Biologia (2021) 76:2079–2092
https://doi.org/10.1007/s11756-021-00704-1

ORIGINAL ARTICLE

Evaluation of endopolyploidy patterns in selected *Capsicum* and *Nicotiana* species (*Solanaceae*)

Viera Fráková[1] · Lukáš Koprivý[1,2] · Marianna Paľová[1] · Vladislav Kolarčik[1] · Pavol Mártonfi[1,2]

Received: 30 March 2020 / Accepted: 1 February 2021 / Published online: 2 March 2021

Abstract

Endopolyploidy has arisen countless times in angiosperms, and endopolyploidisation is an important genetic feature in many plant species. It is generated through a process called endoreduplication, where a mitotic cell cycle shifts into an endocycle, and DNA replication occurs without chromosome separation and cytokinesis. It has been well established that endopolyploidy plays a vital role during plant growth and development and in various stress responses. Many agriculturally important plant families are polysomatic, including the *Solanaceae*. To better understand and characterise polysomatic species within the *Solanaceae*, we studied endopolyploidy in mature vegetative and reproductive organs (root, stem, lower leaf petiole, lower leaf lamina, flower pedicel, calyx, corolla, pistil and stamen tissue) of representative diploids within the genus *Capsicum*, i.e. *C. annuum*, *C. baccatum*, *C. chinense* and diploid and tetraploids within the genus *Nicotiana*, i.e. *N. rustica* (4x), *N. sylvestris* (2x) and *N. tabacum* (4x), by means of flow cytometry. The presence of 2C–16C nuclei (rarely 32C) was detected, and the degree of endopolyploidisation was expressed using four different parameters for each organ analysed. In vegetative organs, the endoreduplication index (EI) reached a maximum of 0.84 on average for roots of *C. baccatum*, whereas the lowest values (EI < 0.10) were detected in the leaf lamina of the same species. Among the reproductive organs investigated in *N. tabacum*, EI values for pistils were higher than for stamens. When the diploid and polyploid *Nicotiana* species were compared, diploid *N. sylvestris* possessed a higher endopolyploidy level than the polyploids *N. rustica* and *N. tabacum*. In this study, we also determined genome size for each of the investigated species, which ranged from 5.51 picograms (pg) in *N. sylvestris* to 10.43 pg in *N. rustica*.

Keywords Endoreduplication · Flow cytometry · Genome size · Hot pepper · Polysomaty · Tobacco

Introduction

Ploidy level, the number of chromosome sets in a cell nucleus, is typically identified as an organism-level trait. In a diploid organism, there are two chromosome sets in a cell nucleus. However, polyploidy, with more than two chromosome sets in a nucleus, is relatively common or even prevalent in some plant lineages (Husband et al. 2013; Van de Peer et al. 2017). However, ploidy level may vary even within the same individual, e.g. most of the cells of the organism are diploid, but some tissues or group of cells may contain higher numbers of chromosome sets. This state of mixed ploidy tissues is known as endopolyploidy or somatic polyploidy, and it is a result of repeated genome multiplication without cytokinesis (D'Amato 1964; Nagl 1976; Smulders et al. 1994).

Endopolyploidy is generated through various processes, which include endomitosis (prevalent in animals), endoreduplication (dominant in plants) and progressively partial endoreplication (D'Amato 1989; Joubès and Chevalier 2000; Frawley and Orr-Weaver 2015; Trávníček et al. 2015; Leitch and Dodsworth 2017). During endomitosis, mitosis occurs with the absence of cytokinesis. The results of repeating endomitoses are multilobulated nuclei in cells or multinucleated cells (Abraham et al. 1965; Hasinoff et al. 2000; Frawley and Orr-Weaver 2015; Von Stetina et al. 2018; Kobayashi 2019). Opposite of endomitosis, endoreduplication

✉ Vladislav Kolarčik
vladislav.kolarcik@upjs.sk

[1] Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Mánesova 23, SK-041 54 Košice, Slovak Republic

[2] Botanical Garden, P. J. Šafárik University, Mánesova 23, SK-04352 Košice, Slovak Republic

occurs when a mitotic cell cycle shifts into an endocycle. DNA replication occurs without chromosome separation and cytokinesis, which leads to the formation of polytene chromosomes (Leitch and Dodsworth 2017; Kobayashi 2019). In progressively partial endoreplication, only a part of the genome is replicated during the synthetic S phase; however, this mechanism has only so far been documented in the *Orchidaceae* (Trávníček et al. 2015).

Whole organism endopolyploidy level (EL) varies among plant species and plant lineages, and while endopolyploidy may be absent in some plants, e.g. gymnosperms, it can be prevalent in others, e.g. bryophytes and angiosperms (Barow and Meister 2003; Bainard and Newmaster 2010; Bainard et al. 2011a; Bainard et al. 2012). Some estimates indicate that 90 % of angiosperms may be polysomatic, but the occurrence of polysomaty is limited to within certain groups or families (Nagl 1976; Barow and Meister 2003). Some plant families, e.g. the *Brassicaceae* or *Fabaceae*, are polysomatic, whereas others only exhibit polysomaty rarely, e.g. the *Asteraceae* (Joubès and Chevalier 2000; Larkins et al. 2001; Barow and Meister 2003; Jovtchev et al. 2007; Bainard et al. 2012). Endopolyploidy occurs irrespective of organism ploidy level and is present in both diploids and polyploids; however, it is not absolutely independent of organism cytotype, and it commonly occurs less frequently in polyploids than in diploids (Mishiba and Mii 2000; Jovtchev et al. 2007; Pacey et al. 2020a).

