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Comparison of the morphology, growth and development of diploid and autotetraploid 'Hanfu' apple trees

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ABSTRACT

Artificial induction of polyploidy is an efficient technique for improving the biological properties and developing new varieties of many plants. Autotetraploid plants were generated through *in vitro* chromosome doubling of the 'Hanfu' apple cultivar (*Malus* × *domestica*) using colchicine treatment. In the present study, we analysed and compared the differences in the morphological and biological characteristics of diploid and autotetraploid 'Hanfu' apple cultivars from 2009 to 2014. We found that the autotetraploid plants flowered at 5 years of age, which was 2 years later than in the diploid plants. Compared with the diploid plants, the autotetraploid plants showed retarded growth and significant dwarfing characteristics before flowering, but their growth was restored after flowering. The stomata were larger and the leaves were not only larger and heavier but also darker green in the autotetraploid than in the diploid plants. The chlorophyll content of the leaves was significantly higher and the photosynthetic efficiency and fluorescence performance were increased in the autotetraploid plants. Additionally, the volume and size of the flowers, fruits and seeds were greater and the soluble solid contents were higher in the autotetraploid than its diploid counterparts. In this study, we provide the first detailed analysis of the morphological and biological characteristics associated with growth and development in diploid and autotetraploid 'Hanfu' apple, and the results will make a meaningful contribution to the breeding of polyploid apples.

1. Introduction

Polyploidization is a natural phenomenon in many plants and plays a critical role in plant variation and evolution (Ramsey and Schemske, 1998; Wendel, 2000). Polyploidy is commonly defined as the occurrence of three or more complete sets of chromosomes in a single cell and is classified as autopolyploidy (involving homologous chromosomes) or allopolyploidy (involving two distinct genomes) according to its origin (Ramsey and Schemske, 1998; Dhooghe et al., 2011). Genome sequence analysis and large EST (Expressed Sequence Tag) datasets have shown that chromosome doubling events have occurred one or more times during the evolution of almost all angiosperm lineages (Birchler and Veitia, 2010; Liao et al., 2016).

Due to chromosome doubling, polyploid plants exhibit many characteristics that are superior to those of diploids with respect to morphological changes, genetic adaptability and tolerance to environmental stresses (Stanys et al., 2006; Allario et al., 2011; Dhooghe et al., 2011; Sattler et al., 2016). The most important characteristic associated with polyploidy is enlarged plant organs (the 'gigas' effect), including larger flowers, heavier fruits and increased leaf thickness and area; changes in stomatal size and density have also been reported in many plants (Sehepper et al., 2004; Leitch and Leitch, 2008; Sun et al., 2009; Allario et al., 2011; Van Laere et al., 2011; Tan et al., 2015). Induction of polyploidy can also improve the resistance of plants to biotic and abiotic stresses, especially salt and drought tolerance (Riddle et al., 2006; Xiong et al., 2006; Xue et al., 2015; Zhang et al., 2015).

Apple is one of the most important fruits in the world. Most apple cultivars are diploid ($2n = 2x = 34$), but many polyploid species also exist (Comai, 2005; Pereira-Lorenzo et al., 2007). These triploid ($2n = 3x = 51$) apple cultivars, such as 'Jonagold', 'Mutsu', 'Hokkaido', 'Sekaiichi', 'Winesap', and 'Gravenstein', also have a large planting area because of their excellent characteristics, including relatively high production and large fruit size (Dhooghe et al., 2011; Sedov et al., 2014). Polyploid breeding plays an important role in apple breeding. However, nature-induced polyploidy is difficult to find. Thus, artificially induced polyploidy is becoming a common way to improve

