Flora 219 (2016) 25–34

Contents lists available at ScienceDirect

Flora

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

Microsporogenesis and meiotic abnormalities in different 'Oblačinska' sour cherry (*Prunus cerasus* L.) clones

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ARTICLE INFO

Article history:
Received 25 May 2015
Accepted 23 December 2015
Edited by Alessio Papini
Available online 31 December 2015

Keywords:
Chromosomes
Cytomixis
Meiosis
Pollen germination
Prunus cerasus
Sour cherry

ABSTRACT

The Oblačinska sour cherry (*Prunus cerasus*) is a mixture of different clones with similar tree and fruit characteristics that is indigenous to and widely planted in commercial orchards in Serbia. Sour cherry, including Oblačinska sour cherry clones, exhibits irregular meiosis which may contribute to low fruit set in some selections. The goal of this study was to examine the process of microsporogenesis and to determine if meiosis and its anomalies effect the *in vitro* pollen germination and pollen tube length in four 'Oblačinska' sour cherry clones that differ in fruit set and yields. All clones displayed varying degrees of chromosomal abnormalities in all meiosis sub-phases. The abnormalities became evident from late pachytene with more than half of the pollen mother cells (PMC) showing abnormal conjugation of chromosomes in metaphase I. The lowest number of PMCs with laggards was in clone III/9 and the highest in clone XIII/1. In second division, the univalent and multivalent association was observed at metaphase-II, the lagging and stickiness in anaphase-II, and the phenomenon persisted up to the microspore stage. In all four 'Oblačinska' clones, PMCs exhibited cytomixis phenomena, however, it was only observed in the second experimental year. Cytomixis differed among the four clones but was equally frequent in all stages of meiosis. The syncytia formed, most often consisted of 2–3 PMCs that were at the same phase of meiosis, and exhibited common cytoplasm and occasionally nuclear fusion. *In vitro* pollen germination and pollen tube length significantly differed between the clones. Most probably abnormalities during meiosis, regardless of the good results of pollen germination, influenced the reduced potential clones XI/3 and XIII/1.

1. Introduction

Microsporogenesis is a genetically controlled physiological, biochemical, and morphological processes where the final product is a tetrad of haploid microspores. Numerous abnormalities of meiosis can occur in plants which can result in a loss of fertility and overall reproductive efficiency (Rai et al., 2010). The most common abnormality is irregular conjugation of chromosomes such as the occurrence of univalents in diakinesis or metaphase I. Other meiotic abnormalities observed during microsporogenesis are chromosome stickiness, mixoploidy, chromosome fragmentation and failure of cytokinesis. Some abnormalities can cause chromosome elimination during microsporogenesis (Adamowski et al., 1998), the absence of collecting bivalents on the equatorial plate at metaphase I, degeneration or spindle break down (Pagliarini, 2000).

Cytomixis is an anomaly that has been identified in plant meiosis, especially in microsporogenesis, a century ago. This phenomenon is characterized by a migration of chromatin/chromosomes between proximate meiocytes (pollen mother cells, MPC) through cytoplasmic channels or intercellular bridges. In most cases it is detectable in microsporocytes (Kumar et al., 2010) and never in megasporocytes (Mursalimov et al., 2013). Cytomixis is potentially a means of maintaining heterozygosity of gametes and an additional tool for phylogenetic evolution of the karyotype by reducing or increasing the basic set of chromosomes (Cheng et al., 1987). Thus, fertile unreduced pollen grains which are '2n' may play a vital role in the sexual polyploidization of species (Kim et al., 2009). Furthermore, cytomixis has been considered to be a characteristic of genetically unbalanced plants such as

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http://dx.doi.org/10.1016/j.flora.2015.12.009
0367-2530/

hybrids, mutants and aneuploids (Rai et al., 2010). In all studied species, number of cells where cytomixis appeared is small, but it increased the pollen sterility (Pagliarini, 2000).

Factors that influence the frequency of cytomixis is pathogen attack, temperature, the use of pesticides or antibiotics, abnormal behavior due to mutagenic agents, plant fitness, presence of a mutant gene and pollution (Mandal et al., 2013). So far, cytomixis, is a well established phenomenon reported in large array of plants (Bellucci et al., 2003; Bhat et al., 2006; Kumar et al., 2010). However, all those facts are connected with herbaceous plants, while for woody perennial plants, such as fruits, reports are scarce except for plum, peach, almond, hazelnut and mulberry (Dwivedi et al., 1988; Kostritsyna and Soldatov, 1991; Lagerstedt, 1977; Soodan and Waffai, 1987).

The 'Oblačinska' sour cherry (Prunus cerasus L.) is a mixture of different clones with similar fruit and tree characteristics which is the most widely planted cultivar in commercial Serbian orchards (Ceroviæ and Radièeviæ, 2008). Accounting for 7.7% of total fruit production, with 8.7 million trees and production of 74,656 MT in 2012, Serbia is in sixth place in the world for the sour cherry production (FAOStat, 2012). Long-term cultivation in diverse agro-ecologic conditions and the use of various types of propagation (both by suckers and by seeds) has caused the 'Oblačinska' sour cherry to become a mixture of numerous genotypes (Rakonjac et al., 2010). According to several authors, who studied 'Oblačinska' populations, the highest variability among genotypes was mainly found in pomological and technological traits, including maturing time, yield, size, and fruit quality (Rakonjac et al., 2010; Fotirić Akšić et al., 2013).