Determining how closely associated endopolyploidy is with physiological processes of plant growth and development remains an emerging field as technology improves. What has been thoroughly established is that cells of higher ploidy levels contain nuclei with higher DNA content. Furthermore, due to the physical constraint on minimum nuclei size, endopolyploidy is strongly correlated with nuclear and cellular size and with organ size in some plants (Melaragno et al. 1993; Cookson et al. 2006; Agulló-Antón et al. 2013; Kladnik 2015; Robinson et al. 2018). Increased cell size and gene expression are components related to increased growth, and endopolyploidy commonly arises in developmentally and metabolically active cells and tissues, such as vascular tissue, nutritive tissue, cotyledons and pericarp (Barow and Meister 2003; Kladnik et al. 2006; Bourdon et al. 2012; Sabelli et al. 2013; Bhosale et al. 2018; Kobayashi 2019), and in highly specialised cells, such as trichomes, antipodal cells, leaf bladder cells and tapetum (Melaragno et al. 1993; Weiss and Maluszynska 2001; Bartoli et al. 2017; Barkla et al. 2018). Conversely, endopolyploidy is an obstacle for the proper function of some specialised cells and is not commonly reported in meristematic, guard or gametic cells (Melaragno et al. 1993; Trávníček et al. 2015; Skaptsov et al. 2017).

An increase in the frequency of endopolyploidy has been documented as being associated with various biological processes and stress responses, but not necessarily related to development. Plant stress responses to UV-B or gamma irradiation can trigger a switch into an endocycle (Gegas et al. 2014; Zedek et al. 2016). Stress-associated endopolyploidy can also occur as part of a response to increased salinity (Ceccarelli et al. 2006; Bennici et al. 2008), whereas opposite effects have been observed in water deficiency experiments (Artlip et al. 1995; Cookson et al. 2006). Plant infection by nematodes can cause the formation of highly endopolyploid cells, where such cells serve as source for nematode nourishment (de Almeida Engler and Gheysen 2013; Smant et al. 2018). Plant root colonisation by arbuscular mycorrhizal fungi can also lead to the enhancement of EL (Lingua et al. 2001; Bainard et al. 2011b).

Generally, our knowledge of the functional roles of endopolyploidy is limited and has been mainly acquired from model polysomatic plants such as *Arabidopsis thaliana* (L.) Heynh., *Solanum lycopersicum* L. and *Zea mays* L. (Chevalier et al. 2014; Li et al. 2019; Musseau et al. 2020; Pacey et al. 2020a). Therefore, studies of different plants may reveal many new functions and roles of endopolyploidy, e.g. in *Mesembryanthemum crystallinum* L. (Barkla et al. 2018). Basic broad searches for endopolyploidy presence in various non-model plant groups are not common (e.g. Barow and Meister 2003; Bainard et al. 2012). Additionally, efforts to perform these studies are highly valuable, because EL may also be an important organismal feature in addition to the number of chromosomes or ploidy level.

Previous studies on endopolyploidy in solanaceous crops (Barow and Meister 2003; Galbraith 2014) revealed that *Nicotiana tabacum* L. and *Capsicum annuum* L. show polysomaty in some of their organs. According to these studies, petal, sepal, stamen, carpel, pedicel, upper leaf, lower leaf and cotyledon tissues of *N. tabacum* showed low and moderate EI (0.11 to 0.72). Furthermore, the fruit pericarp of *C. annuum* showed incredibly high levels (presence of up to 256 C cells) of endopolyploidisation in their cells, while the leaves of *C. annuum* were not polysomatic.

In this study, we investigated endopolyploidy within the *Solanaceae*, primarily focusing on *N. tabacum* and *C. annuum.* Moreover, we investigated EL in seven vegetative and two reproductive organs, most of which had not been previously analysed for *C. annuum*. We also studied four other taxa, *C. baccatum* L., *C. chinense* Jacq., *N. rustica* L. and *N. sylvestris* Speg., which have not had a survey for the presence of endopolyploidy published to date. Our results confirm that all of these investigated species are polysomatic. In addition, we have found that the EL is comparable among them and documented the association between EL and organism ploidy level in *Nicotiana*.

Materials and methods

Plant material

Plants used in the flow cytometry analyses (FCM) were cultivated from commercially available seeds (www.

semenaonline.sk) of *Capsicum annuum* 'Citrina' (diploid, 2n = 24; Rice et al. 2015), *C. baccatum* 'Aji' (diploid, 2n = 24; Rice et al. 2015), *C. chinense* 'Dorset Naga' (diploid, 2n = 24; Rice et al. 2015), *Nicotiana rustica* (tetraploid, 2n = 48; Rice et al. 2015), *N. sylvestris* (diploid, 2n = 24; Rice et al. 2015) and *N. tabacum* (tetraploid, 2n = 48; Rice et al. 2015). Plants were sown in 15 cm pots with a common horticultural substrate and grown in the laboratory under the controlled conditions of 20°C, 60 % humidity and 12 h photoperiod cycle (12 h light/12 h darkness).

Analyses of endopolyploidy were performed on fully developed, mature organs of 2-month-old plants. Since various species required different conditions to reach maturity, not all species were investigated at anthesis. Individuals of *N. sylvestris* only reached the rosette stage, and they showed delayed development. Delays in development also occurred with some individuals of *C. baccatum.* Since not all tissue types were available for all investigated species, analyses were performed on three to nine organ tissues of each of the six investigated species (Table 1). The investigated organ tissues included root, stem, lower leaf petiole, lower leaf lamina, flower pedicel, calyx, corolla (tube and limb analysed separately in *N. tabacum*), pistil and stamen tissue.

Genome size analyses

The samples for genome size analyses were prepared from leaf tissue using a two-step procedure, which consisted of nuclear isolation and staining steps using propidium iodide as a DNA intercalator (Doležel and Göhde 1995; Loureiro et al. 2007). The internal standardisation method was used (Doležel et al. 2007) with the internal reference standards *Zea mays* 'CE-777' (2C = 5.43 pg; Lysák and Doležel 1998) for *Capsicum* L. species and *Solanum pseudocapsicum* L. (2C = 2.59 pg; Temsch et al. 2010) for *Nicotiana* L. species to minimise distortion in the data measurements.