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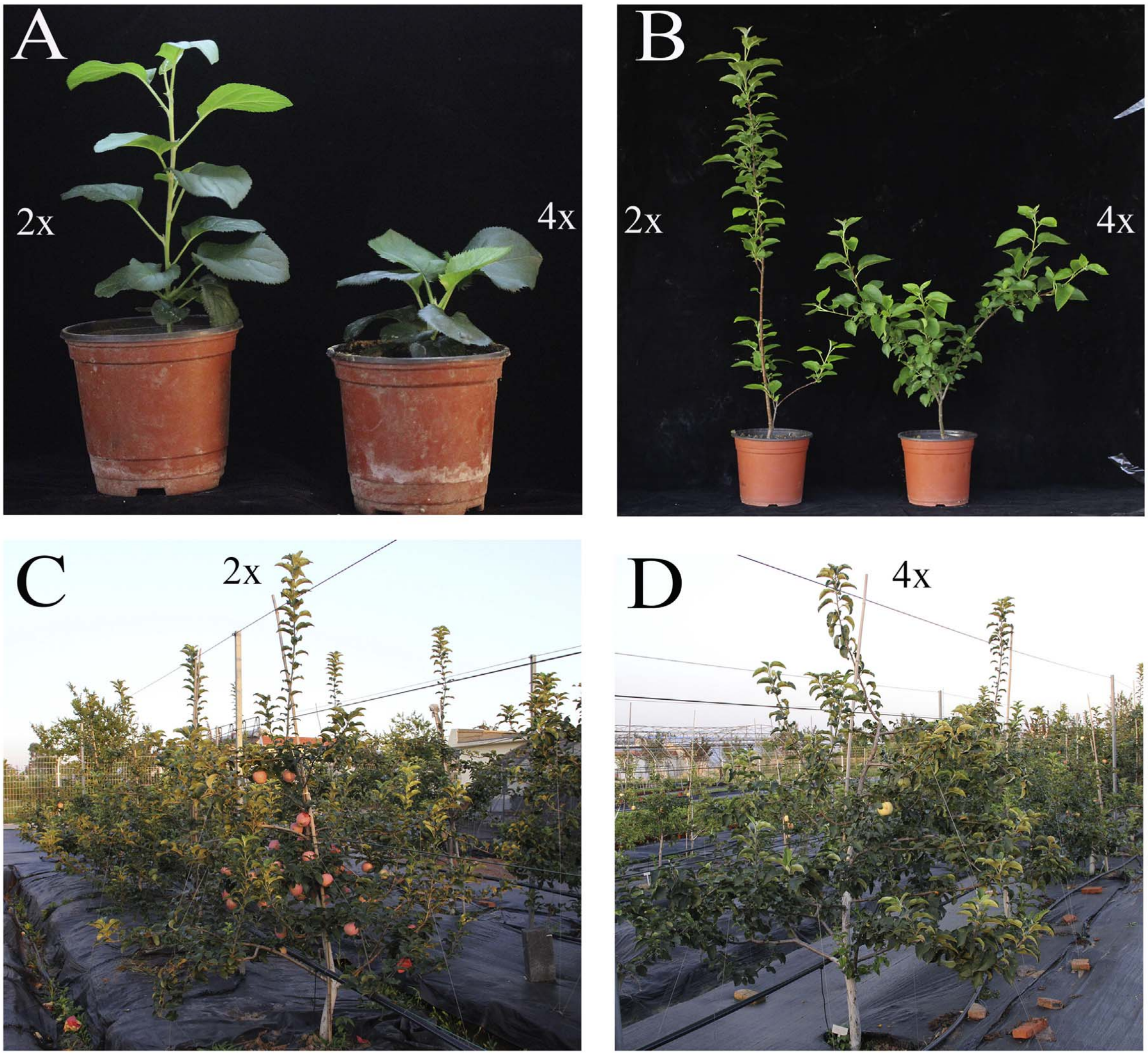

Fig. 1. Vigour of diploid and autotetraploid 'Hanfu' apple trees in different years. A: One-year old diploid and autotetraploid seedlings; B: Two-year old diploid and autotetraploid plants; C–D: Five-year old diploid and autotetraploid plants.

biological properties and develop new apple varieties. Due to the slow growth rate of woody plants, most studies have compared diploid and polyploid apple plants as seedlings, and few studies have explored their differences during later growth and development stages (Liu et al., 2006; Dhooghe et al., 2011; Xue et al., 2015; Zhang et al., 2015).

In a previous study, apples of the 'Hanfu' cultivar were treated with colchicine to induce autotetraploid ($2n = 4x = 68$) *in vitro* (Xue et al., 2015). After transplantation into the field, the autotetraploid and diploid plants showed many differences. In the present study, we analysed and compared the biological and morphological characteristics of leaf, flower, fruit and tree vigour between diploid and autotetraploid apple plants during the entire period of growth and development for the first time. The results of our study have the potential to contribute meaningfully to the breeding of polyploid apples.

2. Materials and methods

2.1. Plant material

Autotetraploid apple plants were induced from leaves of 'Hanfu' trees via colchicine treatment in the Fruit Molecular Biology Laboratory at Shenyang Agricultural University in 2008 (Xue et al., 2015). Both diploid and autotetraploid plants derived from tissue culture were subcultured for 45 days. After rooting, all of the seedlings were transferred to a climate-controlled growth chamber in 2009 (the temperature was maintained at 24 ± 2 °C, with a 12 h photoperiod and a relative humidity of 70%), and transplanted into the field simultaneously two month later. The biological and morphological characteristics were investigated from 2009 to 2014 and compared between the autotetraploid and diploid plants, which were managed under identical conditions without any pruning.

2.2. Leaf characteristics

Several leaf characteristics were determined and compared between the diploid and autotetraploid plants, including leaf morphology, epidermal cell structure, ultrastructure, chlorophyll contents, and photosynthetic and fluorescence parameters. Mature leaves were used in this study. Ten typical individuals were selected, and three biological replicates were performed.

The leaf epidermal cells were observed using the nail varnish method, as described by Huang et al. (2011). The sections were examined with an Olympus BX-41 microscope (Japan), and the obtained images were numerically converted with Photoshop 6.0. Leaf numbers 5 and 6 from the top of the plants were chosen for the measurement of chlorophyll contents, which was performed using the method of Li (2003).

Leaf gas exchange was measured using a portable photosynthesis