Meiotic irregularities during micro-sporogenesis that can result in different levels of infertility was previously studied by Dys (1984), Dirlewanger et al. (2007), Chudíková et al. (2012) and Iordache (2013). In cherries, varying degree of irregularities at meiosis observed in microsporogenesis has been correlated with pollen germination *in vitro* (Popovska et al., 2005). Therefore, an understanding of the microsporogenesis process in sour cherry and its relationship to pollen viability is important when choosing the most productive sour cherry cultivar for commercial production. The objective of this study was to analyze cytogenetic characteristics of meiosis in different the clones 'Oblačinska' sour cherry clones that differ in fruit set and yields and to provide insight which anomalies and in what frequencies occur. Also, the goal was to prove does anomalies have impact on *in vitro* pollen germination and pollen tube length in those clones. A more precise definition of these factors will help to assess the clone fertility and will contribute to an understanding of which clone has the highest fruit set potential.

2. Materials and methods

Twigs bearing flower buds were collected from trees of four 'Oblačinska' sour cherry clones from the Experimental Station 'Radmilovac', located 8 km North-East of Belgrade (44°45′N and 20°35′E, at 135 m altitude), which belongs to the Faculty of Agriculture, University of Belgrade. The collection orchard was established in 1993. Planting distance was 4 m × 2 m. The soil is classified as Eutric Cambisol. The trees were trained as spindle bush, under non-irrigated standard cultural practices.

After five years four of the 41 'Oblačinska' clones were chosen for analysis based on their fruit set and yields. Accessions II/2 and III/9 were distinguished by their high fruit set and high yields; while accessions XI/3 and XIII/1 exhibited the lowest values fruit set and fruit yield.

In two consecutive years (2006–2007) flower buds were successively removed over several days at the first signs of swelling (approximately a month before beginning of flowering). In 2006, the flower buds removal was done in interval between 9th and 12th March, while in 2007 during last three days of February. Temperature and rainfall oscillations before and during the experiment was showed in Fig. 1, Fig. 2, respectively.

The flower primordia were excised and fixed in the rapid fixative (absolute ethyl alcohol and glacial acid = 3:1) and held in solution for 1 day. The fixed material was then rinsed in 70% ethyl alcohol and refrigerated at +4 °C. The anthers, *i.e.* PMCs (pollen mother cells) were stained for microscopic examination with 1% aceto-carmine following the standard squash technique. The preparations with a glass cover were heated slightly over a flame to increase chromosome stainability.

The pollen used in this study was collected from 50 flowers from each clone. The flower sampling was done randomly from all cardinally-oriented branches with different directions around the canopy at the balloon stage (1–2 days before opening). Anthers were removed and stored at 20 °C for 24 h.

In vitro germination of freshly collected pollen was tested on a germination medium containing agar (0.3%) and sucrose (14%). Petri dishes were kept in the laboratory at room temperature (20 ± 2 °C) for 24 h. When the length of the pollen tube exceeded its diameter, the pollen was considered germinated. For pollen germinability at least 500 pollen grains were observed and counted, and used to calculate and germination rate, respectively.

Examination of all stages in meiosis and pollen germination was done under the Leica DM LS microscope (Leica Microsystems, Wetzlar, Germany). Pollen tube length was measured for all four genotypes using the 'Leica IM 100′ program. Tests for all traits included, for both years, were done in three replicates, where each included 150 PMCs or 200 pollen grains.

2.1. Statistic analysis

The meiosis analysis was performed in two-factorial analysis of variance (ANOVA). The significances of the individual differences for the investigated factors (clone, year and interaction clone × year) were determined using the least significant difference (LSD 0.05 = 95 % confidence). Statistical analyses were conducted using STATISTICA for Windows 6.0 (StatSoft Inc., Tulsa, Okla).

3. Results

The beginning of meiosis was clearly observable from pachytene where a netlike structure was readily discernible, with chromosomes filling the whole lumen of the nucleus (Fig. 3a). In all four clones, irregular configurations of conjugated chromosomes were apparent at diakinesis. At metaphase I (Fig. 3b) or later in metaphase II (Fig. 3c) conjugated chromosomes configurations were lined up on the equatorial plate where they could be seen even more clearly. In metaphase I the percentage of PMCs with 16 normal pairing homologus chromosomes, *i.e.* bivalents, varied between all studied clones and interaction clone × year, which was proved by ANOVA (Table 1). Clone II/2 had the highest number of PMCs with bivalents in both years (48.2% and 59.9%, respectively), while clone XIII/1 showed the lowest values (30.8% and 36.1%, respectively). PMCs with differing abnormal conjugation of chromosomes, *i.e.* the pairing configurations in the form of trivalents, quadrivalents, the absence of pairing (univalents) or even combination of several different configurations (1 univalent + 1 trivalent, 2 univalents +1 trivalent, 2 univalents + 1 quadivalent) (data not show) were observed at this stage (Fig. 3d). They occurred in statistically different numbers of PMCs. Years of study did not have any influence to bivalent formation, but showed strong effect on abnormal conjugation of homologous chromosomes (Table 1).