Approximately 0.75 cm^2 of young leaf tissue of each sample and the standard were chopped together with a razor blade in a Petri dish in 1 mL of ice-cold general-purpose buffer (GPB) [0.5 mM spermine × 4 HCl, 30 mM sodium citrate, 20 mM MOPS (4-morpholine propane sulfonate), 80 mM KCl, 20 mM NaCl, 0.5 % (v/v) Triton X-100, pH 7.0] (Loureiro et al. 2007) supplemented with 3 % PVP (polyvinylpyrrolidone). Each suspension was then filtered through 42 μm nylon filter, and each sample was subsequently incubated for approximately 0.5 h with β-mercaptoethanol (final concentration 2 μL/mL), RNase (30 μg/mL) and propidium iodide (30 μg/mL). Three individuals from each of the six species were measured three times on three different days for a total of nine measurements per species (Greilhuber and Obermayer 1997), except for the *N. tabacum* accession, where only two replicates of sufficient quality [coefficients of variation (CV) < 5.5 %] were measured.

Measurements for genome size were performed using a Partec CyFlow ML (Partec Gmbh, Münster, Germany) flow cytometer, which is housed at the Institute of Biological and Ecological Sciences, P. J. Šafárik University in Košice (Slovakia). The flow cytometry machine is equipped with a 532 nm (150 mW) green laser and 590 nm band-pass optical filter. FloMax ver. 2.70 software (Partec Gmbh, Münster, Germany) was used for flow cytometry analyses and final evaluation of all measurements. Histograms of the data were displayed on a linear scale (x-axis). At least 5 000 nuclei per measurement were collected, and the CV of the G_0/G_1 peaks of both the samples and the internal standards did not exceed 5.5 %. The estimation of the quantity of DNA in each sample was based on the value of the G_0/G_1 peak means calculated using the following equation from Doležel and Bartoš (2005):

$$\text{Amount of sample DNA} = \text{Amount standard DNA used} \times \left[\frac{(\text{sample } G_0/G_1 \text{ peak mean})}{(\text{standard } G_0/G_1 \text{ peak mean})}\right]$$

Determining endopolyploidy using flow cytometry

Flow cytometry techniques were used to determine the nuclei frequency of ploidy classes, such as 2C, 4C, 8C, etc. The protocol for FCM preparation was used as described by Kocová et al. (2017). In brief, we applied a chopping technique using a razor blade to release nuclei from a given organ into 1 mL of ice-cold GPB + 3 % PVP. Nuclei filtering, staining and FCM measurements followed as described above for genome size determination. Log-scaled fluorescence intensity histograms were inspected for the symmetry of peaks and proportion of background debris. Flow cytometry records with skewed peaks or high amounts of background debris were excluded from final analyses.

Evaluation of endopolyploidy level

Endopolyploidy level was assessed through various approaches. The number of nuclei for each ploidy level class (2C, 4C, 8C, etc.) was recorded, and four different indices were calculated based on the recorded number of nuclei of each ploidy level class. Mean C value (Lemontey et al. 2000) also referred to as mean C-level (Jovtchev et al. 2007) represents the mean ploidy of cells of a tissue and is calculated according to the formula:

$$\text{MCV} = \frac{[(2 \times \text{n2C}) + (4 \times \text{n4C}) + (8 \times \text{n8C}) + (16 \times \text{n16C})]}{(\text{n2C} + \text{n4C} + \text{n8C} + \text{n16C})}$$

Table 1 Survey of endopolyploidy level (EL) in species and organs investigated in this study

