XV Overall Polish *in vitro* Culture and Plant Biotechnology Conference

Biotechnological utilization of *in vitro* culture variability

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The conference is dedicated to Prof. dr hab. Maciej Zenkteler

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Session II	**New tools in plant biotechnology**
Session III	**Haploids and chromosome analysis**
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Poster no. 3

Evaluation of the nuclear DNA content/ploidy level and some morphological traits of the rootstocks for cherry trees collected in the gene resources of the Research Institute of Horticulture in Skierniewice

MAŁGORZATA PODWYSZYŃSKA*, MIROSŁAW SITAREK, AGNIESZKA MARASEK-CIOŁAKOWSKA

Research Institute of Horticulture, Skierniewice, Poland

* Malgorzata.Podwyszynska@inhort.pl

Genome size is a fundamental parameter in many genetic and biological studies involving genome organisation, species relationships, and breeding works. Knowledge of the ploidy level can be useful in evaluating reproductive and somatic compatibility, an important parameter in scion and rootstock breeding programs. The evaluation of the nuclear DNA content was performed in order to determine the ploidy level of rootstocks of genus *Prunus* used in the growing of sour and sweet cherries. At the first stage of the research, the conditions of the flow cytometry analysis (with the application of propidium iodide for DNA staining) (FCM/PI) were optimized: the type of buffer for nuclei extraction and incubation time as well as the selection of internal standards (genotypes with a known nuclear DNA content). Repeatable results and relatively good-quality histograms were obtained using Partec extraction buffer with 1% PVP addition, with incubation time longer than 50 min. The best internal standard for the FCM analysis of cherry rootstocks with a nuclear DNA content from about 0.7 to 1.4 pg proved to be *Solanum lycopersicum* (2C = 1.96 pg), whose peaks 2C and 4C did not coincide with the peaks of the genotypes tested, but overlapped partially with the peaks of *Prunus* genotypes with larger genomes tested. For rootstock genotypes with a nuclear DNA contents from 1.8 to 2.3 pg, *Glycine max* (2.91 pg) or *Zea mays* (2C = 5.44 pg) were selected as the internal standards. At the second stage, the optimized method and well-chosen internal standards allowed to assess with high precision the nuclear DNA content/ploidy level of 20 *Prunus* genotypes of the rootstocks for cherries and sweet cherries. For the reference *Prunus* genotypes of the known chromosome number, 2C DNA values was 0.68 pg for diploid *P. cerasifera* var. *divaricata* Led. "Anna", the value of 1.18 pg for triploid "GiSelA 3" (*P. cerasus* "Schattenmorelle" × *P. canescens*) and 2.16 pg for hexaploid *P. domestica* "Eruni". In nine rootstocks for sour cherry and sweet cherry trees ("F 12/1", "PiKu 1", "PiKu 3", "INRA SL 64", "Maxma Delbard 14 Brokforest", "Ferci SL 405", "GM 79", "LC-13", "L-2"), 2C DNA values ranged from 0.74 to 0.86 pg indicating their diploid chromosome number. Nine rootstocks were identified as triploids ("Colt", "GiSelA 3", "GiSelA 5", "GiSelA 6", "P-HL A", "P-HL C", "PiKu 4", "VSL 1" and "VSL 2") with their 2C DNA contents from 1.03 to 1.24 pg. Two rootstocks were considered to be tetraploids ("WC-13" and "LC 52") having 2C DNA of 1.39 pg. The ploidy level of the genotypes of sour cherry and sweet cherry rootstocks was evaluated in relation to the morphological and agronomical traits.

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