Evaluation of nuclear DNA content / ploidy level and some morphological traits of the rootstocks for cherry trees

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INTRODUCTION

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 basic chromosome number in *Prunus* is x = 8. Somatic chromosome number of various *Prunus* species vary from diploid to hexaploid. Most of the species are diploid (2n = 2x = 16), e.g., *P. dulcis*, *P. armeniaca*, *P. avium*, *P. canescens*, *P. cerasifera*, *P. lannesiana*, *P. mahaleb*, *P. munsoniana*, *P. persica*, *P. pumila* and *P. tomentosa*. Some of these species (*P. avium*, *P. cerasifera* and *P. spinosa*) occur in different polyploid forms. There are also several tetraploids (2n = 2x = 32) such as *P. cerasus*, *P. fruticosa*, *P. maackii* and hexaploids (2n = 2x = 48), *P. domestica* and *P. domestica* var. *insititia*. Some of these *Prunus* species have been used for selecting improved rootstock genotypes or served as parental genotypes for creation hybrid rootstock cultivars combining desired traits.

AIM

In this study, 2C DNA value (somatic nuclear DNA content) / ploidy level was evaluated of *Prunus* rootstocks in relation to stomata and leaf sizes and agronomical trait such as vigor growth. In the first stage of the research, the conditions of flow cytometry analysis (with application of propidium iodide for DNA staining) (FCM/PI) were optimized.

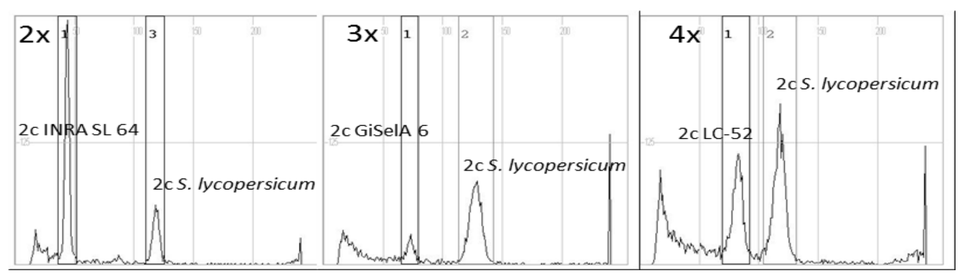
MATERIAL AND METHODS

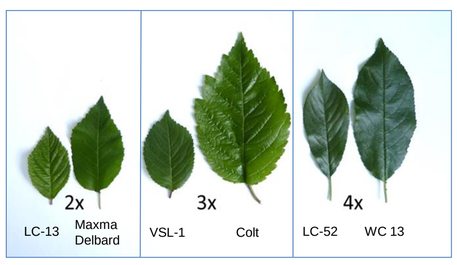
Plant material. Twenty *Prunus* genotypes of rootstocks for cherry and sweet cherry, gathered in gene resources of the Research Institute of Horticulture (Skierniewice, Poland).
Evaluation of nuclear DNA content. In the first stage of the research, the conditions of flow cytometry analysis were optimized. Analysis of genome size was done using FCM/PI. Samples were taken in mid-July from six leaves collected randomly from each genotype. Leaf tissue was chopped together with a piece of plant internal standard in a Petri dish in nuclei isolation Galbraith's buffer or Partec buffer to which propidium iodide (50 µg mL^{-1}) and RNasa (50 µg mL^{-1}) were added. As the internal standards, the young leaves were used of *Solanum lycopersicum* 'Stupicke' (2C DNA = 1.96 pg), *Glycine max* 'Polanka' (2C DNA = 2.91 pg) or *Zea mays* CE-777 (2C DNA = 5.43 pg). Optimization of FCM/PI analysis was performed with reference plants of *Prunus* genotypes (external standards) with the known chromosome numbers: diploid *P. cerasifera* var. *divaricata* Led. 'Anna' (Myrobalan) (2n = 2x = 16), triploid sweet cherry rootstock GiSelA 3 (*P. cerasus* 'Schattenmorelle' × *P. canescens*) (2n = 3x = 24) and haxaploid *P. domestica* 'Eruni' (2n = 6x = 48). The samples were filtered through a 30 µm filter and incubated for 30–60 min in room temperature. The fluorescence of the nuclei was measured using analyser CyFlow Ploidy (Partec, Germany). The 2C DNA content of a sample was calculated as the sample peak mean divided by the mean of the standard plant peak and multiplied by the amount of DNA of the standard plant.
Phenotype evaluation. Leaves were collected in mid-June. Leaf area was measured for randomly collected 30 leaves, with optical planimeter Delta-T Devices. Stomata length was measured using light microscopy, according to the procedure of Dyki and Habdas (1996).

RESULTS

- 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 FCM analysis of the rootstocks for cherries with a nuclear DNA content from about 0.7 to 1.4 pg proved to be *Solanum lycopersicum* (2C = 1.96 pg), of which peaks 2C and 4C did not coincide with the peaks of the genotypes tested.
- In nine rootstocks for cherry trees, 2C DNA values ranged from 0.55 to 0.86 pg indicating their diploid chromosome number (F 12/1, PiKu 1, Piku 3, INRA SL 64, 'Maxma Delbard 14 Brokforest', 'Ferci SL 405', GM 79, LC-13, L-2). Nine rootstocks were identified as triploids (e.g.. 'Colt', GiSelA 3, GiSelA 6, P-HL A, PiKu 4, and VSL 2) with their 2C DNA contents from 1.03 to 1.24 pg. Two rootstocks were considered tetraploid (WC-13 and LC 52) having 2C DNA of 1.39 pg.
- Genotypes differ significantly in the leaf size and stomata length.
- Stomata size tended to increase with the ploidy level. Among 10 genotypes of the smaller stomata (18.9–24.9 µm), there were eight diploids and two triploids while within cherry rootstocks of larger stomata (25.0–30.8 µm), there were two diploids, six triploids and two tetraploids. However, the tendency of the increase in the leaf size along with the increase in the ploidy level was not so clear. The smallest leaves were observed for diploid LC-13 (13.3 cm2) and triploid VSL-2 (15.1 cm2) while the largest leaves, 48.3 and 57.8 cm2, were found for triploids, P-HL C and 'Colt', respectively. In turn, tetraploid WC 13 and LC-52 had leaves of intermediate size.

