance allele (Close et al. 2006; Talon and Gmitter Jr 2008).

9.6 Conclusion

Poncirus is found in all citrus-growing regions of the world. It is known to have originated in China, where it is also used for its medicinal properties. In certain parts of Europe, it is grown as an ornamental plant. It is propagated by seeds and is a useful rootstock for citrus. Its genetic signatures of resistance to major diseases (such as CTV, nematode, etc.) and environmental stresses (such as salinity, temperature, etc.)

have been exploited by citrus breeders in their various rootstock improvement programs. It is also highly amenable to plant tissue culture techniques. Haploid plants have been produced via anther culture, hybrid embryo rescue has been utilized to produce triploids, and somatic hybridization has given rise to tetraploid plants in *Poncirus*. These haploids, triploids, and tetraploids are highly beneficial for citrus scion and rootstock breeding programs. It has also been genetically transformed to establish and standardize the protocol for use in citrus improvement.

Poncirus has been extensively used as a parent in intergeneric crosses with citrus. This has given rise to optimum polymorphism, which has facilitated the construction of a number of molecular genetic linkage maps. The identification, tagging, and cloning of the economically important genes will provide new information and gene targets for genetic manipulation, and hence will be of great use in citrus genetic improvement. The mapping populations of the intergeneric crosses between citrus and *Poncirus* have also been used to construct BAC libraries, develop ESTs, used in microarrays, and to develop sequence-based maps. Their gene sequence divergence, synteny, orientation, and possible probable functions have been annotated and compared. These genomic resources will have a great impact on the whole-genome sequencing of citrus. As the whole citrus genome is sequenced, subsequent exploration, comparison, and utilization of that data would be beneficial for the genetic improvement of *Poncirus*.

References

Asins MJ, Bernet GP, Ruiz C, Cambra M, Guerri J, Carbonell EA (2004) QTL analysis of citrus tristeza virus-citradia interaction. Theor Appl Genet 108:603–611

Ballester A, Cervera M, Pena L (2007) Efficient production of transgenic citrus plants using isopentenyl transferase positive selection and removal of the marker gene by site-specific recombination. Plant Cell Rep 26:39–45

Befu M, Kitajima A, Yang XL, Hasegawa K (2000) Classification of 'Tosa-Buntan' pummelo (*Citrus grandis* [L.] Osb.), 'Washington' navel orange (*C. sinensis* [L.] Osb.) and trifoliate orange (*Poncirus trifoliata* [L.] Raf.) chromosomes using young leaves. J Jpn Soc Hortic Sci 69:22–28

Bernet GP, Asins MJ (2003) Identification and genomic distribution of *gypsy*-like retrotransposons in *Citrus* and *Poncirus*. Theor Appl Genet 108:121–130

Bowman KD, Wutscher HK, Kaplan DT, Chaparro JX (1999) A new hybrid citrus rootstock for Florida: US-852. Proc FL State Hortic Soc 112:54–55

Brasileiro-Vidal AC, dos Santos-Serejo JA, Soares Filho WS, Guerra M (2007) A simple chromosomal marker can reliably distinguish *Poncirus* from *Citrus* species. Genetica 129:273–279

Cai Q, Guy CL, Moore GA (1994) Extension of the genetic linkage map in *Citrus* using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation responsive loci. Theor Appl Genet 89:606–614

Cervera M, Pina JA, Juarez J, Navarro L, Pena L (1998a) *Agrobacterium*-mediated transformation of citrange: factors affecting transformation and regeneration. Plant Cell Rep 16:271–278

Cervera M, Juarez J, Navarro A, Pina JA, Duran-Vila N, Navarro L, Pena L (1998b) Genetic transformation and regeneration of mature tissues of woody fruit plants bypassing the juvenile stage. Transgenic Res 7:51–59

Cervera M, Juarez J, Pina JA, Navarro L, Pena L (1998c) *Agrobacterium*-mediated transformation of citrange: factors affecting transformation and regeneration. Plant Cell Rep 18:271–278

Cervera M, Ortega C, Navarro A, Navarro L, Pena L (2000a) Generation of transgenic citrus plants with the tolerance-to-salinity gene *HAL2* from yeast. J Hortic Sci Biotechnol 75:26–30

