doi: 10.17221/7/2016-HORTSCI

Effect of polyploidization on morphology in two apple (*Malus* × *domestica*) genotypes

Niek Hias[1]*, Leen Leus[2], Mark W. Davey[3], Stijn Vanderzande[4], Johan Van Huylenbroeck[2], Johan Keulemans[1]

[1]*Department of Biosystems, Katholieke Universiteit Leuven, Leuven, Belgium*
[2]*Plant Sciences Unit, Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium*
[3]*Bayer Crop Science, Gent, Belgium*
[4]*Department of Horticulture, Washington State University, Pullman, USA*

**Corresponding author: nick.hias@biw.kuleuven.be*

Abstract

Hias N., Leus L., Davey M.W., Vanderzande S., Van Huylenbroeck J., Keulemans J. (2017): Effect of polyploidization on morphology in two apple (*Malus* × *domestica*) genotypes. Hort. Sci. (Prague), 44: 55–63.

Because polyploidy often results in enhancement of desirable properties, artificial genome doubling is commonly used in agri- and horticultural crop breeding programs. In this study genome doubling was induced in two apple genotypes. The effect on vegetative morphological and physiological traits of the plants was then comprehensively determined by comparing the obtained tetraploid apple plants with their diploid counterparts. Out of 17 different physio- and morphological characteristics, 15 were significantly affected in one or both genotypes. The response of these 15 characteristics also appeared to have been caused by two effects; 10 of the 15 characteristics exhibited a common response to ploidy change over both genotypes while five traits showed a genotype-specific response to polyploidization. Tetraploid leaves also exhibited a darker leaf colour, which could be correlated to a higher pigment concentration. Furthermore, the results also show a decreased elongation rate and leaf size in tetraploids, which is suggested to be due to the observed lower cell density in the polyploid apple plants.

Keywords: chromosome doubling; diploid; growth; leaf parameters; tetraploid

Polyploidy is frequently found in agricultural and horticultural crops because it allows increased genetic variation and often possesses properties superior to their diploid counterparts (Van Laere et al. 2011). Through gene-dosage and epigenetic effects, together with nuclear enlargement, polyploidy causes genetic changes that result in pronounced phenotypic alterations (Comai 2005; Sun et al. 2009; Van Laere et al. 2011; Allario et al. 2013; Hao et al. 2013). Morphological effects commonly associated with polyploidy are bigger flowers, an altered leaf length-to-width ratio and changes in size and density of stomata (Sun et al. 2009; Van Laere et al. 2011; Trojak-Goluch, Skomra 2013; Tan et al. 2015). Other frequently occurring characteristics include darker-coloured green leaves and a more compact growth habit (Kermani et al. 2003; Liu et al. 2007; Allario et al. 2011).

Most apple cultivars are diploids ($2n = 2x = 34$). Nevertheless, triploid cultivars ($2n = 3x = 51$) do exist; their use dates back at least to the 17th century when natural triploids were selected for their high yield and large fruit size (e.g. cv. 'Gravenstein'). The beneficial characteristics of triploid fruit are also supported by their relatively high occurrence. For example, a study by Pereira-Lorenzo et al. (2006)

Supported by Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

doi: 10.17221/7/2016-HORTSCI

found that 29% of the local cultivars in northern Spain are triploid. A study (Defra 2010) at East Malling Research (East Malling, UK) also showed that out of 2,165 apple accessions in the Defra National Fruit Collection, 304 (14%) were triploid, although the frequency of polyploid formation in flowering plants is only about 0.001% (Comai 2005). Tetraploids are more rare in apple, however; they are mainly artificially created and used by breeders as a means to develop triploid genotypes (Vaarama 1946; Sedysheva, Gorbacheva 2013; Sedov et al. 2014).

Notwithstanding existing literature (Xue et al. 2015), still little is known about the successful creation and morphological alterations of artificially induced polyploid apple plants. This study aims at inducing genome doubling in several genotypes to investigate the potential role of polyploidy in the response of *Malus* × *domestica* to biotic and abiotic stress. In a later phase, the tetraploids will be used as breeding material to create triploid varieties. The work presented here focuses on the polyploidization event of two different diploid *Malus* × *domestica* genotypes and compares morphological and physiological characteristics of the newly created tetraploids to their diploid predecessors.

MATERIAL AND METHODS

Plant material and polyploidization. Two apple genotypes were polyploidized: the commercial cv. 'Gala' and the genotype coded G40 from the breeding germplasm of Better3fruit (Belgium). Tetraploid plants were obtained after chromosome doubling in tissue culture by stirring axillary buds (3–4 mm) during 2 days in a MS multiplication medium based on Dobránszki et al. (2000) supplemented with colchicine (10 mM). Obtained tetraploid and diploid control plants were rooted in vitro and finally acclimatized in the greenhouse. Ploidy analyses were performed after induction of polyploidy in tissue culture and, as a second control, on the rooted greenhouse plants on a flow cytometer Cyflow Space (Partec, Münster, Germany) as described by Dhooghe et al. (2009).

