



Biotechnology of Fruit and Nut Crops

2nd Edition

Edited by Richard E. Litz, Fernando Pliego-Alfaro
and Jose Ignacio Hormaza



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Contents

| | |
|---|------|
| List of Contributors | ix |
| Preface | xiii |
| <i>Richard E. Litz, Fernando Pliego-Alfaro and Jose Ignacio Hormaza</i> | |
| 1 Actinidiaceae | 1 |
| 1.1 Actinidia spp. Kiwifruit | 1 |
| <i>Manuel Rey, Yolanda Ferradás, Óscar Martínez and María Victoria González</i> | |
| 2 Anacardiaceae | 19 |
| 2.1 Anacardium occidentale Cashew | 19 |
| <i>Smitha Hegde, Shashikiran Nivas and Leo D'Souza</i> | |
| 2.2 Mangifera indica Mango | 27 |
| <i>Richard E. Litz and Jose Ignacio Hormaza</i> | |
| 2.3 Pistacia vera Pistachio | 44 |
| <i>Ahmet Onay, Yelda Özden Çiftçi, Hülya Akdemir Koç, Veysel Süzener and Engin Tilkat</i> | |
| 3 Annonaceae | 65 |
| 3.1 Annona spp. Atemoya, Cherimoya, Soursop and Sugar Apple | 65 |
| <i>Jose Ignacio Hormaza, Elisabeth Carmona, Isabel María González-Padilla, Nerea Larrañaga, Jorge Lora, Alicia Talavera and Carlos López Encina</i> | |
| 4 Arecaceae | 79 |
| 4.1 Cocos nucifera Coconut | 79 |
| <i>S.W. Adkins, J. Biddle, Q.T. Nguyen and M. Foale</i> | |
| 4.2 Elaeis guineensis Oil Palm | 92 |
| <i>Alain Rival</i> | |
| 4.3 Phoenix dactylifera Date Palm | 107 |
| <i>Yuval Cohen</i> | |
| 5 Bromeliaceae | 118 |
| 5.1 Ananas comosus Pineapple | 118 |
| <i>Mike K. Smith and José Ramón Botella</i> | |
| 6 Caricaceae | 131 |
| 6.1 Carica papaya Papaya | 131 |
| <i>Maureen M.M. Fitch</i> | |
| 7 Clusiaceae | 154 |
| 7.1 Garcinia mangostana Mangosteen | 154 |
| <i>Rekha Chaudhury and S.K. Malik</i> | |
| 8 Ebenaceae | 164 |
| 8.1 Diospyros kaki Persimmon | 164 |
| <i>Qing-Lin Zhang, Takuya Tetsumura, Ryutaro Tao and Zheng-Rong Luo</i> | |