Cervera M, Pina JA, Juarez J, Navarro L, Pena L (2000b) A broad exploration of a transgenic population of citrus: stability on gene expression and phenotype. Theor Appl Genet 100:670–677

Cervera M, Navarro A, Navarro L, Pena L (2006) Retransformation of *APETALA1* (*AP1*) early-flowering citrus plants as a strategy to rapidly evaluate transgenes addressing fruit quality traits. In: Teixeira da Silva JA (ed) Horticulture, ornamental and plant biotechnology: advances and topical issues. Global Science Books, London, pp 117–123

Chen C, Bowman KD, Choi YA, Dang PM, Nageswara Rao M, Huang S, Soneji JR, McCollum TG, Gmitter FG Jr (2008) EST-SSR Genetic Maps for *Citrus sinensis* and *Poncirus trifoliata*. Tree Genet Genomes 4:1–10

Close TJ, Wanamaker S, Lyon M, Mei G, Davies C, et al (2006) A GeneChip R for Citrus. In: Plant and animal genome XIV conference, 14–18 Jan 2006, San Diego, CA, USA, W82, p 26

Cristofani M, Machado MA, Grattapaglia D (1999) Genetic linkage maps of *Citrus sunki* Hort. ex. Tan. and *Poncirus trifoliata* (L.) Raf. and mapping of citrus tristeza virus resistance gene. Euphytica 109:25–32

Deng XX, Deng ZA, Xiao SY, Zhang WC (1992a) Pollen derived plantlets from anther culture of Ichang papeda hybrids No. 14 and trifoliate orange. Proc Int Soc Citric 1:190–192

Deng ZA, Deng XX, Zhang WC, Wan SY (1992b) A preliminary report on gametosomatic fusion in citrus. Proc Int Soc Citric 1:170–172

Deng Z, Huang S, Xiao S, Gmitter FG Jr (1997) Development and characterization of SCAR markers linked to the citrus tristeza virus resistance gene from *Poncirus trifoliata*. Genome 40:697–704

Deng Z, Huang S, Ling P, Chen C, Yu C, Weber CA, Moore GA, Gmitter FG Jr (2000) Cloning and characterization of the NBS-LRR class resistance-gene candidate sequence in citrus. Theor Appl Genet 101:814–822

Deng Z, Huang S, Ling P, Yu C, Tao Q, Chen C, Wendell MK, Zhang HB, Gmitter FG Jr (2001) Fine genetic mapping and BAC contig development for the citrus tristeza virus resistance gene locus in *Poncirus trifoliata* (Raf.). Mol Genet Genom 265:739–747

Dominguez A, Cervera M, Perez R, Romero J, Fagoaga C, Cubero J, Lopez MM, Juarez J, Navarro L, Pena L (2004) Characterization of regenerants obtained under selective conditions after *Agrobacterium*-mediated transformation of citrus explants reveals production of silenced and chimeric plants at unexpected high frequencies. Mol Breed 14:171–183

Durham RE, Liou PC, Gmitter FG Jr, Moore GA (1992) Linkage of restriction fragment length polymorphisms and isozymes in Citrus. Theor Appl Genet 84:39–48

Endo T, Shimada T, Fujii H, Kobayashi Y, Araki T, Omura M (2005) Ectopic expression of an *FT* homolog from *Citrus* confers an early flowering phenotype on trifoliate orange (*Poncirus trifoliata* L. Raf.). Transgenic Res 14:703–712

Fagoaga C, Lopez C, Moreno P, Navarro L, Flores R, Pena L (2005) Viral-like symptoms induced by the ectopic expression of the p23 gene of *Citrus tristeza virus* are citrus-specific and do not correlate with the pathogenicity of the virus strain. Mol Plant Microbe Interact 18:435–445

Fagoaga C, Tadeo FR, Iglesias D, Huerta L, Lliso I, Vidal AM, Talon M, Navarro L, García-Martínez JL, Pena L (2007) Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture. J Exp Bot 58:1407–1420

Fang DQ, Federici CT, Roose ML (1998) A high-resolution linkage map of the citrus tristeza virus resistance gene region in *Poncirus trifoliata* (L.) Raf. Genetics 150:883–890