Plant material for morphological characterization. After *ex vitro* acclimatization own-rooted di- and tetraploid 'Gala' ('Gala'-2*x* and 'Gala'-4*x*, respectively) and G40 (G40-2*x* and G40-4*x*, respectively) plantlets were transferred to a 2 l container with ready-made soil (DCM Pepi 3, Grobbendonk, Belgium) and grown for 10 months under controlled greenhouse conditions: day/night temperature 18/14°C and day/night relative humidity 70/60%; additional lighting (120 µmol/m²·s when daylight intensity < 457 µmol/m²s). Shortly after winter dormancy (2 months outside the greenhouse), the plants were cut back to three buds above ground and transferred again to the mentioned greenhouse conditions. After budburst, one shoot per plant was left to grow for 47 days until the start of the experiment.

Shoot growth parameters. Six plants per genotype and per ploidy level were used to determine length and diameter at the base and top of the main shoot at 47, 53, 60, 72 and 87 days post budburst (dpb). Using the Smalian's sectional volume (*SV*, cm³) formula, the stem volume of the shoot was calculated (Achten et al. 2010):

$$SV = \frac{A_1 + A_2}{2} \times L$$

where: A_1 – area of the shoot base based on the base diameter (cm²); A_2 – area of the shoot top based on top diameter (cm²); L – shoot length (cm)

Shoot length at the different time points followed a linear increase, therefore elongation rates were determined by the slope of the linear regression line.

Leaf parameters. Mean leaf area was determined from digital photographs using an in-house developed image analysis software for all mature leaves of three plants per genotype and per ploidy level.

Mature leaves were fixed for microscopic analysis and stored in a 10/5/50 vol. % solution of Formalin-Acetic-Alcohol (FAA) until analysis. Hand-cut transverse sections were stained in a 0.5% Safranin O solution for 10 seconds. Images were taken with a light microscope (Olympus BX 40 with Colorview Soft Imaging System camera, Aartselaar, Belgium) and analysed using the UTHSCSA ImageTool (http://compdent.uthscsa.edu/dig). Thickness of all leaf cell tissues was determined in one fully matured leaf per plant using four plants per genotype and per ploidy level.

Leaf colour was measured with a portable spectrophotometer (CM-700d, Konica-Minolta, Zaventem, Belgium). Colour is represented through L^* (luminosity), a^* and b^* (colour-opponent dimensions, red to green and yellow to blue, respectively) values (three measurements per leaf on two mature

doi: 10.17221/7/2016-HORTSCI

leaves per plant on five own-rooted trees per genotype and ploidy level).

Stomatal characteristics were determined using Jones' (2013) nail-varnish method (micro relief method); three epidermal imprints were taken of one fully matured leaf for three plants per genotype and ploidy level. Stomatal density was determined by counting the number of stomata under a light microscope (Olympus BX 40 with Colorview Soft Imaging System camera). Guard cell length was determined for 10 stomata per epidermal strip on one microscopic field. Stomatal area was calculated using the area formula of an ellipse (A, μm²):

$$A = \pi / 4 \times L \times W$$

where: L – guard cell length (μm); W – guard cell width (μm)

Chlorophyll *a*, *b* and the total carotenoid content were spectrophotometrically determined according to Wellburn (1994). Pigments were measured on the same leaves used for the spectrophotometric analysis.

The difference in cell number between di- and tetraploid plants of own-rooted 'Gala' and G40 plants was determined as a ratio of nuclei counted in the diploid and tetraploid plants. Cells were counted on a flow cytometer. Samples were prepared as described by Dhooghe et al. (2009). From 3 plants, leaf discs of 4 mm diameter were taken in 10 biological replicates and the weight per disc was determined. For the diploid plants, two discs of the same young leaf were used: one in a separate sample to evaluate the number of dividing cells and one in a pooled sample combining leaf discs from both ploidy levels. By subtracting the percentage of dividing diploid nuclei from the total number of nuclei in the tetraploid peak measured in the pooled sample, a ratio of diploid nuclei/g FW (fresh weight) over tetraploid nuclei/g FW can be determined per leaf.

Statistical analysis. Statistical analyses were performed in JMP Pro®, Version 11. Data were subjected to a one-way analysis of variance (ANOVA). Main and interaction effects were determined using a two-way ANOVA effects test.