Organ	No	Nuclei ploidy classes					Endocycles	Endopolyploidy parameters			
		2C [%]	4C [%]	8C [%]	16C [%]	32C [%]	N_{max}	EI	MCV	E4P	≥4C [%]
C. annuum											
root	7	41.07±6.60	54.04±6.98	4.87±2.94	0.02±0.06	-	3	0.64±0.08	3.38±0.19	4.34±0.20	58.93±6.60
stem	7	72.19±6.95	27.03±6.74	0.78±0.48	-	-	2	0.29±0.07	2.59±0.15	4.11±0.07	27.81±6.95
petiole	7	65.26±11.05	31.45±8.61	3.25±2.69	0.05±0.13	-	3	0.38±0.14	2.83±0.33	4.34±0.21	34.74±11.05
lamina	7	93.40±3.66	6.54±3.68	0.06±0.15	-	-	2	0.07±0.04	2.13±0.07	4.04±0.11	6.60±3.66
peduncle	7	75.72±4.73	23.60±4.60	0.67±0.38	-	-	2	0.25±0.05	2.51±0.10	4.11±0.07	24.28±4.73
calyx	7	75.39±2.83	23.10±2.55	1.51±0.67	-	-	2	0.26±0.03	2.55±0.07	4.24±0.09	24.61±2.83
corolla	7	94.63±1.35	5.37±1.35	-	-	-	0	0.05±0.01	2.11±0.03	4.00±0.00	5.37±1.35
C. baccatum											
root	6	21.64±5.43	72.49±4.76	5.87±1.99	-	-	2	0.84±0.07	3.80±0.16	4.30±0.09	78.36±5.43
stem	6	76.67±6.25	22.75±5.81	0.58±0.62	-	-	2	0.24±0.07	2.49±0.14	4.09±0.08	23.33±6.25
petiole	6	49.63±2.86	46.95±2.12	3.41±0.91	-	-	2	0.54±0.04	3.14±0.09	4.27±0.06	50.37±2.86
lamina	6	94.33±1.18	5.67±1.18	-	-	-	0	0.06±0.01	2.11±0.02	4.00±0.00	5.67±1.18
C. chinense											
root	7	39.53±8.79	53.69±8.97	6.76±0.58	0.01±0.03	-	3	0.67±0.09	3.48±0.17	4.46±0.08	60.47±8.79
stem	7	70.37±3.18	28.42±3.01	1.21±0.74	-	-	2	0.31±0.04	2.64±0.08	4.16±0.09	29.63±3.18
petiole	7	70.26±11.55	27.05±11.77	2.69±1.75	-	-	2	0.32±0.12	2.70±0.24	4.52±0.68	29.74±11.55
lamina	7	92.85±3.04	7.05±2.93	0.09±0.25	-	-	2	0.07±0.03	2.15±0.07	4.04±0.10	7.15±3.04
peduncle	2	62.42	35.80	1.78	-	-	2	0.39	2.82	4.19	37.58
calyx	2	71.06	26.55	2.39	-	-	2	0.31	2.67	4.32	28.94
corolla	2	89.04	10.96	-	-	-	1	0.11	2.22	4.00	10.96
N. rustica											
root	7	41.22±4.74	56.32±4.72	2.46±1.42	-	-	2	0.61±0.05	3.27±0.12	4.17±0.09	58.78±4.74
stem	7	66.20±3.82	32.49±3.81	1.31±0.62	-	-	2	0.35±0.04	2.73±0.08	4.16±0.07	33.80±3.82
petiole	7	64.79±11.48	32.34±8.64	2.87±4.68	-	-	2	0.38±0.15	2.82±0.39	4.26±0.33	35.21±11.48
lamina	7	91.01±5.21	8.74±5.21	0.25±0.45	-	-	2	0.09±0.05	2.19±0.11	4.11±0.20	8.99±5.21
peduncle	7	70.85±5.96	27.54±6.03	1.50±0.47	0.11±0.20	-	3	0.31±0.06	2.66±0.11	4.27±0.14	29.15±5.96
calyx	7	71.10±5.04	25.65±4.42	2.41±0.57	0.78±0.21	0.07±0.11	4	0.33±0.06	2.79±0.16	4.71±0.16	28.90±5.04
corolla	7	88.61±2.50	11.39±2.50	-	-	-	1	0.11±0.03	2.23±0.05	4.00±0.00	11.39±2.50
N. sylvestris											
root	6	56.15±9.64	40.74±8.57	3.11±1.63	-	-	2	0.47±0.11	3.00±0.24	4.27±0.15	43.85±9.64
petiole	6	46.03±12.75	39.57±7.66	12.34±3.76	2.07±2.33	-	3	0.70±0.21	3.82±0.66	5.29±0.50	53.97±12.75
lamina	6	54.97±11.50	41.23±11.47	3.72±1.51	0.08±0.13	-	3	0.49±0.12	3.06±0.25	4.37±0.20	45.03±11.50
N. tabacum											
root	6	44.69±5.56	53.32±5.55	2.00±0.21	-	-	2	0.57±0.06	3.19±0.11	4.15±0.02	55.31±5.56
stem	6	67.41±7.37	31.88±6.79	0.72±0.68	-	-	2	0.33±0.08	2.68±0.17	4.08±0.07	32.59±7.37
petiole	6	60.43±6.10	25.58±3.26	13.01±3.94	0.99±1.42	-	3	0.55±0.12	3.43±0.40	5.56±0.51	39.57±6.10
lamina	6	85.42±8.89	13.70±8.50	0.88±0.47	-	-	2	0.15±0.09	2.33±0.19	4.27±0.11	14.58±8.89
peduncle	4	53.28±4.78	45.56±4.65	1.16±0.26	-	-	2	0.48±0.05	2.98±0.10	4.10±0.02	46.72±4.78
calyx	4	81.18±8.20	18.43±7.78	0.39±0.46	-	-	2	0.19±0.09	2.39±0.18	4.06±0.07	18.82±8.20
corolla tube	4	60.86±0.49	39.05±0.52	0.09±0.18	-	-	2	0.39±0.01	2.79±0.01	4.01±0.02	39.14±0.49
corolla limb	4	78.26±7.51	21.58±7.53	0.16±0.32	-	-	2	0.22±0.08	2.44±0.15	4.03±0.06	21.74±7.51
pistil	4	73.81±4.33	25.64±4.35	0.54±0.63	-	-	2	0.27±0.04	2.55±0.09	4.08±0.10	26.19±4.33
stamen[a]	4	37.25±5.88	62.24±5.34	0.51±0.60	-	-	2	0.63±0.06	3.28±0.14	4.03±0.04	62.75±5.88

a – endopolyploidy level for stamen tissue may be underestimated, because many of 2C nuclei may be nuclei of pollen generative cells arrested in the G_2 phase of cell cycle

Number of samples (No) and mean ± error estimate (standard deviation) are given for percentage of nuclei ploidy classes 2C–32C and four endopolyploidy parameters: endoreduplication index (EI), mean C value (MCV), mean ploidy of endoreduplicated nuclei (E4P) and estimate of initiation of the endocycle (≥4C). Maximal number of endocycles (N_{max}) in an plant organ is given

where n2C, n4C, n8C… are nuclei counts of corresponding ploidy level classes (2C, 4C, 8C…).

The endoreduplication index (Bainard et al. 2012) or cycle value (Barow and Meister 2003) indicates the mean number of endoreduplication cycles per nucleus of the cells and follows the equation:

$$EI = \frac{[(0 \times n2C) + (1 \times n4C) + (2 \times n8C) + (3 \times n16C)]}{(n2C + n4C + n8C + n16C)}$$

The parameter E4P is calculated according to the original parameter E6P proposed by Dilkes et al. (2002) for

endospermal tissue and represents mean ploidy of endoreduplicated cells of a tissue using the equation:

$$E4P = \frac{[(4 \times n4C) + (8 \times n8C) + (16 \times n16C)]}{(n4C + n8C + n16C)}$$

The proportion of cells that underwent at least one endocycle is simply the proportion of cells with > 2C level (given as a percentage). Again, it is a modification of the original parameter %E (proportion of > 3C cells) proposed by Dilkes et al. (2002) for endosperm using the equation:

$$\geq 4C = 100 \times \frac{(n4C + n8C + n16C)}{(n2C + n4C + n8C + n16C)}$$

In addition, the maximal number of endocycles in a sample that occurred during endopolyploidisation, as inferred from FCM histograms, is reported. In this study, when at least three peaks were recorded on a histogram, the maximal number of endocycles corresponded to n–1, where n is the maximal recorded number of peaks. However, when only two peaks of 2C and 4C nuclei were present, one endocycle was registered only if 4C nuclei exceeded 10 % in an organ that was fully developed, and mitotic activity was excluded or at minimal level.

