

S01O01

Citron germplasm in Yunnan, China

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The center of origin and diversity for citron (*Citrus medica*) is Southwestern China, Northeastern India, and adjacent regions of Southeast Asia, but very little is known about citron germplasm resources in this area. Citron germplasm resources are rapidly being eroded as forests are cleared for development and farmers abandon citron cultivation for more profitable crops. Starting in 2008, researchers for the Chinese Citron Germplasm Project travelled around Yunnan and other provinces of China, observing both cultivated and wild citron. We collected information about germplasm, horticultural practices, and economic uses, as well as cuttings and seeds, which were used to establish a germplasm collection with 30 accessions in Jianshui, Yunnan. Wild citrons typically have smaller fruits and thinner rinds than commercial cultivars; it is likely that over the centuries farmers have selected for a thick rind, as the most economically valuable part, for preserving and eating fresh. The majority of common (non-fingered) citrons grown in Yunnan have thick, sweet albedos, but no juice vesicles. Some grow as large as 15 kg, making them the largest known citrus fruits. Common citron cultivars include 'Large Wrinkled', 'Small Wrinkled', 'Pillow', 'Sour', 'Water', 'Dog Head', 'Bullet', 'Yunnanensis', 'Oblate', and 'Stigma Persistens'. Fingered (Buddha's Hand) citrons are grown primarily as ornamental plants and to be dried for use in Chinese Traditional Medicine, but also for candying. Many distinct cultivars exist, which vary in flower color, size, thickness of fingers, and the point at which the fingers branch out from the body of the fruit. Fruit types of fingered citron include the common, 'Octopus', 'Fist', and 'Half-and-Half'. There is also a cultivar native to southern Sichuan, 'Muli' citron, which is intermediate between fingered and common citron.

S01O02

Cryopreservation of citrus for long-term conservation

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More than 850 varieties of *Citrus*, *Fortunella*, and *Citrus*-related species are maintained within the USDA-ARS National Plant Germplasm System and the University of California Citrus Variety Collection. These genetic resources are held within duplicated field, screenhouse, and greenhouse collections. Plant materials are at risk of disease infestations, abiotic stresses, and natural disasters. We have developed a cryopreservation method by which we can back-up citrus genetic resources for the long term at liquid nitrogen temperatures. We excise shoot tips directly from greenhouse or screenhouse source plants, surface sterilize, and then treat with cryoprotectants. These solutions dehydrate and allow the shoot tips to survive liquid nitrogen exposure. Plants are recovered by micrografting thawed shoot tips onto 'Carrizo' seedling rootstocks. Plants representing diverse species exhibit a high regrowth (more than 50%), and can be transferred to greenhouse conditions within months. Cryopreserved plants did not revert to a juvenile state. Accessions of *Citrus aurantifolia*, *Citrus clementina*, *Citrus celebica*, and *Citrus paradisi* all flowered within 13 to 15 months after cryoexposure. These methods are also applicable for disease eradication using cryotherapy techniques.

S01O03

Genetic diversity and population structure of the mandarin germplasm revealed by nuclear and mitochondrial markers analysis

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Mandarins (*C. reticulata*) are considered as one of the four main species involved in the origin of cultivated citrus. However, the classification of the mandarin germplasm is still controversial and numerous cases of introgression from other species are known or suspected in this germplasm. The main objective of this work was to analyze the genetic diversity structure of mandarin germplasm and its relationship with the other citrus species. Fifty

microsatellite (SSR) markers, 25 Insertion-Deletion (InDel) nuclear markers and four mitochondrial InDel markers were genotyped for 223 accessions. 'Structure' software was applied on nuclear data to check and quantify potential interspecific introgressions in the mandarin germplasm, mainly the pummelo and papeda genomes. Within the mandarin germplasm without identified introgression, seven clusters were revealed by 'Structure' analysis. Five of them should be true basic mandarin groups and the other two include genotypes of known or supposed hybrid origin. The contributions of these seven groups to the mandarin genotypes were estimated. The mitochondrial InDel analysis revealed eight mitotypes, in which the mandarin germplasm was represented in four of them. In this work, new insights in the organization of mandarin germplasm and its structure have been found, and different mandarin core collections were determined. This will allow a better management and use of citrus germplasm collections and to perform genetic association studies.

S01004

Study on genetic diversity of 39 Citron germplasm resources with SCoT and ISSR markers

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The genetic relationships and diversity of 39 citron (*Citrus medica*) germplasm resources were examined by SCoT and ISSR markers in this study. Of the 80 SCoT primers screened, 9 primers gave reproducible, polymorphic DNA amplification patterns with a total 69 amplified bands. The polymorphic percentage was 76.8%. Of the 100 ISSR primers screened, 9 primers gave reproducible, polymorphic DNA amplification patterns with a total 84 of amplified bands. The percentage of polymorphic band was 77.4%. An UPGMA relationship tree was established based on the similarity coefficients. At the coefficient level of 0.85 for SCoT and 0.77 for ISSR, the 39 germplasm resources were divided into six groups and five groups, respectively. The application of SCoT and ISSR markers is discussed in this paper.

S01005

Origin of dwarf, thornless-type trifoliolate orange varieties deduced from genome-wide genotyping analysis

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Trifoliolate orange (*Poncirus trifoliata*) is commonly used as a rootstock in Japan. Several varieties that are different for tree height or tree morphology are recognized. However, origin and phylogenetic relationship among them are not evaluated. We compared genotypes of three varieties, a thornless type trifoliolate orange that lacks thorns at the leaf axil and two dwarf types, 'Hiryu' ('Flying dragon') and 'Unryu' ('Cloudy dragon'), to a reference accession of trifoliolate orange in our citrus collection. Genotyping analysis with 289 genome wide SSR markers gave 265 valid genotypes. Among them, 'Hiryu' lost five loci but 96.2% of genotypes were matched to those of the reference. In contrast, the thornless-type lost 4 loci and 78.8% of genotypes were matched to those of the reference, and 'Unryu' lost 6 loci and 81.4% of genotypes were matched to those of wild type. Assignment of SSR marker genotypes to a citrus linkage map revealed that genotypes of 'Hiryu' were well conserved among all linkage groups. However, loss of heterozygosity (LOH) was observed at various regions of linkage groups for the thornless-type and 'Unryu'. Ratios of homozygous loci of the reference, the thornless-type, 'Hiryu' and 'Unryu' were 70.3%, 88.5%, 69.1%, and 84.9%, respectively. These observations suggested that 'Hiryu' is a sport of trifoliolate orange but the thornless-type and 'Unryu' resulted from trifoliolate orange by self-pollination and mutation at particular loci.

S01006

Multilocus haplotyping by parallel sequencing to decipher the interspecific mosaic genome structure of cultivated citrus

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Recent studies support the theory that four basic taxa (*Citrus medica*, *Citrus maxima*, *Citrus reticulata* and *Citrus micrantha*) have generated all cultivated *Citrus* species. It is supposed that the genomes of most of the actual