Forner JB, Forner-Giner MA, Alcaide A (2003) Forner-Alcaide 5 and Forner-Alcaide 13: two new citrus rootstocks released in Spain. HortScience 38(4):629–630

Garcia R, Asins MJ, Forner J, Carbonell EA (1999) Genetic analysis of apomixis in *Citrus* and *Poncirus* by molecular markers. Theor Appl Genet 99:511–518

Garcia R, Asins MJ, Carbonell EA (2000) QTL analysis of yield and seed number in Citrus. Theor Appl Genet 101:487–493

Gentile A, LaMalfa S, Deng ZN, Domina F, Nicolosi E, Tribulato E (1998) Transgenic citrus: first experiences with *Rol* genes. Rivisti di Frutticoltura e di Ortofloricoltura 61:59–61

Germana MA (2007) Haploidy. In: Khan I (ed) Citrus: genetics, breeding and biotechnology. CABI, Cambridge, MA, USA, pp 167–196

Germana MA, Scarano MT, Crescimanno FG (1996) First results on isolated microspore culture of *Citrus*. Proc Int Soc Citric 2:882–885

Ghorbel R, Juarez J, Navarro L, Pena L (1999) Green fluorescent protein as a screenable marker to increase the efficiency of generating transgenic woody fruit plants. Theor Appl Genet 99:350–358

Gmitter FG Jr, Xiao SY, Huang S, Hu XL, Garnsey SM, Deng Z (1996) A localized linkage map of the citrus tristeza virus resistance gene region. Theor Appl Genet 92:688–695

Gmitter FG Jr, Chen C, Nageswara Rao M, Soneji JR (2007) Citrus fruits. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 4, Fruits and nuts. Springer, Berlin, Germany, pp 265–279

Gonzalez-Arnao MT, Engelmann F, Urra C, Morenza M, Rios A (1988) Cryopreservation of citrus apices using the encapsulation-dehydration technique. CryoLetters 19:177–182

Grosser JW (2003) Somatic hybridization in citrus – a relevant technique for variety improvement in 21st century. Acta Hort 622:491–497

Grosser JW, Louzada ES, Gmitter FG Jr, Chandler JL (1994) Somatic hybridization of complementary citrus rootstocks. HortScience 29(7):812–813

Grosser JW, Mourao-Fo FAA, Gmitter FG Jr, Louzada ES, Jiang J, Baergen K, Quiros A, Cabasson C, Schell JL, Chandler JL (1996) Allotetraploid hybrids between *citrus* and seven related genera produced by somatic hybridization. Theor Appl Genet 92:577–582

Grosser JW, Jiang J, Mourao-Fo FAA, Louzada ES, Baergen K, Chandler JL, Gmitter FG Jr (1998) Somatic hybridization, an integral component of citrus cultivar improvement: I Scion improvement. HortScience 33:1057–1059

Grosser JW, Ollitrault P, Olivares-Fuster O (2000) Somatic hybridization in citrus: an effective tool to facilitate variety improvement. In Vitro Dev Cell Biol Plant 36:434–449

Gutierrez-E MA, Luth D, Moore GA (1997) Factors affecting *Agrobacterium*-mediated transformation in *Citrus* and production of sour orange (*Citrus aurantium* L.) plants expressing the coat protein gene of citrus tristeza virus. Plant Cell Rep 16:745–753

Hidaka T (1984) Induction of plants from anthers of Trovita orange (*Citrus sinensis* Osbeck). J Jpn Soc Hortic Sci 53:1–5

Hidaka T, Yamada Y, Shichijo T (1979) *In vitro* differentiation of haploid plants by anther culture in *Poncirus trifoliata* (L.) Raf. Jpn J Breed 29:248–254

Iwanami T, Shimizu T, Ito T, Hirabayashi T (2004) Tolerance to *Citrus mosaic virus* in transgenic trifoliate orange lines harboring capsid polyprotein gene. Plant Dis 88:865–868

Jarrell DC, Roose ML, Traugh SN, Kupper RS (1992) A genetic map of citrus based on the segregation of isozymes and RFLPs in an intergeneric cross. Theor Appl Genet 84:49–56