Principal component analysis (PCA, performed as described in Kocová et al. 2017) and exploratory data analysis were performed to get an overview of the variation in ploidy classes and endopolyploidy parameters (mean and standard deviation are presented). Analysis of numerical variance (ANOVA) was used to test for significant differences between means of different species. Correlation analysis (Pearson correlation coefficient) was applied to test for relationships among various endopolyploidy indices and ploidy classes and between our data and published data (Barow and Meister 2003) for *N. tabacum*. Data analyses and graphs were produced using packages *gplots* ver. 3.0.1.1 (Warnes et al. 2019), *ggplot2* ver. 2.2.1 (Wickham 2009), *PerformanceAnalytics* ver. 1.5.3 (Peterson and Carl 2019) and *vegan* ver. 2.5-4 (Oksanen et al. 2019) in R ver. 3.5.3 environment (R Core Team 2019).

Results

Genome size differences among the six species were statistically significant (ANOVA, $F_{5, 17} = 2764$, $p < 0.001$) and separated into five different groups, where *C. annuum* and *C. chinense* had an almost identical genome size and formed one group (Fig. 1). Genome size varied among *Capsicum* species from 6.99 pg in *C. chinense* to 7.41 in *C. baccatum* and among *Nicotiana* species from 5.51 pg in *N. sylvestris* to 10.43 pg in *N. rustica*.

The presence of 2C – 16C nuclei (Figs. 2a and 3a; Table 1) were measured using flow cytometry in all the species investigated irrespective of genus assignment. Furthermore, 32C nuclei were recorded in two samples of calyces of *N. rustica* (0.19 and 0.27 % of 32C nuclei). 2C nuclei most frequently occurred in leaf lamina and corolla tissue (94.63 % on average in *C. annuum* corolla tissue) and less frequently in root tissues (21.64 % in *C. baccatum* root) and specifically the stamen tissue of *N. tabacum* (37.25 %). 2C nuclei frequency had a strong negative correlation with 4C nuclei frequency. This leads to observations such as 4C nuclei most frequently occurring in root tissues (72.49 % in *C. baccatum* root) and specifically the stamen tissue of *N. tabacum* and less frequently in corolla or leaf lamina tissues (5.37 % in *C. annuum* corolla tissue). Other ploidy classes were observed less frequently. Moreover, 8C was the highest in petiole tissue of *N. tabacum* (13.01 % on average), whereas 16C nuclei in petiole tissue of *N. sylvestris* were only 2.07 % on average.

Parameters EI, MCV and ≥4C were highly correlated ($r > 0.90$), which indicates that they likely reflect similar features of endopolyploidy. However, E4P was less correlated to the other three parameters ($r = 0.31$, 0.44 and 0.56, respectively). Further direct comparison of EI vs. E4P using biplot analysis allowed for the identification of a group of samples that differed in either EI or E4P. For instance, the E4P parameter emphasises number of endoreduplicated nuclei, also known as mean ploidy of endoreduplicated nuclei (Dilkes et al. 2002), and was able to differentiate between highly similar standard EI parameter samples, e.g. the petiole tissue of *N. sylvestris* from root tissue of *C. annuum* (Fig. 4).

The standard parameter EI, which is preferred in comparative endopolyploidy studies, always reached < 1.0, e.g. a maximum of 0.96 for leaf petiole tissue of *N. sylvestris* and 0.84 on average for *C. baccatum* root (Table 1). The lowest values (EI < 0.10), which were commonly found in non-polysomatic organs such as the leaf lamina and corolla of *Capsicum* species with some exceptions (Figs. 2a and 3a).

Root, leaf petiole and leaf lamina vegetative organs were available for all the studied species and allowed for analyses to at least partially differentiate the species (Fig. 2). Statistically significant differences (at least $p < 0.05$) in EI values were identified among species for all investigated vegetative organs, including sterile floral organs (Figs. 2a and 3a); root (ANOVA, $F_{5, 38} = 15.78$, $p < 0.001$), stem (ANOVA, $F_{4, 32} = 3.31$, $p < 0.05$), leaf petiole (ANOVA, $F_{5, 38} = 6.94$, $p < 0.001$), leaf lamina (ANOVA, $F_{5, 38} = 39.15$, $p < 0.001$), pedicel (ANOVA, $F_{2, 17} = 24.56$, $p < 0.001$), calyx (ANOVA, $F_{2, 17} = 7.59$, $p < 0.01$) and corolla (ANOVA, $F_{3, 21} = 89.01$, $p < 0.001$). In corolla tissue, we determined that there was EI variability for both the corolla tube and limb in *N. tabacum* (Fig. 3a and 3c). Interestingly, there was no consistent pattern in EI values for specific organs; however, *C. baccatum* and *N. sylvestris* were clearly different from their congeners (Fig. 2b), as revealed in PCA analysis, which retrieved first two PC axes accounting together