RESULTS

Growth characteristics: leaf area, shoot elongation, volume and internode length

Several morphological traits were determined on di- and tetraploid plants of both genotypes at different time points. Although trends seem visible (Table 1), a two-way ANOVA did not show significant interactions between genotype and ploidy level for elongation rate and shoot volume. In G40 the shoot elongation rate decreased significantly after polyploidization (one-way ANOVA; $P < 0.01$), but no significant decrease was observed in 'Gala' (Table 1). Also in shoot volume, a significant decrease of over 50% was observed for G40-4*x* compared to G40-2*x* (one-way ANOVA; $P < 0.05$). Again, 'Gala' showed no significant differences between 2*x* and 4*x* plants. The internode length was found to be similar between both genotypes and ploidy levels, with no significant effect of neither genotype nor ploidy level.

Table 1. Growth characteristics at different time points (days post budburst (dpb)) of G40 and 'Gala' in di- and tetraploid form with mean values and interaction effects of genotype and ploidy

Genotype	Length 87 dpb (cm)	Internode length 47 dpb (cm)	Elongation rate (cm/day)	Smalian volume increase (cm³/day)	Mature leaf area (cm²)
G40-2*x*[w]	77.7 ± 4.41**	2.7 ± 0.180	0.85 ± 0.065**	0.19 ± 0.025*	32.664 ± 1.42*
G40-4*x*	46.7 ± 3.70	2.5 ± 0.311	0.50 ± 0.059	0.092 ± 0.022	23.477 ± 2.11
'Gala'-2*x*	89.7 ± 4.16	2.9 ± 0.144	0.96 ± 0.094	0.22 ± 0.00090	25.278 ± 0.928
'Gala'-4*x*	77.3 ± 4.50	2.9 ± 0.181	0.75 ± 0.123	0.20 ± 0.036	23.748 ± 1.30
Genotype[z]	0.0002**	0.291	0.0516	0.0198*	0.0453*
Ploidy	0.0001**	0.807	0.0039**	0.0341*	0.0073**
Genotype*ploidy	0.0579	0.605	0.4023	0.1469	0.0342*

[w]within one genotype one-way ANOVA test (*$P \leq 0.05$; **$P \leq 0.01$; n = 5–6; ± SE); [z]two-way ANOVA effect test (*$P \leq 0.05$; **$P \leq 0.01$)

doi: 10.17221/7/2016-HORTSCI

Table 2. Mean thickness of the different leaf cell layers and mean nuclei density ratio (2*x*/4*x*) of G40 and 'Gala' in di- and tetraploid form with main and interaction effects of genotype and ploidy on cell layer thickness

Genotype-Ploidy	Adaxial epidermis	Palisade parenchyma	Spongy parenchyma	Abaxial epidermis	Total leaf thickness	Nuclei density ratio
	(μm)					
G40-2*x*[w]	28.31 ± 1.20	68.30 ± 5.12	101.57 ± 5.42*	18.84 ± 0.39	214.10 ± 10.71*	2.43 ± 0.34
G40-4*x*	31.17 ± 3.44	86.30 ± 6.06	124.80 ± 5.00	21.27 ± 1.64	256.85 ± 12.92	
'Gala'-2*x*	19.54 ± 0.69**	59.06 ± 1.79*	66.36 ± 0.98*	17.93 ± 0.14*	161.19 ± 0.48*	2.41 ± 0.24
'Gala'-4*x*	27.57 ± 0.64	68.52 ± 2.71	88.13 ± 8.19	19.26 ± 0.47	199.74 ± 11.27	
Genotype[z]	0.0065**	0.0083**	<0.0001**	0.122	0.0001**	–
Ploidy	0.014*	0.0076**	0.0016**	0.0535	0.0017**	–
Genotype*ploidy	0.20	0.34	0.90	0.55	0.839	–

[w]within one genotype one-way ANOVA test (*$P \leq 0.05$; **$P \leq 0.01$; $n = 5$–6; ± SE); [z]two-way ANOVA effect test (*$P \leq 0.05$; ** $P \leq 0.01$; $n = 4$; ± SE)

Leaf parameters. Mature leaf area was significantly affected by the interaction between genotype and ploidy (two-way ANOVA; $P < 0.05$), showing a significant genotype-specific response to the genome doubling (Table 1). Polyploidization led to a 28% decrease in leaf area for G40, whereas no significant decrease was observed in 'Gala'. On average, leaves were 0.040 mm thicker in tetraploid genotypes compared to their diploid progenitor. For G40 and 'Gala' this represented an increase in thickness of 17 and 20%, respectively (Table 2). Both genotype and ploidy had significant effects on thickness of the different cell layers, with exception of the abaxial epidermis (two-way ANOVA; $P < 0.05$). No significant interaction was determined, thus the effect of genotype and ploidy level seemed independent from each other (Table 2). For both genotypes, thickness of the different cell layers increased significantly after polyploidization (one-way ANOVA; $P < 0.05$; Table 2). The adaxial epidermis in G40-4*x* showed a 10% increase compared to its diploid counterpart, while 'Gala'-4*x* increased 41%. The abaxial epidermis showed a comparable increase of 13% and 7% for both G40-4*x* and 'Gala'-4*x*, respectively. For G40, a relatively large increase was observed in both palisade and spongy mesophyll compared to the other layers. For 'Gala' the increase in palisade mesophyll was relatively low, while spongy mesophyll showed a high increase. Both genotypes in their tetraploid form showed a 24% increase in total mesophyll. For both genotypes the density of nuclei in diploid plants was more than double the density observed in tetraploid plants (Table 2).