Kaneko K, Okafuji Y, Matsumoto O (1995) Production of somatic hybrid plants between *Citrus* and *Poncirus trifoliata* by electro-fusion. Bull Yamaguchi Agric Exp Stat 46:73–79

Kaneyoshi J, Kobayashi S (1999) Characteristics of transgenic trifoliate orange (*Poncirus trifoliata* Raf.) possessing the *rolC* gene of *Agrobacterium rhizogenes* Ri plasmid. J Jpn Soc Hortic Sci 68:734–738

Kaneyoshi J, Kobayashi S, Shigemoto N, NakamuraY Y, Doi Y (1994) A simple and efficient gene transfer system of trifoliate orange. Plant Cell Rep 13:541–545

Kayim M, Ceccardi TL, Berretta MJG, Barthe GA, Derrick KS (2004) Introduction of a citrus blight-associated gene into Carrizo citrange [*Citrus sinensis* (L.) Osbc. × *Poncirus trifoliata* (L.) Raf.] by *Agrobacterium*-mediated transformation. Plant Cell Rep 23:377–385

Kijas JMH, Thomas MR, Fowler JCS, Roose ML (1997) Integration of trinucleotide microsatellites into a linkage map of Citrus. Theor Appl Genet 94:701–706

Kobayashi S, Ohgawara T (1988) Production of somatic hybrid plants through protoplast fusion in citrus. J Agric Rev Quart 22:181–188

Kobayashi S, Nakamura Y, Kaneyoshi J, Higo H, Higo K (1996) Transformation of kiwifruit (*Actinidia chinensis*) and trifoliate orange (*Poncirus trifoliata*) with a synthetic gene encoding the human epidermal growth factor (*hEGF*). J Jpn Soc Hortic Sci 64:763–769

Krueger RR, Navarro L (2007) Citrus germplasm resources. In: Khan I (ed) Citrus: genetic, breeding and biotechnology. CABI, Wallingford, UK, pp 45–140

LaMalfa S, Gentile A, Deng ZN, Domina F (2000) Citrus genetic transformation with a vital reporter gene: expression of green fluorescent protein in Troyer citrange. Ital Hortic 7:17–21

Le BV, Ha NT, Hong LTA, Van KTT (1999) High frequency shoot regeneration from trifoliate orange (*Poncirus trifoliata* L. Raf.) using the thin cell layer method. C R Acad Sci Ser 3 (Sci Vie 322):1105–1111

Ling P, Yu C, Deng Z, Chen C, Huang S, Wendell MK, Gmitter FG Jr (1999) Citrus genome mapping with AFLP markers. In: Plant and animal genome VII conference, 17–21 Jan 1999, San Diego, CA, USA, P189

Ling P, Duncan LW, Deng Z, Dunn D, Xu X, Huang S, Gmitter FG Jr (2000) Inheritance of citrus nematode resistance and its linkage with molecular markers. Theor Appl Genet 101:1010–1017

Louzada ES, Grosser JW, Gmitter FG Jr, Nielsen B, Chandler JL (1992) Eight new somatic hybrid citrus rootstocks with potential for improved disease resistance. HortScience 27 (9):1033–1036

Luro F, Laigret F, Lorieux M, Ollitrault P (1996) Citrus genome mapping with molecular markers: two maps obtained by segregation analysis of progeny of one intergeneric cross. Proc Int Soc Citric 2:862–866

Machado MA, Amaral AM, Astua JF, et al (2007) Analysis of citrus transcriptome: CitEST in Brazil. In: Plant and animal genome XV conference, 13–17 Jan 2007, San Diego, CA, USA, p 77

Medina-Urrutia V, Lopez-Madera KF, Serrano P, Ananthakrishnan G, Grosser JW, Guo W (2004) New intergeneric somatic hybrids combining amblycarpa mandarin with six trifoliate/ trifoliate hybrid selections for lime rootstock improvement. HortScience 39(2):355–360

Mestre PF, Asins MJ, Carbonell EA, Navarro L (1997) New gene(s) involved in the resistance of *Poncirus trifoliata* (L.) Raf. to citrus tristeza virus. Theor Appl Genet 95:691–695

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregating analysis: a rapid method to detect markers in specific genomic regions using segregant populations. Proc Natl Acad Sci USA 88:9828–9832