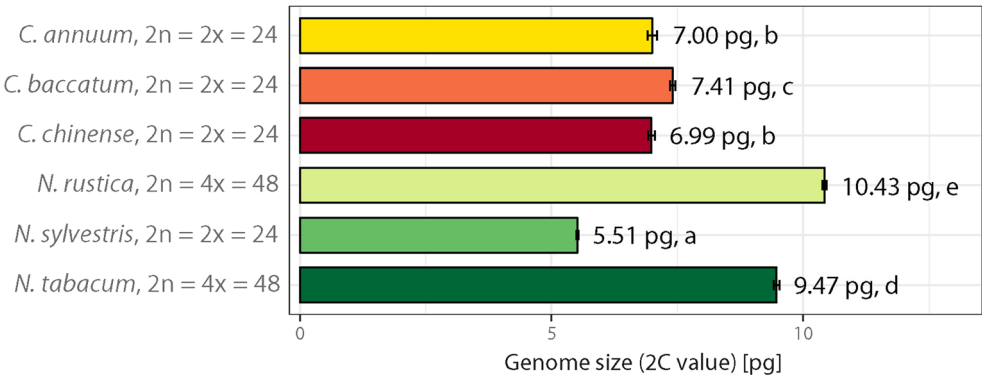

Fig. 1 Genome size variation (2C value) for investigated species of *Capsicum* and *Nicotiana*

for almost 89 % of the total variation. A significant difference was also found between two fertile floral organs of *N. tabacum*, the stamen and pistil (t-test, $t = -9.37$, $df = 5.31$, $p < 0.001$, Fig. 3b). Unfortunately, reproductive organs were unavailable for some species, limiting the extent of the conclusions that can be made with respect to species similarity in reproductive organs.

In comparison to other studies, correlation analysis revealed that there was a strong association between our data and data obtained by Barow and Meister (2003) for *N. tabacum* ($r = 0.97$, $p < 0.01$, $n = 6$), which increases our confidence in these findings and the conclusions drawn from the present data.

Discussion

Endopolyploidy is widespread among angiosperms (D'Amato 1984; Barow and Meister 2003; Bainard et al. 2012); it plays a prominent role in developmental processes (Kudo and Kimura 2001; Barow and Meister 2003; Kocová et al. 2014; Rewers and Sliwinska 2014; Straková et al. 2014; Skaptsov et al. 2017). It is commonly found in at least one cell or tissue type, usually with a specialised function, e.g. suspensor, tapetum, endosperm or leaf bladder cells (Nagl 1976; D'Amato 1984; Barow and Meister 2003). However, in addition to these specialised cells and tissues, endopolyploidy commonly occurs in various plant organs, particularly in vegetative organs, such as roots, stems and leaves. It has only been consistently documented in a few families that may be considered 'polysomatic families'. Endopolyploidy in such families occurs at a high level, which means that 8C nuclei are commonly present, and the proportion of 2C nuclei is lower than that of polyploid nuclei. Many economically important plant families, such as the *Brassicaceae*, *Fabaceae*, *Amaranthaceae* and *Cucurbitaceae*, may be referred to as polysomatic families. Due to the impact that endopolyploidy has on plant physiology and crop plants, it is important to increase the extent of our knowledge of the overall presence of endopolyploidy among plants.

Endopolyploidy in Solanaceae

The *Solanaceae* family contains several economically important species. Solanaceous species are distributed worldwide and are widely known, e.g. potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), bell pepper (*C. annuum*), eggplant (*Solanum melongena* L.) and tobacco (*N. tabacum*). Barow and Meister (2003) reported the *Solanaceae* as a polysomatic plant family, which was later confirmed in several studies. For instance, pericarp tissue of tomato is now known to be highly polysomatic, containing cells of ploidy levels up to 512C (Cheniclet et al. 2005; Chevalier et al. 2014). In addition, tubers of *S. tuberosum* and other related species were identified as highly polysomatic, reaching a maximum 64C in *S. candolleanum* Berthault (EI = 2.62, Laimbeer et al. 2017). It has also been found that mature leaf tissue of both diploid and tetraploid plants of *Hyoscyamus niger* L. is endopolyploid (EI ~ 0.4, Weber et al. 2008).

Only a few studies focusing on the endopolyploidisation of *Capsicum* and *Nicotiana* have been published. A study by Gilissen et al. (1996) reported the presence of endopolyploidy in stem cells of *N. tabacum*. Barow and Meister (2003) showed endoreduplication (EI values above 0.1) in four and eight organ types of *Capsicum frutescens* L. and *N. tabacum*, respectively. Ogawa et al. (2010) focused on revealing the degree of polysomaty in fruit tissues of *Capsicum* species, and they showed very high levels of endopolyploidisation in the pericarp tissue of *C. annuum* cv. 'Édes alma', reaching a maximum of 256C.

Several studies have identified endopolyploidy in various tissues of seeds of *C. annuum* during development (Lanteri et al. 1993, Lanteri et al. 1994, Portis et al. 1999, Rewers and Sliwinska 2014). In the developing embryo, cells with 4C ploidy levels were present in considerable amounts, while the mature embryo in dry seed possessed almost only 2C cells (4C < 10 %). Endosperm cells during development, as well as endosperm cells in mature seed, frequently had 6C ploidy level.

In this study, endopolyploidy patterns were evaluated across a sample of the *Solanaceae*. Endopolyploidy was

