Environmental and Experimental Botany 71 (2011) 152–157

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

First stages of microspore reprogramming to embryogenesis through anther culture in *Prunus armeniaca* L.

María Antonietta Germanà[a], Benedetta Chiancone[a], Diego Padoan[a], Ivett Bárány[b], María-Carmen Risueno[b], Pilar S. Testillano[b,*]

[a] Dipartimento SENFIMIZO, Università degli Studi di Palermo, Viale delle Scienze 11, 90128 Palermo, Italy
[b] Centro de Investigaciones Biológicas (CSIC), C/Ramiro del Maeztu, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form
17 November 2010
Accepted 25 November 2010

Keywords:
Apricot
Cell structure
Cytochemistry
Microspore embryogenesis

ABSTRACT

Prunus armeniaca L. is a worldwide known species, very important particularly in the Mediterranean basin. Microspore embryogenesis through *in vitro* anther culture is a widely used method to obtain haploid and doubled haploid (DHs) plants which are being routinely used in breeding programmes for new superior cultivar development in many crops. Haploid–diploidization through gametic embryogenesis allows single-step development of complete homozygous lines from heterozygous parents. In the case of fruit crops, with long reproductive cycle, a high degree of heterozygosity, large size, and, often, self-incompatibility, there is no way to obtain haploidization through conventional methods. Induction of microspore embryogenesis *in vitro* is switched by a stress treatment. In many species, heat or cold stress has been reported to trigger pollen embryogenesis, the response being genotype dependent. In the present work we analyzed whether microspore reprogramming could be induced in apricot cultivars by cold stress through anther culture. We report the development of an *in vitro* anther culture protocol in *P. armeniaca* L. and analyse the response of several cultivars to stress treatments and culture media for inducing pollen embryogenesis. Results showed the formation of multicellular pollen and proembryos. The effect of two culture media in the embryogenic response was also analyzed, being the responses genotype-dependent. Monitoring of the cellular changes on the microspores was performed by structural and confocal microscopy analyses. Results indicated that the reprogramming of the microspore and the first steps of the embryogenic pathway have been achieved in different varieties of *P. armeniaca*, which constitutes a crucial step in the design of protocols for the regeneration of microspore-derived embryos and DH plants, for future potential applications in breeding programmes of this economically important fruit tree.

1. Introduction

Among the biotechnological tools that can greatly help fruit crop breeding, haploid and doubled haploid production, through gametic embryogenesis, allows the single-step development of complete homozygous lines from the heterozygous parents, increasing the efficiency of perennial crop breeding programmes. Haploid plants are sporophytes carrying the gametic chromosome number (*n* instead of 2*n*), and doubled haploids (DHs) are haploids that underwent, spontaneous or induced, chromosome duplication. Haploid and DHs have a potential use in mutation research, selection, genetic analysis, transformation and in the production of homozygous cultivars also required to utilize as parental lines for F1 hybrids (heterosis). Fruit crops are characterized by the high heterozygosity of the genomes, the long duration of generation cycle with a long juvenile period, the large size, and, often, the self-incompatibility, and, for these reasons, there is no way to produce homozygous breeding lines through conventional methods that involve several generations of selfing. Considerable research has been carried out since the 1970s to obtain haploids for fruit tree breeding, through gametic embryogenesis, but, not always, they were successful (Ochatt and Zhang, 1996; Maluszynski et al., 2003; Germanà 2006; Srivastava and Chaturvedi, 2008).

Haploids can be mainly induced by regeneration from the male gamete (pollen embryogenesis) or from the female gamete (gynogenesis), and, particularly, *in vitro* anther or isolated microspore cultures are the most effective and widely used methods of producing haploids and DHs. Haploid production from *Datura innoxia* anther *in vitro* cultured was first reported by Guha and Maheshwari (1964), since then pollen embryogenesis has been reported for more than 250 species, belonging to 100 genera and more than 40 families, including *Cruciferae* and *Gramineae* [1], while many

* Corresponding author.
E-mail address: testillano@cib.csic.es (P.S. Testillano).

0098-8472/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2010.11.011