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| | | |
|------|---|-----|
| 9 | <i>Ericaceae</i> | 191 |
| 9.1 | <i>Vaccinium</i> spp. Blueberry and Cranberry <i>Guo-Qing Song</i> | 191 |
| 10 | <i>Fagaceae</i> | 206 |
| 10.1 | <i>Castanea</i> spp. Chestnut <i>Scott A. Merkle, Francisco Javier Viéitez, Elena Corredoira and John E. Carlson</i> | 206 |
| 11 | <i>Juglandaceae</i> | 238 |
| 11.1 | <i>Carya illinoensis</i> Pecan <i>Wagner Vendrame and Hazel Wetzstein</i> | 238 |
| 11.2 | <i>Juglans regia</i> Walnut <i>Patrick J. Brown, Charles A. Leslie and Abhaya Dandekar</i> | 246 |
| 12 | <i>Lauraceae</i> | 258 |
| 12.1 | <i>Persea americana</i> Avocado <i>Fernando Pliego-Alfaro, Elena Palomo-Ríos, José Angel Mercado, Clara Pliego, Araceli Barceló-Muñoz, Rodolfo López-Gómez, Jose Ignacio Hormaza and Richard E. Litz</i> | 258 |
| 13 | <i>Malvaceae</i> | 282 |
| 13.1 | <i>Theobroma cacao</i> Cacao <i>Antonio Figueira and Danielle Camargo Scotton</i> | 282 |
| 14 | <i>Musaceae</i> | 314 |
| 14.1 | <i>Musa</i> Banana and Plantain <i>Mike K. Smith, Mathieu Rouard, Julie Sardos and Nicolas Roux</i> | 314 |
| 15 | <i>Myrtaceae</i> | 330 |
| 15.1 | <i>Psidium guajava</i> Guava <i>Manoj K. Rai and Uma Jaiswal</i> | 330 |
| 16 | <i>Oleaceae</i> | 343 |
| 16.1 | <i>Olea europaea</i> Olive <i>Eddo Rugini, Luciana Baldoni, Cristian Silvestri, Roberto Mariotti, Isabel Narváez, Niccolò Cultrera, Valerio Cristofori, Muhammad Ajmal Bashir, Soraya Mousavi, Elena Palomo-Ríos, José Angel Mercado and Fernando Pliego-Alfaro</i> | 343 |
| 17 | <i>Oxalidaceae</i> | 377 |
| 17.1 | <i>Averrhoa carambola</i> Carambola <i>Richard E. Litz</i> | 377 |
| 18 | <i>Passifloraceae</i> | 381 |
| 18.1 | <i>Passiflora</i> spp. Passionfruit <i>Diego Ismael Rocha, Diego Silva Batista, Fábio Gelape Faleiro, Marcelo Rogalski, Leonardo Monteiro Ribeiro, Maria Olívia Mercadante-Simões, Roxana Yockteng, Maurecilne Lemes da Silva, Wellington Santos Soares, Marcos Vinícius Marques Pinheiro, Túlio Gomes Pacheco, Amanda de Santana Lopes, Lyderson Facio Viccini and Wagner Campos Ottoni</i> | 381 |
| 19 | <i>Rosaceae</i> | 409 |
| 19.1 | <i>Eriobotrya japonica</i> Loquat <i>Maria del Mar Naval, Manuel Blasco and Maria Luisa Badenes</i> | 409 |
| 19.2 | <i>Fragaria</i> × <i>ananassa</i> Strawberry <i>Pablo Ric-Varas, Elena Palomo-Ríos, Antonio J. Matas, Fernando Pliego-Alfaro and José Angel Mercado</i> | 418 |
| 19.3 | <i>Malus</i> × <i>domestica</i> Apple <i>Magda-Viola Hanke, Henryk Flachowsky, Andreas Peil and Ofere Francis Emeriemen</i> | 440 |

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| | | |
|-------|---|------------|
| 19.4 | <i>Prunus persica</i> Peach and Nectarine | 474 |
| | <i>Rosa M. Pérez-Clemente, Gabino Ríos, Maria Luisa Badenes and Luis A. Cañas</i> | |
| 19.5 | <i>Prunus armeniaca</i> Apricot | 496 |
| | <i>Nuria Alburquerque, David Ruiz, Lorenzo Burgos and Cesar Petri</i> | |
| 19.6 | <i>Prunus domestica</i> Plum | 512 |
| | <i>Cesar Petri, David Ruiz, M. Faize, Lorenzo Burgos and Nuria Alburquerque</i> | |
| 19.7 | <i>Prunus</i> spp. Cherry | 532 |
| | <i>Paula M. Pijut</i> | |
| 19.8 | <i>Prunus dulcis</i> syn. <i>Prunus amygdalus</i> Almond | 561 |
| | <i>Pedro M. Barros, Pedro Martínez-Gómez, Ossama Kodad, Ana Paula Farinha and M. Margarida Oliveira</i> | |
| 19.9 | <i>Pyrus</i> spp. Pear and <i>Cydonia</i> spp. Quince | 581 |
| | <i>Elisabeth Chevreau, Kate Evans, David Chagné and Sara Montanari</i> | |
| 19.10 | <i>Rubus</i> spp. Cane Fruit | 606 |
| | <i>Julie Graham and Nikki Jennings</i> | |
| 20 | <i>Rutaceae</i> | 621 |
| 20.1 | <i>Citrus</i> | 621 |
| | <i>Vicente Febres, Leandro Peña, Svetlana Y. Folimonova and Gloria Moore</i> | |
| 21 | <i>Sapindaceae</i> | 645 |
| 21.1 | <i>Dimocarpus longan</i> Longan and <i>Litchi chinensis</i> Litchi | 645 |
| | <i>Guillermo Padilla, Simon H.T. Raharjo, Richard E. Litz and Jose Ignacio Hormaza</i> | |
| 22 | <i>Vitaceae</i> | 655 |
| 22.1 | <i>Vitis</i> spp. Grape | 655 |
| | <i>Sadanand A. Dhekney, A.T. Basford, V.E. Chhatre, M.B. Rosenberg, C. Claflin, S.K. Sessions, Z.J. Li and Dennis J. Gray</i> | |
| | Index | 675 |