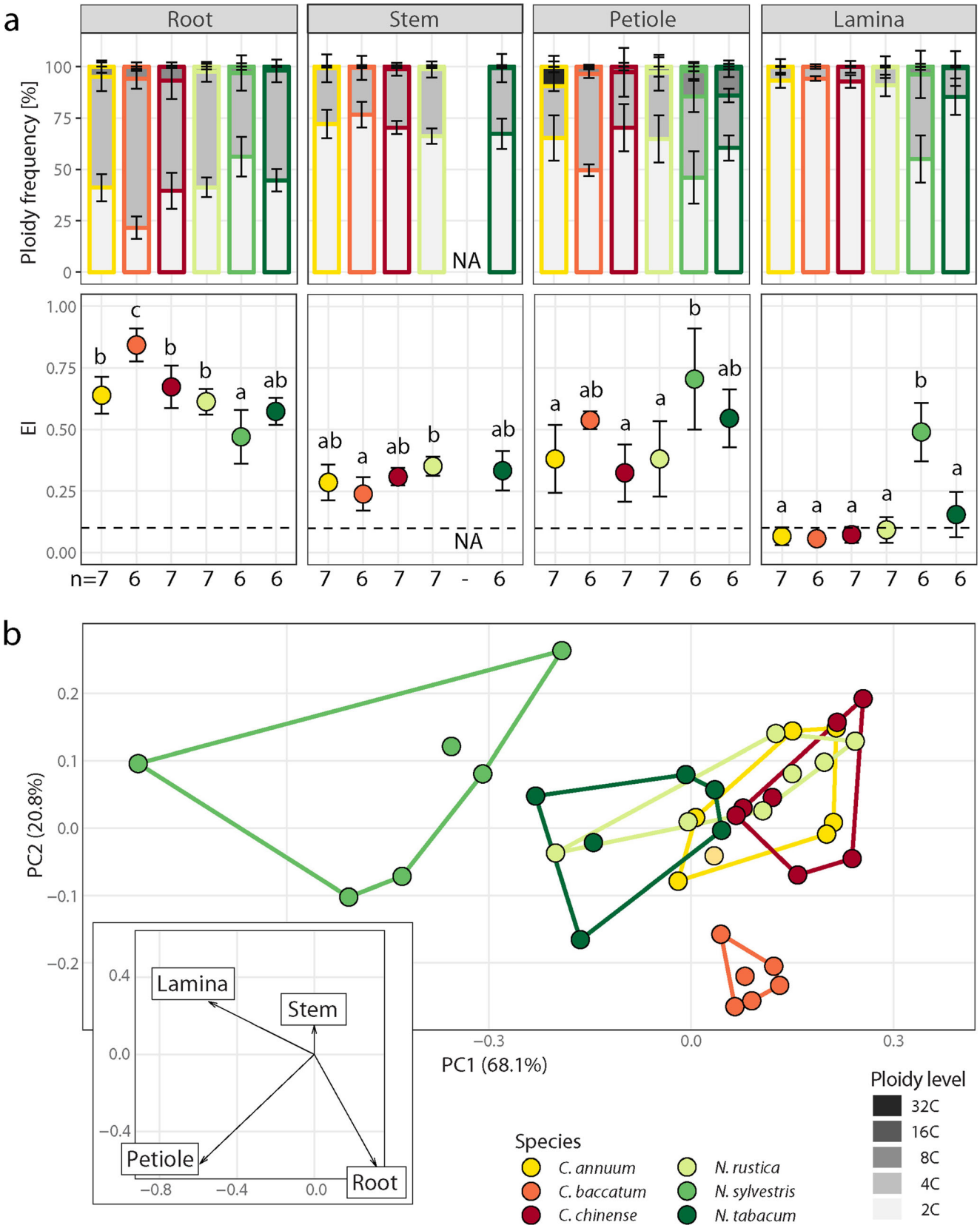

Fig. 2 Endopolyploidy analysis among samples of vegetative organs for six investigated species of *Capsicum* and *Nicotiana*. **a** Proportion of nuclei classes and endoreduplication index. Dashed line represents trashold value of EI = 0.1 (tissues with EI < 0.1 are not considered to be endopolyploid). NA, not applied. **b** PCA analysis of EI. Statistically homogeneous groups are denoted by lowercase letters