After *in vitro* acclimatization, tetraploid plants exhibited a clearly visible darker leaf coloration

Fig. 1. Tetraploid (2*n* = 4*x*) (left) and diploid (2*n* = 2*x*) (right) plants of 'Gala' post acclimatization

doi: 10.17221/7/2016-HORTSCI

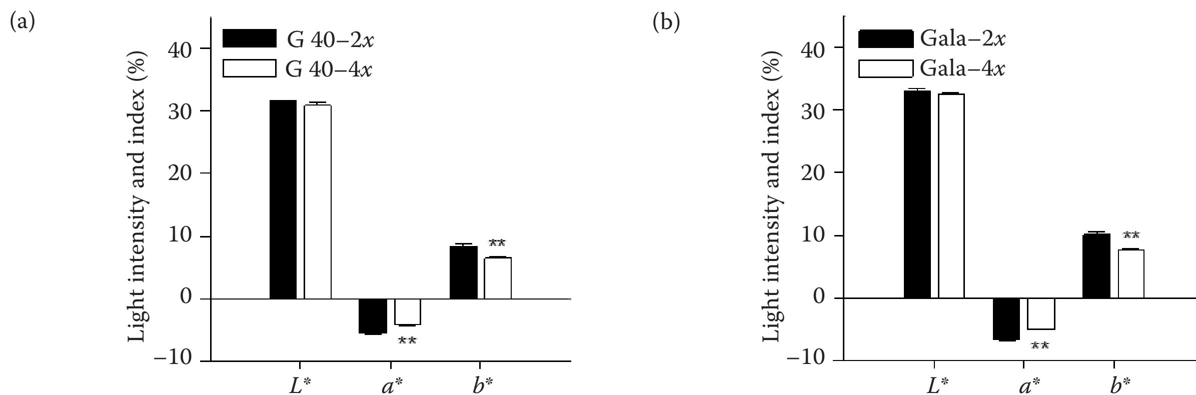

Fig. 2. Mean values of L^* (luminosity), colour component a^* (red-green), b^* (yellow-blue) colour values of G40 (a) and 'Gala' (b) in di- and tetraploid form
$^*P \leq 0.05$; $^{**} P \leq 0.01$; $n = 3$; ± SE

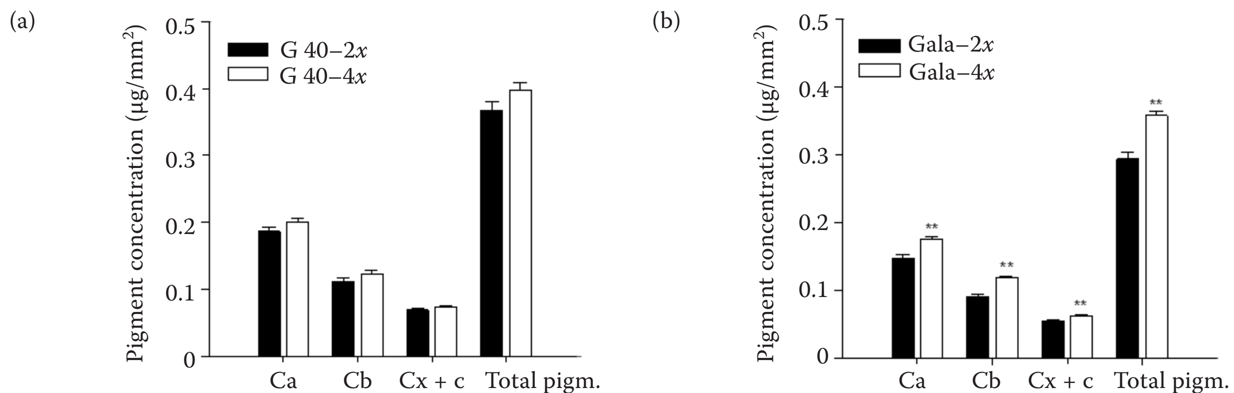

Fig. 3. Chlorophyll a (Ca), chlorophyll b (Cb), carotenoids (Cx + c) and total pigment concentration (Total pigm.) for di- and tetraploid forms of genotype G40 (a) and 'Gala' (b)
$^*P \leq 0.05$; $^{**}P \leq 0.01$; $n = 3$; ± SE

compared to their diploid progenitors (Fig. 1). This colour difference was less visible on older plants, but spectrophotometric analysis still showed a significant difference in the a^* and b^* indices of the $L^*a^*b^*$– colour values in both genotypes (one-way ANOVA; $P < 0.001$; Fig. 2), staying in the negative range of these indices this represents a shift in the horizontal a^*–b^* colour plane from a perceptual light green for the diploid plants to a darker colour for the tetraploid plants for the a^* index and from light yellow to darker yellow for the b^* index.