16.1 *Olea europaea* Olive

Eddo Rugini,¹ Luciana Baldoni,² Christian Silvestri,¹ Roberta Mariotti,² Isabel Narváez,³ Niccolò Cultrera,² Valerio Cristofori,¹ Muhammad Ajmal Bashir,¹ Soraya Mousavi,² Elena Palomo-Ríos,³ José Angel Mercado³ and Fernando Pliego-Alfaro³

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1. Introduction

1.1. Botany and history

The olive (*Olea europaea* L.) belongs to the *Oleaceae* family, which is divided into two subfamilies, *Jasminoidaceae* and *Oleideae*, based on chromosome number. The olive is included within the *Oleideae* subfamily, *Olea* genus, *Olea* subgenus and *Olea europaea* species. The family comprises *c.* 25 genera and 600 species distributed in temperate and tropical regions (Besnard *et al.*, 2009). Besides the olive, other known species in the *Oleaceae* native to Europe include ash (*Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl.), privet (*Ligustrum vulgare* L.) or phyllirea (*Phillyrea angustifolia* L., *P. media* L. and *P. latifolia* L.). A few species are cultivated or used as ornamentals, e.g. jasmine (*Jasminum fruticans* L.), lilac (*Syringa vulgaris* L.), *Fraxinus ornus* L., *Forsythia × intermedia* Zabel and *Osmanthus fragrans* Lour. Only *O. europaea* is cultivated for its edible fruit. Following a thorough revision by Green (2002), the genus *Olea* includes 33 species and 9 subspecies. Six of these subspecies form the *Olea* subsection or complex: (i) *O. europaea* ssp. *europaea* (2n) of the Mediterranean region, where cultivated (*O. europaea* L. ssp. *europaea* var. *sativa*) and wild olive, oleaster, (*Olea europaea* ssp. *europaea* var. *sylvestris*) are included; (ii) *O. europaea* ssp. *laperrinei* (2n) and (3n) (Batt. and Trab.), Ciferri, of the Sahara massifs; (iii) *O. europaea* ssp. *cerasiformis* (4n) (Webb. and Berth.) Kunk. and Sund., of the Madeira Islands; (iv) *O. europaea* ssp. *guanchica* (2n), of the Canary Islands; (v) *O. europaea* ssp. *maroccana* (6n) (Greuter and Burdet) of

Morocco; and (vi) *O. europaea* ssp. *cuspidata* (2n) (Wall. Ciferri), of Asia (China, India, Pakistan, Iran, south Arabia) and south-east Africa (Green and Wickens, 1989; Green, 2002).