Miller JE, Martiz JGJ, Froneman IJ, Koekemoer PJJ (2003) Potential citrus cultivars in South Africa's scion and rootstock development pipeline. Proc Int Soc Citric 3:62–65

Miranda M, Ikeka F, Endo T, Morigushi T, Omura M (1997) Comparative analysis on the distribution of heterochromatin in *Citrus, Poncirus* and *Fortunella* chromosomes. Chrom Res 5:86–92

Molinari HBC, Bespalhok JCF, Kobayashi AK, Pereira LFP, Vieira LGE (2004a) *Agrobacterium tumefaciens*-mediated transformation of Swingle citrumelo (*Citrus paradisi* Macf. × *Poncirus trifoliata* L. Raf.) using thin epicotyl sections. Sci Hortic 99:379–385

Molinari HBC, Marur CJ, Bespalhok JCF, Kobayashi AK, Pileggi M, Leite Junior RP, Pereira LFP, Vieira LGE (2004b) Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* Osb. *Poncirus trifoliata* L. Raf.) overproducing proline. Plant Sci 167:1375–1381

Moore GA, Jacono CC, Neidigh JL, Lawrence SD, Cline K (1992) *Agrobacterium*-mediated transformation of citrus stem segments and regeneration of transgenic plants. Plant Cell Rep 11:238–242

Moraes AP, Mirkov TE, Guerra M (2008) Mapping the chromosomes of *Poncirus trifoliata* Raf. by BAC-FISH. Cytogenet Genom Res 121:277–281

Navarro L, Olivares-Fuster O, Juarez J, Aleza P, Pina JA, Ballester-Olmos JF, Cervera M, Fagoaga C, Duran-Vila N, Pena L (2004) Applications of biotechnology to citrus improvement in Spain. Acta Hortic 632:221–234

Nicotra A (2001) Mandarin-like hybrids of recent interest for fresh consumption. Problems and ways of control. In: Proceedings of China/FAO citrus symposium, 14–17 May 2001, Beijing, Peoples Republic of China, pp 15–24

Ohgawara T, Kobayashi S, Ohgawara E, Uchimiya H, Ishii S (1985) Somatic hybrid plant obtained by protoplast fusion between *Citrus sinensis* and *Poncirus trifoliata*. Theor Appl Genet 71:1–4

Ohgawara T, Kobayashi S, Ishii S, Yoshinaga K, Oiyama I (1991) Fertile fruits obtained by somatic hybridization: navel orange (*Citrus sinensis*) and Troyer citrange (*C. sinensis* × *Poncirus trifoliata*). Theor Appl Genet 81:141–143

Ollitrault P, Allent V, Luro F (1996) Production of haploid plants and embryogenic calli of clementine (*Citrus reticulata* Blanco) after in situ parthenogenesis induced by irradiated pollen. Proc Int Soc Citric 2:913–917

Ollitrault P, Vanel F, Froelicher Y, Dambier D (2000) Creation of triploid citrus hybrids by electrofusion of haploid and diploid protoplasts. Acta Hortic 535:191–198

Pena L, Cervera M, Juarez J, Ortega C, Pina JA, Duran-Vila N, Navarro L (1995a) High efficiency *Agrobacterium*-mediated transformation and regeneration of citrus. Plant Sci 104:183–191

Pena L, Cervera M, Juarez J, Navarro A, Pina JA, Duran-Vila N, Navarro L (1995b) *Agrobacterium*-mediated transformation of sweet orange and regeneration of transgenic plants. Plant Cell Rep 14:616–619

Pena L, Martin-Trillo M, Juarez J, Pina JA, Navarro L, Martinez-Zapater JM (2001) Constitutive expression of *Arabidopsis LEAFY* and *APETALA*1 genes in citrus reduces their generation time. Nat Biotechnol 19:263–267

Pena L, Cervera M, Fagoaga C, Romero J, Ballester A, Soler N, Pons E, Rodríguez A, Peris J, Juarez J, Navarro L (2008) Citrus. In: Kole C, Hall TC (eds) Compendium of transgenic crop plants, vol 5, Transgenic tropical and subtropical fruits and nuts. Wiley-Blackwell, West Sussex, UK, pp 1–61

