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Short communication

Improved technique for counting chromosomes in almond

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Abstract

In almond [*Prunus dulcis* (Mill.) D.A. Webb], there are few chromosome studies. In this work, different methods described for slide preparation and staining of chromosomes in other *Prunus* species have been evaluated using almond root tips. By modifying and updating traditional methods, an improved technique for counting chromosomes has been developed. Root tips were first placed in cold water for 4 h at 0 °C and treated with 0.2% colchicine for 3 h at 5 °C. They were then fixed for 24 h at 5 °C in methanol, propionic acid, chloroform (6:3:2), and stored in 70% ethanol. This optimized protocol allowed the identification of chromosomes in the different stages of mitosis late prophase being the best stage for chromosome counting.

Keywords: Almond; *Prunus dulcis*; Mitosis; Chromosomes

1. Introduction

Chromosome studies are an important prerequisite for fruit genetic and breeding studies (Lespinasse et al., 1976; Schuster, 1996). These techniques have been used in *Prunus* species in the study of interspecific crosses (Salesses and Bonherta, 1993), the characterization of germplasm with an unknown ploidy level (Hesse, 1971; Toyama,

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1974), and the establishment of taxonomic and phylogenetic relationships (Singh et al., 1984; Soodan et al., 1988). *Prunus* species are characterized by small chromosomes that are difficult to karyotype (Hesse, 1971; Oginuma, 1987; Salesses and Bonherta, 1993; Schuster, 1996). In these species, chromosome studies by slide preparation and staining have been reported mainly from apricot (*P. armeniaca* L.) and peach [*P. persica* (L.) Batsch] (Jelenkovic and Harrington, 1972; Kliphuis and Barkoudah, 1977; Medeira and Warden, 1986; Warden and Medeira, 1986; Salesses and Bonherta, 1993; Yamamoto et al., 1999; Gostjeva, 2001). In the case of almond [*P. dulcis* (Mill.) D.A. Webb], there are few studies, those in many cases reported in publications with limited readership showing results of poor quality in terms of observation and counting of chromosomes (Kliphuis and Barkoudah, 1977; Singh et al., 1984; Soodan et al., 1988).

In order to optimize a technique for counting chromosomes in almond, different treatments described for slide preparation and staining of chromosomes in other *Prunus* species, were assayed using root tips.

2. Materials and methods

Chromosome studies were carried out in meristematic cells of root tips (1 cm in length) of seedlings from 'Nonpareil' almond cultivar. Seedlings were grown in 5 l pots in a greenhouse at a temperature between 25 and 30 °C with a photoperiod of 16 h.

2.1. Preparation of samples

Samples were prepared by incubation in cold water for 4 h at 0 °C. After the incubation in cold water, a treatment in 0.2% colchicine for 3 h at 5 °C (Martens and Reisch, 1988; Harandi and Ghaffari, 2001) was applied to some samples. Root tips were collected during the morning and directly placed into the cold water.

2.2. Fixation of samples

Two different fixation solutions were used:

- (A) ethanol, acetic acid (3:1) (Lespinasse et al., 1976; Medeira and Warden, 1986);
- (B) methanol, propionic acid, chloroform (6:3:2) (Owen and Miller, 1993; Harandi and Ghaffari, 2001).

Root tips were incubated in the described fixation solutions for 24 h at 4 °C, and then washed in distilled water and preserved in ethanol 70% at 4 °C.

2.3. Hydrolysis

Root tips were hydrolyzed in 1N HCl at 60 °C, assaying six different times (5, 10, 20, 30, 40 and 60 min) according with the times previously assayed in *Prunus* species by different authors.