Also pigment concentration was affected by polyploidization in 'Gala', but not in G40 (Fig. 3; one-way ANOVA; $P < 0.05$). Leaf pigment content was also found to be positively correlated with the a^* index of the colour measurements ($r = 0.72$; $P < 0.0001$; Fig. 4).

With exception of the stomatal coverage (stomatal area/leaf area) all stomatal properties were significantly affected by ploidy but not by genotype (two-way ANOVA; $P < 0.05$). Interaction effects

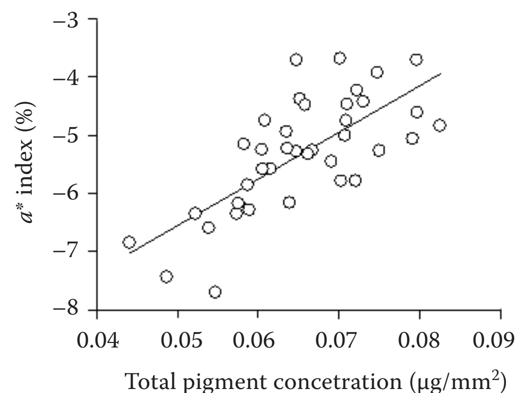

Fig. 4. Relationship between a^* colour component and the total pigment concentration
$r = 0.72$; $P < 0.0001$

doi: 10.17221/7/2016-HORTSCI

Table 3. Stomatal characteristics of both genotypes in di- and tetraploid form with main and interaction effects of genotype and ploidy

Genotype	Density (#/mm²)	Area (μm²)	Length (μm)	Width (μm)	Stomatal area/leaf area (mm²/cm²)
G40-2*x*[w]	226.13 ± 4.85**	176.53 ± 7.17**	20.25 ± 0.43**	11.07 ± 0.22*	4.00 ± 0.26
G40-4*x*	145.94 ± 9.45	358.78 ± 32.84	26.48 ± 0.24	17.21 ± 1.69	5.27 ± 0.78
'Gala'-2*x*	187.75 ± 3.58**	268.08 ± 6.83	21.62 ± 0.46	15.77 ± 0.16	5.03 ± 0.06
'Gala'-4*x*	156.20 ± 2.79	317.06 ± 48.33	23.70 ± 1.62	16.73 ± 1.55	4.98 ± 0.84
Genotype[z]	0.082	0.4248	0.4466	0.1051	0.547
Ploidy	<.0001**	0.0045**	0.001**	0.0152*	0.330
Genotype*ploidy	0.0088**	0.0547	0.046*	0.0558	0.293

[w]within one genotype one-way ANOVA test (*$P \leq 0.05$; **$P \leq 0.01$; $n = 3$; ± SE); [z]two-way ANOVA effect test (*$P \leq 0.05$; **$P \leq 0.01$)

between genotype and ploidy occurred for stomatal density and stomatal length (two-way ANOVA; $p < 0.01$ and $P < 0.05$ respectively; Table 3). The stomatal density for both apple genotypes was significantly lower (one-way ANOVA; $P < 0.01$) in the tetraploids (Table 3). The stomatal area showed a substantial increase in both tetraploids, especially for G40-4x, where the stomatal area doubled (Table 3). Mean stomatal coverage (percentage stomatal area/unit leaf area) was marginally higher for G40-4*x* compared to the G40-2*x* plants, but not significant.

DISCUSSION

In our experiments, a comprehensive comparison of different morphological properties of artificially created tetraploid apple plants to their diploid predecessors was performed. Mitotic chromosome doubling of the diploid genotypes 'Gala' and G40 resulted in clear physio- and morphological alterations.

Similar changes due to polyploidization were reported in other plants, e.g. leaf colour and anatomy in *Citrus* spp. (Allario et al. 2011), leaf anatomy in *Lonicera* (Li et al. 2009), cell anatomy in *Lolium* (Sugiyama 2005) and leaf morphology and colour in *Rosa* (Kermani et al. 2003). The variation in leaf colour in our study could be significantly correlated with the pigment concentration per mm² leaf area. Tetraploid plants of both genotypes expressed a higher pigment content, especially for chlorophyll *a* and *b*. Since these pigments are known to absorb the blue and red light of the spectrum (French et al. 1956), the deeper green colour of the tetraploid plants could be explained at least partially by their higher pigment concentration.