Radhamani J, Chandel KPS (1992) Cryopreservation of embryonic axes of trifoliate orange (*Poncirus trifoliata* [L.] RAF.). Plant Cell Rep 11(4):372–374

Roose ML, Schwarzacher T, Heslop-Harrison JS (1998) The chromosomes of *Citrus* and *Poncirus* species and hybrids: Identification of characteristic chromosomes and physical mapping of *r*DNA loci using in situ hybridization and fluorochrome banding. J Hered 89(1):83–86

Roose ML, Feng D, Cheng FS, Tayyar RI, Federici CT, Kupper RS (2000) Mapping the citrus genome. In: Goren R, Goldschmidt EE (eds) Proceedings of international society of horticultural sciences (ISHS), Leuven, Belgium. Acta Hortic 535:25–32

Ruiz C, Asins MJ (2003) Comparison between *Poncirus* and *Citrus* genetic linkage maps. Theor Appl Genet 106:826–836

Sankar AA, Moore GA (2001) Evaluation of inter-simple sequence repeat analysis for mapping in Citrus and extension of the genetic linkage map. Theor Appl Genet 102:206–214

Siviero A, Cristofani M, Furtado EL, Garcia AAF, Coelho ASG, Machado MA (2006) Identification of QTLs associated with citrus resistance to *Phytophthora* gummosis. J Appl Genet 47:23–28

Talon M, Gmitter FG Jr (2008) Citrus genomics. Int J Plant Genom Article ID 528361. doi:10.1155/2008/528361

Tan M, Song J, Deng XX (2007) Production of two mandarin trifoliate orange hybrid populations *via* embryo rescue with verification by SSR analysis. Euphytica 157:155–160

Torres AM, Mau-Lastovicka T, Williams TE, Soost RK (1985) Segregation distortion and linkage of Citrus and *Poncirus* isozyme genes. J Hered 76:289–294

Tozlu I, Guy CL, Moore GA (1999a) QTL analysis of Na^+ and Cl^- accumulation related traits in an intergeneric BC_1 progeny of *Citrus* and *Poncirus* under saline and nonsaline environments. Genome 42:692–705

Tozlu I, Guy CL, Moore GA (1999b) QTL analysis of morphological traits in an intergeneric BC1 progeny of *Citrus* and *Poncirus* under saline and nonsaline environments. Genome 42:1020–1029

Tusa N, Geraci G, Radogna L (1992) New strategies for citrus rootstock improvement by means of protoplast fusion. Proc Int Soc Citric 1:177–179

Wang Q, Batuman O, Bar-Joseph M, Gafny R (2000) Cryopreservation of in vitro 'Troyer' citrange shoot tips. Proc Int Soc Citric 3:210

Weber CA, Moore GA, Deng Z, Gmitter FG Jr (2003) Mapping freeze tolerance quantitative trait loci in a *Citrus grandis* × *Poncirus trifoliata* F1 pseudo-testcross using molecular markers. J Am Soc Hortic Sci 128:508–514

Wong WS, Li GG, Ning W, Xu ZF, Hsiao WL, Zhang LY, Li N (2001) Repression of chilling-induced ACC accumulation in transgenic citrus by over-production of antisense 1-aminocyclopropane-1-carboxylate synthase RNA. Plant Sci 161:969–977

Yang ZN, Ye XR, Choi S, Molina J, Moonan F, Wing RA, Roose ML, Mirkov TE (2001) Construction of a 1.2-Mb contig including the cistrus tristeza virus resistance gene locus using a bacterial artificial chromosome library of *Poncirus trifoliata* (L.) Raf. Genome 44:382–393

Yang ZN, Ye XR, Molina J, Roose ML, Mirkov TE (2003) Sequence analysis of a 282-kilobase region surrounding the citrus tristeza virus resistance gene (*Ctv*) locus in *Poncirus trifoliata* L. Raf. Plant Physiol 131:482–492

Yu C, Huang S, Chen C, Deng Z, Ling P, Gmitter FG (2002) Factors affecting *Agrobacterium* mediated transformation of sweet orange and citrange. Plant Cell Tiss Org Cult 71:147–155