An improved method for inducing prometaphase chromosomes in plants

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Abstract

Background: Detailed karyotyping using metaphase chromosomes in melon (Cucumis melo L.) remains a challenge because of their small chromosome sizes and poor stainability. Prometaphase chromosomes, which are two times longer and loosely condensed, provide a significantly better resolution for fluorescence in situ hybridization (FISH) than metaphase chromosomes. However, suitable method for acquiring prometaphase chromosomes in melon have been poorly investigated.

Results: In this study, a modified Carnoy’s solution II (MC II) [6:3:1 (v/v) ethanol:acetic acid: chloroform] was used as a pretreatment solution to obtain prometaphase chromosomes. We demonstrated that the prometaphase chromosomes obtained using the MC II method are excellent for karyotyping and FISH analysis. We also observed that a combination of MC II and the modified air dry (ADI) method provides a satisfactory mitotic pachytene chromosome preparation with reduced cytoplasmic background and clear chromatids. Moreover, we demonstrated that pachytene and prometaphase chromosomes of melon and Abelia x grandiflora generate significantly better FISH images when prepared using the method described. We confirmed, for the first time, that Abelia x grandiflora has pairs of both strong and weak 45S ribosomal DNA signals on the short arms of their metaphase chromosomes.

Conclusion: The MC II and ADI method are simple and effective for acquiring prometaphase and pachytene chromosomes with reduced cytoplasm background in plants. Our methods provide high-resolution FISH images that can help accelerate molecular cytogenetic research in plants.

Keywords: Prometaphase, Pachytene, Chloroform, FISH, Cucumis melo, Abelia x grandiflora

Background

Chromosome preparation is crucial for cytogenetic studies. Fluorescence in situ hybridization (FISH), a molecular cytogenetic technique, requires properly dispersed metaphase or prometaphase chromosomes for its application. Melon (Cucumis melo L.) belongs to the Cucurbitaceae family and is a diploid species having 2n = 2x = 24 chromosomes [1]. Detailed karyotype analysis in the Cucumis genus, particularly in melon, has been difficult to achieve because of their small chromosome sizes and poor stainability [2, 3]. In addition, the identification of secondary constrictions and the procurement of more detailed chromatid images are also difficult, even when using properly dispersed metaphase chromosomes, because of their highly condensed status. For these reasons, we propose the use of prometaphase chromosomes for FISH analyses in melon. Prometaphase chromosomes are effective and preferable for cytogenetic analyses and identification of individual chromosomes because the chromosomes are easily distinguishable due to the uneven condensation of chromatids fibers along chromosomes [4]. FISH using prometaphase chromosomes has been successfully applied in studies involving Brassica [5], rice [6–8], Catharanthus roseus [9], and Lablab purpureus [10]. However, suitable methods to induce prometaphase chromosomes in other plants have been poorly investigated.

Prometaphase chromosomes in Brassica [5] and rice [7] have been successfully induced using ethanol and acetic acid (3:1) without pretreatment. Other methods to accumulate metaphase and prometaphase chromosomes, such as with ice water (ice) treatment for 24 h [11] or 0.02 M 8-hydroxyquinoline (8-Hq) [12, 13] have also...