Polyploidy decreased stomatal density and increased guard cell length, width and stomatal area. Stomatal coverage remained similar for both ploidy levels. These observations are in accordance with studies on polyploidy in other plants: e.g. apple (Lespinasse, Noiton 1986; Blanke et al. 1994), *Populus* (Cohen et al. 2010), *Citrus* spp. (Allario et al. 2011) and *Arabidopsis* (Del Pozo, Ramirez-Parra 2014). The increase in guard cell length, decrease in stomatal density and increase in overall cell size proves to be strongly correlated with an increase in genome size; this seems to be a general observation in plants (Beaulieu et al. 2008).

In this study, plant growth was characterized in a non-destructive way by measuring the elongation rates of the main shoot, internode length, mature leaf area and by calculating the stem volume. Both G40-4*x* and 'Gala'-4*x* showed a decrease in elongation rate and stem volume compared to their diploid counterparts, while internode length seemed unaffected. The mature leaf area was also significantly lower in G40-4*x* compared to G40-2*x*, while 'Gala'-4*x* and 'Gala'-2*x* leaves were similar in size. The results suggest that artificial genome doubling of diploid apple genotypes decreased plant vigour. Although polyploidization in plants is often associated with increased vigour, e.g., triploid apples (Sedysheva, Gorbacheva 2013) and hexaploid *Hibiscus* (Van Laere et al. 2006, 2011), artificial genome doubling is very frequently associated with a decreased growth rate, e.g., in *Citrus* (Allario et al. 2011; Guerra et al. 2014), *Spathiphyllum* (Van Laere et al. 2011), *Platanus* (Liu et al.

doi: 10.17221/7/2016-HORTSCI

2007), *Solanum phureja* (Stupar et al. 2007), and tetraploid *Zea mays* (Riddle et al. 2006). Several observations show the increased vigour of triploid apple cultivars (Sedysheva, Gorbacheva 2013) and triploid *Zea mays* (Riddle et al. 2006) over diploids but decreased vigour of higher ploidy levels and own observations of an increased vigour in tetraploid azalea; however, decreased vigour of hexaploids compared to diploids (Eeckhaut et al. 2006) suggests that plant species potentially possess an optimal ploidy degree, below or above which its growth will be reduced. Studies on diploid and polyploid *Arabidopsis thaliana* indeed indicate that larger cell size due to an increase in DNA content (C-value), does not necessarily result in larger plant size (Comai 2005). Polyploidy through nuclear enlargement increases the complexity of different processes involved in managing and partitioning of chromosomes and hence can cause alterations in cell division (Comai 2005). It is also generally assumed that a difference in size over equivalent organs within and among various species is mainly the result of cell density rather than cell volume (Mizukami 2001; Guo et al. 2010; Powell, Lenhard 2012). As polyploidization increases cell size, decreases cell density and probably increases cell cycle length due to the increased C-value, it is not hard to imagine that growth rate is potentially decreased in cells with a normal or low metabolic rate, and final organ size has the same size or is even smaller.

Observations of this study, where internode length and leaf size seemed unaffected, and for the latter even lower in the G40 genotype, could then be explained in part by the two-fold decrease in cell density observed in the tetraploid plants compared to the diploids. The molecular mechanisms behind this altered cell density are not yet completely understood; Mizukami (2001) proposes that a ploidy-dependent gene-dosage associated or epigenetically modified gene expression can result in an altered cell proliferation and hence influencing meristematic competence and cell density.

In addition to the main effects exerted by polyploidization, significant interactions were also measured between polyploidization and genotype, which suggests that the effect of polyploidization on certain morphological characteristics also depends on the genotype. Results from a study performed on different maize inbred lines using monoploid, diploid, triploid and tetraploid derivatives showed that for 6 of 13 measured morphological characters a significant interaction occurred between inbred line and ploidy level (Riddle et al. 2006). Our findings partially confirm those of Riddle et al. (2006) who suggest that observed morphological alterations are mainly due to three factors: a common response to ploidy change, a different genetic background and a response to a different ploidy level dependent on genetic background.

