



Supplementary material for:

In vitro induction and primary evaluation of octoploid plants in Saskatoon berry (*Amelanchier alnifolia* Nutt.)

Danuta Kucharska¹, Małgorzata Podwyszyńska¹, Aleksandra Trzewik¹, Agnieszka Marasek-Ciołakowska¹, Stanisław Pluta², Łukasz Seliga²

¹ Department of Applied Biology, The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3 Street, 96-100 Skieriewice,

² Department of Breeding, The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3 Street, 96-100 Skieriewice,

* Correspondence: danuta.kucharska@inhort.pl

Contents

Figure S1. Chromosome counts of *Amelanchier* genotypes. **a.** 'Smoky' ($2n = 4x = 68$). **b.** 'Smoky' S 8-1 ($2n = 8x = 136$). **c.** 'Smoky' S 8-2 ($2n = 8x = 136$). **d.** 'Martin' ($2n = 4x = 68$). **e.** 'Martin' M 8x-1 ($2n = 8x = 136$). **f.** 'Martin' M 8x-2 ($2n = 8x = 136$). **g.** 'Martin' M 8x-3 ($2n = 8x = 136$). **h.** 'Martin' M 8x-4 ($2n = 8x = 136$). Bars represent 5 μ m.

Figure S2. Histograms of nuclear DNA / ploidy level estimation using flow cytometry (FCM-PI) with internal standards: *Solanum lycopersicum* (2C DNA = 1.96 pg) for the *Amelanchier alnifolia* genotypes. **a.** 'Smoky' control plant S4x (2C DNA = 2.28 pg). **b.** 'Smoky' S 8-1 (2C DNA = 4.60 pg). **c.** 'Smoky' S 8-2 (2C DNA = 4.64 pg). **d.** 'Martin' control 4x (2C DNA = 2.29 pg). **e.** 'Martin' M 8x-1 (2C DNA = 4.55 pg). **f.** 'Martin' M 8x-2 (2C DNA = 4.63 pg). **g.** 'Martin' M 8x-3 (2C DNA = 4.57 pg). **h.** 'Martin' M 8x-4 (2C DNA = 4.56 pg).

Table S1. Stages of obtaining, micropropagation, rooting and acclimatization of Saskatoon berry octoploids