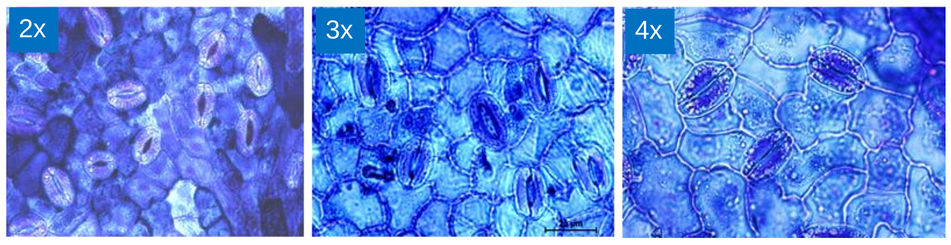
Genotype	Parentage	Nuclear 2C DNA content (pg)	Ploidy level	Leaf area (cm^2)	Stomata length (µm)	Vigour level
LC-13	*P. avium x P. cerasus*	0.55	2x	13.3 k	18.9 kl	Intermediate vigour
Ferci SL 405	*P. mahaleb*	0.75	2x	18.9 h-k	25.7 c-e	Vigorous
INRA SL 64	*P. mahaleb*	0.75	2x	19.7 h-k	25.2 d-f	Vigorous
L-2 (Krymsk 7)	*P. lannesiana*	0.77	2x	15.9 jk	23.7 gh	Intermediate vigour
PiKu 3 (4.83)	*P. pseudocerasus x (P. canescens x P. incisa)*	0.77	2x	21.1 h-j	24.9 d-g	Vigorous
Maxma Delbard 14 Brokforest	*P. avium x P. mahaleb*	0.79	2x	32.2 d-f	24.1 f-h	Vigorous
GM 79 (Camil)	*P. canescens*	0.80	2x	33.6 de	20.1 k	Intermediate vigour
PiKu 1 (4.20)	*P. avium x (P. canescens x P. tomentosa)*	0.81	2x	20.2 h-k	23.4 hi	Weak
F 12/1	*P. avium*	0.86	2x	45.3 b	22.3 ij	Very vigorous
Colt	*P. avium* (F299/2) *x P. pseudocerasus*	1.03	3x	57.8 a	24.5 e-h	Very vigorous
PiKu 4 (4.22)	*(P. canescens x P. tomentosa) x P. avium*	1.12	3x	31.6 d-f	22.2 ij	Intermediate vigour
VSL-1	*P. fruticosa x P. lannesiana*	1.12	3x	22.3 g-i	25.0 d-f	Intermediate vigour
VSL-2 (Krymsk 5)	*P. fruticosa x P. lannesiana*	1.12	3x	15.1 jk	26.1 cd	Vigorous
GiSelA 5 (146/2)	*P. cerasus* 'Schattenmorelle' x *P. canescens*	1.13	3x	29.4 d-f	21.8 j	Intermediate vigour
GiSelA 6 (148/1)	*P. cerasus* 'Schattenmorelle'x *P. canescens*	1.14	3x	29.0 e-g	27.0 bc	Intermediate vigour
GiSelA 3 (209/1)	*P. cerasus* 'Schattenmorelle' *x P. canescens*	1.18	3x	21.0 h-j	26.2 cd	Weak
P-HL A (84)	*P. avium x P. cerasus*	1.20	3x	41.9 bc	25.7 c-e	Intermediate vigour
P-HL C (6)	*(P. avium x P. cerasus)*	1.24	3x	48.3 b	25.2 d-f	Weak
LC-52 (Krymsk 6)	*P. cerasus* 'Lyubskaya' *x (P. cerasus* 'Michurin' *x P. maackii)*	1.39	4x	25.6 fg	30.8 a	Intermediate vigour
'WC-13'	*P. cerasus* 'Vladimirskaya' *x (P. cerasus* 'Michurin' *x P. maackii)*	1.39	4x	36.7 cd	27.8 b	Intermediate vigour





Effect of ploidy level on leaf area (mean values for ploidy levels)

Ploidy level	Leaf area (cm^2)	Stomata length (µm)
2x	24.3 b	23.6 c
3x	32.9 a	24.8 b
4x	31.2 a	29.3 a



diploid GM 79 **triploid VSL-1** **tetraploid LC-52**
Stomata of cherry rootstocks

CONCLUSION

- The certain tendency to increase the size of stomata and leaves along with an increase in the ploidy level is observed within *Prunus* rootstocks genotypes, however, the correlations between these traits are not so evident.
- There is no correlation between ploidy level and vigour of individual rootstocks.
- Stomata and leaf sizes cannot be considered as a morphological marker indicating ploidy level.
- The evaluated nuclear DNA contents/ploidy levels as well as stomata and leaf sizes are the additional, useful for breeders descriptors of *Prunus* rootstock genotypes.

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