In conclusion, 17 morphological characteristics were measured: 5 stomatal characteristics, 6 cell characteristics, 4 growth characteristics, leaf colour and leaf pigment content in the diploid and tetraploid form of 2 apple genotypes. In total, 15 of these properties were significantly affected by ploidy in at least one genotype. Two characteristics, namely internode length and stomatal coverage, were not affected by ploidy in both genotypes. Ten of the 15 properties were altered in both genotypes, mainly due to the effect of ploidy, while for 5 traits a genotype-specific response to polyploidization was observed. These were mainly involved in growth and stomatal characteristics. Based on our observations and other studies, it is suggested that the frequently observed lower growth rate and smaller or unchanged organ size of tetraploids is caused by a decreased cell density, that could be partially compensated by an increased cell volume. The obtained tetraploid genotypes in this study are valuable material to be used in breeding programs to obtain triploid plants.

Acknowledgements

The authors acknowledge Better3Fruit for offering apple genotypes. Pepijn De Raeymaecker (ILVO) for technical assistance and the technical greenhouse staff of KU Leuven and ILVO are also greatly appreciated. Ewaut Kissel helped with the leaf area analyses and Miriam Levenson improved the language in the manuscript, thank you for that.

References

Achten W.M.J., Maes W.H., Reubens B., Mathijs E., Singh V.P., Verchot L., Muys B. (2010): Biomass production and allocation in *Jatropha curcas* L. seedlings under different levels of drought stress. Biomass and Bioenergy, 34: 667–676.

Allario T., Brumos J., Colmenero-Flores J.M., Iglesias D.J., Pina J.A., Navarro L., Talon M., Ollitrault P., Morillon R. (2013): Tetraploid Rangpur lime rootstock increases

doi: 10.17221/7/2016-HORTSCI

drought tolerance via enhanced constitutive root abscisic acid production. Plant, Cell & Environment, 36: 856–868.

Allario T., Brumos J., Colmenero-Flores J.M., Tadeo F., Froelicher Y., Talon M., Navarro L., Ollitrault P., Morillon R. (2011): Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. Journal of Experimental Botany, 62: 2507–2519.

Beaulieu J.M., Leitch I.J., Patel S., Pendharkar A., Knight C.A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytologist, 179: 975–986.

Blanke M.M., Höfer M., Pring R.J. (1994): Stomata and structure of tetraploid apple leaves cultured in vitro. Annals of Botany, 74: 651–654.

Cohen D., Bogeat-Triboulot M-B., Tisserant E., Balzergue S., Martin-Magniette M-L., Lelandais G., Ningre N., Renou J-P., Tamby J-P., Le Thiec D., Hummel I. (2010): Comparative transcriptomics of drought responses in Populus: a meta-analysis of genome-wide expression profiling in mature leaves and root apices across two genotypes. BMC Genomics, 11: 630.

Comai L. (2005): The advantages and disadvantages of being polyploid. Nature Reviews, Genetics 6: 836–46.

Defra. (2010): Fingerprinting the National Apple & Pear Collections. Department for Environment Food and Rural Affairs – Final Project Report: 1–18. Available at http://randd.defra.gov.uk

Del Pozo J.C., Ramirez-Parra E. (2014): Deciphering the molecular bases for drought tolerance in Arabidopsis autotetraploids. Plant, Cell & Environment, 37: 2722–2737.

Dhooghe E., Grunewald W., Leus L., Van Labeke M.C. (2009): In vitro polyploidisation of Helleborus species. Euphytica, 165: 89–95.

Dobránszki J., Abdul-Kader A., Magyar-Tábori K., Jámbor-Benczúr E., Bubán T., Szalai J., Lazányi J. (2000): Single and dual effects of different cytokinins on shoot multiplication of different apple scions. International Journal of Horticultural Science, 6: 76–78.

Eeckhaut T., Van Huylenbroeck J., De Schepper S., Van Labeke MC. (2006): Breeding for polyploidy in Belgian azalea (*Rhododendron simsii* Hybrids). Acta Horticulturae (ISHS), 714: 113–118.

French C.S., Smith J.H., Virgin H.I., Airth R.L. (1956): Fluorescence-spectrum curves of chlorophylls, pheophytins, phycoerythrins, phycocyanins and hypericin. Plant Physiology, 31: 369–374.

Guerra D., Teresa M., Wittmann S., Schwarz S.F., Vitor P., De Souza D., Gonzatto M.P., Weiler R.L. (2014): Comparison between diploid and tetraploid citrus rootstocks: morphological characterization and growth evaluation. Bragantia, 73: 1–7.

Guo M., Rupe M.A., Dieter J.A., Zou J., Spielbauer D., Duncan K.E., Howard R.J., Hou Z., Simmons C.R. (2010): Cell Number Regulator1 affects plant and organ size in maize : implications for crop yield enhancement and heterosis. The Plant Cell, 22: 1057–1073.

Hao G.Y., Lucero M.E., Sanderson S.C., Zacharias E.H., Holbrook N.M. (2013): Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). New Phytologist, 197: 970–978.

Jones H.G. (2013): Plants and Microclimate: a Quantitative Approach to Environmental Plant Physiology. 3rd Ed. Cambridge: Cambridge University press: 129–130.