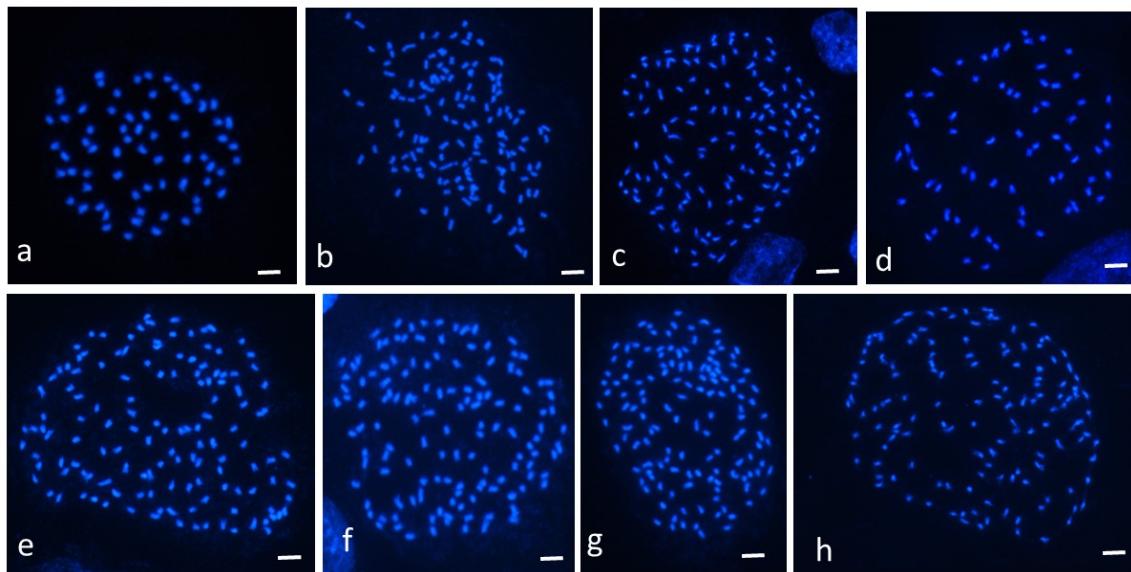


Figure S1. Chromosome counts of *Amelanchier* genotypes. **a.** 'Smoky' ($2n = 4x = 68$). **b.** 'Smoky' S 8-1 ($2n = 8x = 136$). **c.** 'Smoky' S 8-2 ($2n = 8x = 136$). **d.** 'Martin' control ($2n = 4x = 68$). **e.** 'Martin' M 8x-1 ($2n = 8x = 136$). **f.** 'Martin' M 8x-2 ($2n = 8x = 136$). **g.** 'Martin' M 8x-3 ($2n = 8x = 136$). **h.** 'Martin' M 8x-4 ($2n = 8x = 136$). Bars represent 5 μ m.