Studies confirm that olive was present in the Mediterranean region for several thousand years, particularly in the Middle East, before its domestication (Besnard *et al.*, 2013; Newton *et al.*, 2014). Palynological, anthropological and archeological evidence (García *et al.*, 2017) demonstrate the presence of some sporadic forms of olive during the last glaciation (18,000 BC) in the western and eastern Mediterranean regions. Olive was probably domesticated in the Middle East, north of the Dead Sea in the Jordan River valley *c.* 5700–5200 years BC (Zohary and Spiegel-Roy, 1975; Liphshitz *et al.*, 1991; Kaniewski *et al.*, 2012). It has been suggested that oleaster contributed to olive domestication and is continuing to do so (Angiolillo *et al.*, 1999; Besnard *et al.*, 2001; Terral *et al.*, 2004; Carrión *et al.*, 2010; Besnard and Rubio de las Casas, 2016). Thus, the wild olive could be considered as the main progenitor of the cultivated olive, based on similar morphology, ecological requirements and ploidy level (Green, 2002; Besnard and Rubio de las Casas, 2016); however, palaeobotanical, archaeological, historical studies and molecular data have enabled the reconsideration of the biogeography of the wild olive and the history of its cultivation (Besnard and Rubio de las Casas, 2016; Mousavi *et al.*, 2017a). Based on comprehensive samplings, independent research revealed that olive cultivars belong to three main genetic pools that approximately match three geographic areas corresponding to the west (Q1), centre (Q2) and east (Q3) of the Mediterranean region (Haouane *et al.*, 2011; Belaj *et al.*, 2012;

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rate. Inclusion of hormones in post-thaw medium allowed Lynch *et al.* (2007) to maintain growth for 10 weeks. Histological examination of shoot apices showed damage in subapical cells, which could be the reason for the failure of shoot recovery. Results on cryopreservation of shoot tips appear promising for germplasm conservation (Benelli *et al.*, 2013).

Shibli and Al-Juboory (2000) used encapsulation–vitrification or the encapsulation–dehydration procedure to cryopreserve embryogenic cultures of juvenile origin. In both protocols, a dehydration step was required. Survival rates were 68% (encap–vitrif) or 58% (encap–dehyd) which was similar to controls. Sanchez-Romero *et al.* (2009) used the droplet vitrification method to cryopreserve somatic embryos derived from radicles of mature zygotic embryos. Bradai *et al.* (2017) indicated that 1–6 mm somatic embryos were the optimum explants; recovery following cryopreservation was improved when cultures had been grown in liquid medium for 28 days.

Embryogenic ‘Canino’ cultures, consisting of PEMs and somatic embryos at various stages of development, can be cryopreserved by vitrification (Lambardi *et al.*, 2002). Thirty-eight per cent of the cryopreserved cultures can survive. Cryopreserved cultures showed enhanced proliferation and morphogenic potential. The encapsulation–dehydration procedure has been ineffective for cryopreservation of ‘Frantoio’ (Benelli *et al.*, 2001a) and ‘Arbequina’ (Martinez *et al.*, 1999).

Somatic embryos of ‘Canino’ were pretreated with sucrose and incubated in a cryoprotectant mixture. Slow freezing at a controlled rate (0.5°C/min to –35°C prior to plunging into liquid nitrogen) allowed Lynch *et al.* (2011) to successfully recover viable somatic embryos. Pretreatments enhanced the content of endogenous antioxidants, proline and sugar levels in tissues.

8. Conclusions

Molecular markers are being used for evaluating the level and distribution of olive germplasm variability as well as for the

identification, through genetic mapping, of QTLs controlling important agronomic and quality traits. New next-generation sequencing technologies will make a significant impact on molecular olive breeding.

Several olive cultivars have been successfully micropropagated, and the technique is used commercially. Significant progress has also been made to refine somatic embryogenesis from mature phase tissues of elite cultivars. Promising results with respect to medium- and long-term conservation of olive by slow growth storage and cryoconservation will be useful for the establishment of *in vitro* repositories, which could safeguard olive biodiversity.

The potential of manipulating genetic information in a precise manner and the development of improved plants not only provide the opportunity to create novel phenotypes but also facilitate gene function studies for better understanding of different biological mechanisms (Limera *et al.*, 2017). Identification of useful genes and optimization of regeneration protocols for important cultivars remains difficult and is a bottleneck. Sequencing of the olive genome will be a key tool for identifying genes of interest. For cultivars that are recalcitrant *in vitro*, a method is available based on: (i) gene modification in cells close to the root system of *in vitro* plantlets; (ii) transplant of the plantlets in the field; and (iii) selection of mutant suckers spontaneously grown from putative genetically modified cells in the crown area of the plant.

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