Kermani M.J., Sarasan V., Roberts A.V., Yokoya K., Wentworth J., Sieber V.K. (2003): Oryzalin-induced chromosome doubling in Rosa and its effect on plant morphology and pollen viability. Theoretical and Applied Genetics, 107: 1195–1200.

Lespinasse Y, Noiton D. (1986): Contribution á l'étude d'une plante haploïde de pommier (*Malus pumila* Mill.). Etude descriptive et comparaison avec des clones de ploïdie différente. I. – Caractères végétatifs : entre- noeuds, feuilles et stomates. (Contribution to the study of a haploid apple (*Malus pumila*). Descriptive study and comparison with clones of different levels of ploidy. L - Vegetative characters: internodes, leaves and stomata). Agronomie, 6: 659–664.

Li W.D., Biswas D.K., Xu H., Xu C.Q., Wang X.Z., Liu J.K., Jiang G.M. (2009): Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. Functional Plant Biology, 36: 783–792.

Liu G., Li Z., Bao M. (2007): Colchicine-induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. Euphytica, 157: 145–154.

Mizukami Y. (2001): A matter of size: Developmental control of organ size in plants. Current Opinion in Plant Biology, 4: 533–539.

Pereira-Lorenzo S., Ramos-Cabrer A.M., Díaz-Hernández M.B. (2006): Evaluation of genetic identity and variation of local apple cultivars (*Malus × domestica* Borkh.) from Spain using microsatellite markers. Genetic Resources and Crop Evolution, 54: 405–420.

Powell A.E., Lenhard M. (2012): Control of organ size in plants. Current Biology, 22: 360–367.

Riddle N.C., Kato A., Birchler J.A. (2006): Genetic variation for the response to ploidy change in *Zea mays* L. Theoretical and Applied Genetics, 114: 101–111.

Sedov E.N., Sedysheva G.A., Serova Z.M., Gorbacheva N.G. (2014): Autogamy of polyploid apple varieties and forms. Russian Agricultural Sciences, 40: 253–256.

Sedysheva G.A., Gorbacheva N.G. (2013): Estimation of new tetraploid apple forms as donors of diploid gametes for

doi: 10.17221/7/2016-HORTSCI

selection on a polyploidy level. Universal Journal of Plant Science, 1: 49–54.

Stupar R.M., Bhaskar P.B., Yandell B.S., Rensink W.A., Hart A.L., Ouyang S., Veilleux R.E., Busse J.S., Erhardt R.J., Buell C.R., Jiang J. (2007): Phenotypic and transcriptomic changes associated with potato autopolyploidization. Genetics, 176: 2055–2067.

Sugiyama S. (2005): Polyploidy and cellular mechanisms changing leaf size: Comparison of diploid and autotetraploid populations in two species of Lolium. Annals of Botany, 96: 931–938.

Sun Q., Sun H., Li L., Bell R.L. (2009): *In vitro* colchicine-induced polyploid plantlet production and regeneration from leaf explants of the diploid pear (*Pyrus communis* L.) cultivar, 'Fertility'. Journal of Horticultural Science & Biotechnology, 84: 548–552.

Tan F.Q., Tu H., Liang W.J, Long J.M., Wu X.M., Zhang H.Y., Guo W.W. (2015): Comparative metabolic and transcriptional analysis of a doubled diploid and its diploid citrus rootstock (*C. junos* cv. Ziyang xiangcheng) suggests its potential value for stress resistance improvement. BMC Plant Biology, 15: 1–14.

Trojak-goluch A., Skomra U. (2013): Artificially induced polyploidization in *Humulus lupulus* L. and its effect on morphological and chemical traits. Breeding Science, 63: 393–399.

Vaarama A. (1946): Meiosis and polyploid characters in the tetraploid apple variety hibernal. Hereditas, 34: 147–160.

Van Laere K., Van Huylenbroeck J., Van Bockstaele E. (2006): Breeding strategies to increase genetic variability within Hibiscus syriacus. Proceedings of the 22nd International Eucarpia Symposium Section Ornamentals: Breeding for Beauty: 75–81.

Van Laere K., França S.C., Vansteenkiste H., Van Huylenbroeck J., Steppe K., Van Labeke MC. (2011): Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. Acta Physiologiae Plantarum, 33: 1149–1156.

Wellburn A.R. (1994): The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology, 144: 307–313.

Xue H., Zhang F., Zhang Z-H., Fu J-F., Wang F., Zhang B., Ma Y. (2015): Differences in salt tolerance between diploid and autotetraploid apple seedlings exposed to salt stress. Scientia Horticulturae, 190: 24–30.

Received for publication January 15, 2016
Accepted after corrections June 10, 2016