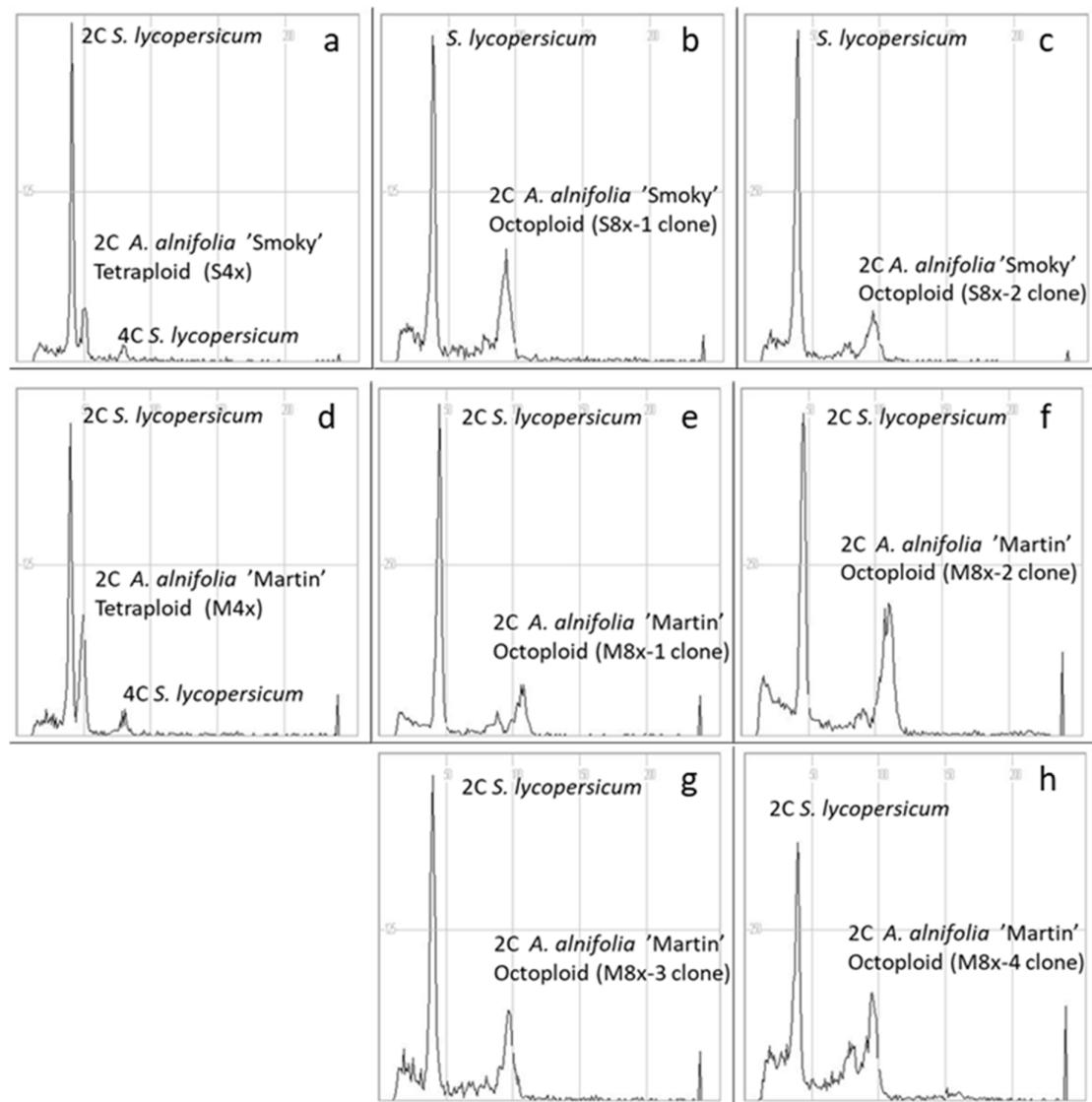


Figure S2. Histograms of nuclear DNA / ploidy level estimation using flow cytometry (FCM-PI) with internal standards: *Solanum lycopersicum* (2C DNA = 1.96 pg) for the *Amelanchier alnifolia* genotypes. **a.** 'Smoky' control plant S4x (2C DNA = 2.28 pg). **b.** 'Smoky' S 8-1 (2C DNA = 4.60 pg). **c.** 'Smoky' S 8-2 (2C DNA = 4.64 pg). **d.** 'Martin' control 4x (2C DNA = 2.29 pg). **e.** 'Martin' M 8x-1 (2C DNA = 4.55 pg). **f.** 'Martin' M 8x-2 (2C DNA = 4.63 pg). **g.** 'Martin' M 8x-3 (2C DNA = 4.57 pg). **h.** 'Martin' M 8x-4 (2C DNA = 4.56 pg).

Table S1. Stages of obtaining, micropropagation, rooting and acclimatization of Saskatoon berry octoploids.

No	Stage	Media (mg L ⁻¹)	Duration and conditions of the subcultures
1.	Micropropagation of Saskatoon berry	<u>Basic medium (BM):</u> MS (salts and vitamins), 325 CaCl ₂ , 175 MgSO ₄ , 1.0 BA, 1.0 GA ₃ , 0.1 IAA, 30 g L ⁻¹ sucrose, 6 g L ⁻¹ plant agar (Duchefa), pH 5.6	4 weeks <u>standard conditions (SC):</u> growth room, 23 °C, 16 h photoperiod
2.	Induction of octoploids	BM supplemented antimitotics: colchicine 125, 250, trifluralin 50, 100, oryzalin 5, 10, APM 5, 10, the control - BM	2 weeks (6 days in the darkness, 8 days in SC)
3.	Detection of octoploids	1 st subculture - BM 2 nd subculture - BM	4 weeks in SC 4 weeks in SC (checking the ploidy of all regenerated shoots by flow cytometry).
4.	Micropropagation of octoploids	BM	4 weeks in SC (confirmation of octoploid status of shoots)
5.	Cooling of octoploids	BM	1-3 months cold room, 4 °C, darkness
6.	<i>In vitro</i> rooting of octoploids	<u>Stage I:</u> ½ MS salts and vitamins, 1.0 IBA, 30 g L ⁻¹ sucrose, 6 g L ⁻¹ plant agar, pH 5.6; <u>Stage II:</u> ½ MS salts and vitamin, 4 g L ⁻¹ active carbon (Duchefa), 30 g L ⁻¹ sucrose, 6 g L ⁻¹ plant agar, pH 5.6	five days in darkness 4 weeks in SC
7.	Acclimatization of octoploids	shading and keeping high humidity spraying 10 GA ₃ foliar fertilization 0.4% Npk 18-18-18 (Kristalon Yara Poland)	greenhouse, during the first 3 weeks, the next day after planting, from the 10th day every week