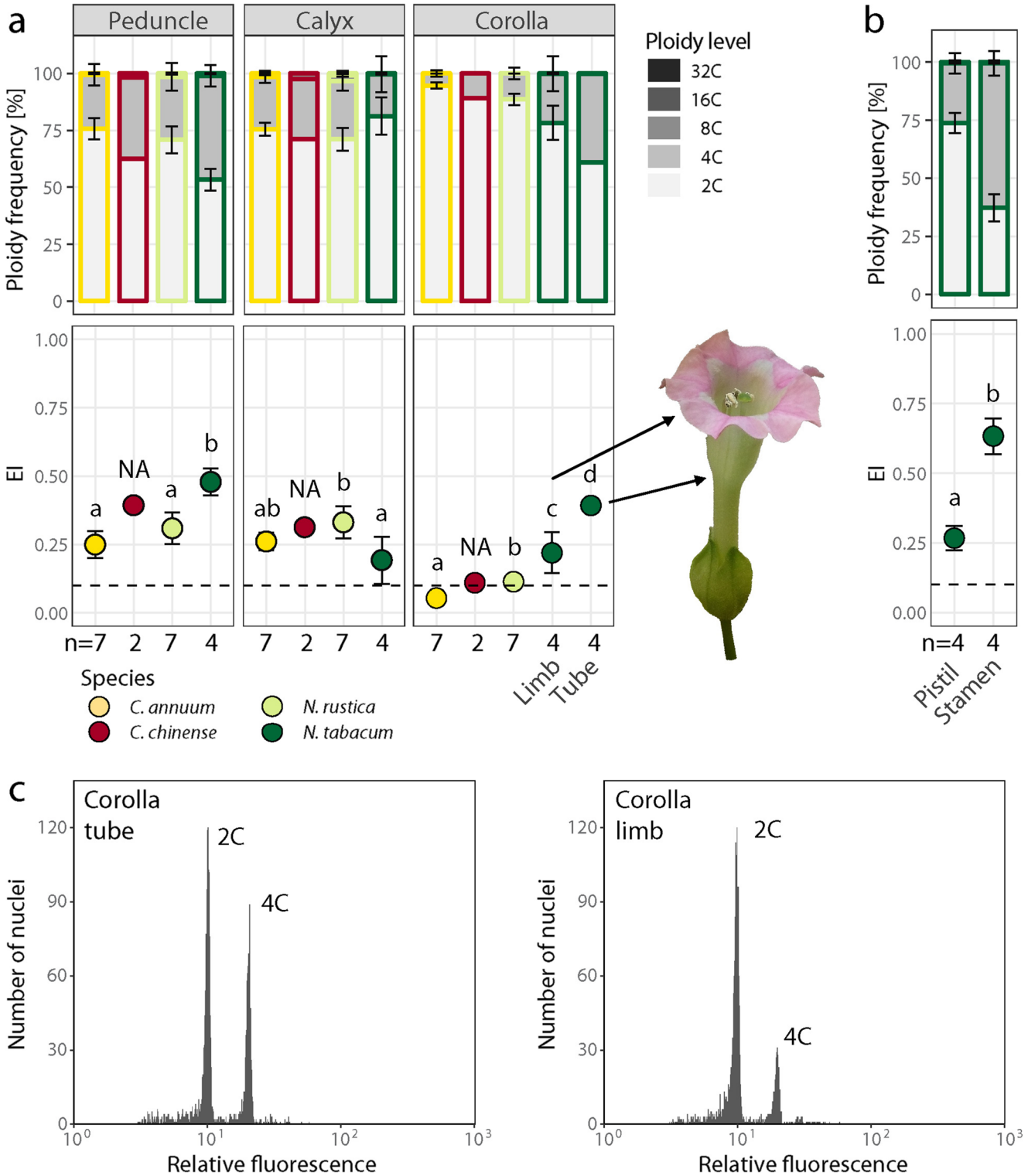

Fig. 3 Endopolyploidy analysis among samples of floral organs for four investigated species of *Capsicum* and *Nicotiana*. Endopolyploidy level in four species with details for corolla parts and fertile floral organs in *N. tabacum*. **a** Proportion of nuclei classes and endoreduplication index in sterile floral organs, note that corolla tube and corolla limb were analysed separately for *N. tabacum*; for *C. chinense*, only n = 2 flowers were analysed and only mean value (without error bars) is plotted. NA, not applied. **b** Variation in endoreduplication index of fertile floral organs, pistil and stamen of *N. tabacum*. Dashed line represents trashold value of EI = 0.1 (tissues with EI < 0.1 are not considered to be endopolyploid). **c** Representative histograms for corolla tube and corolla limb of *N. tabacum*

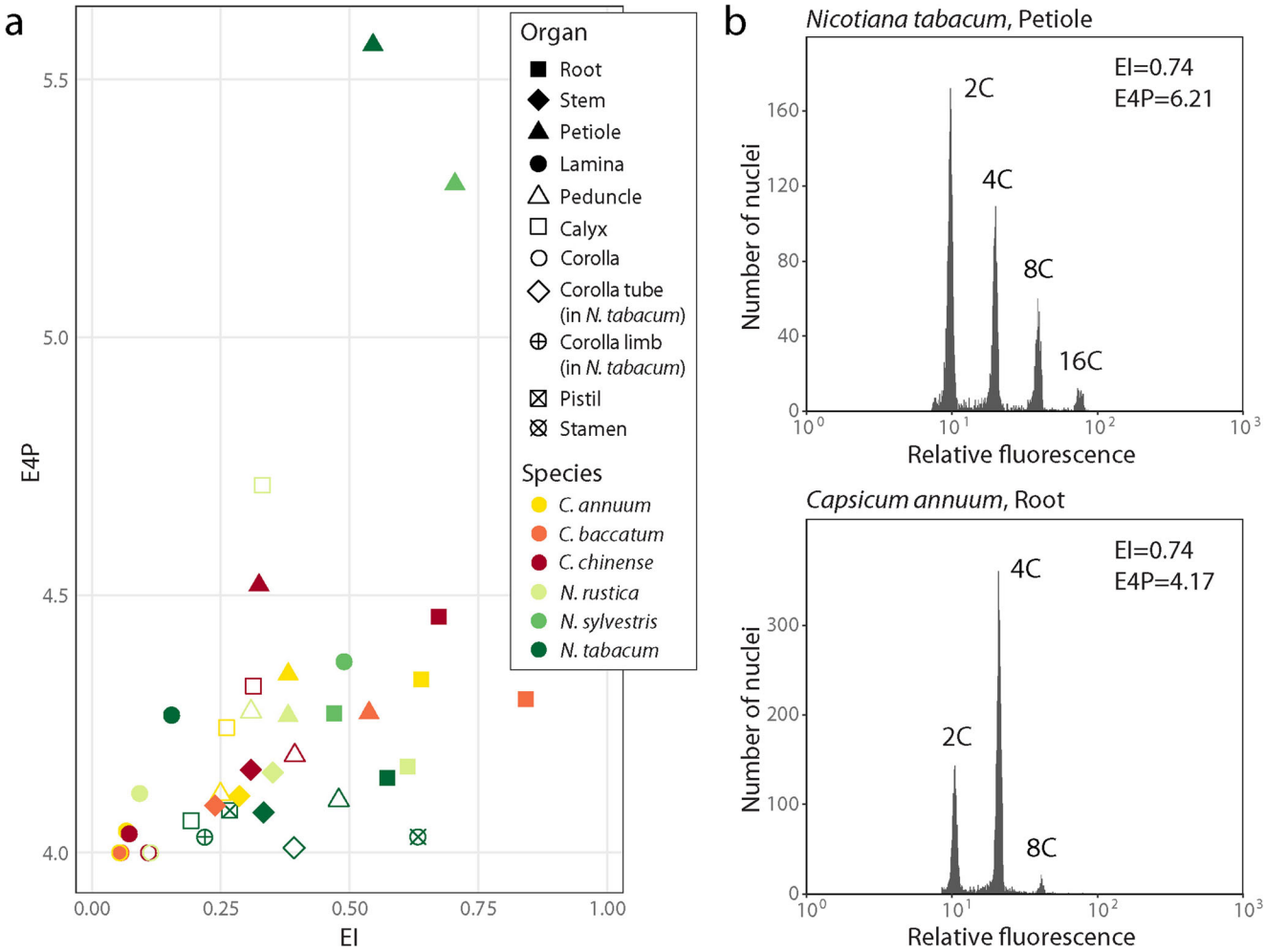

Fig. 4 Relationship between endoreduplication index and E4P parameter. **a** Biplot of per organ means for six species of EI vs. E4P parameters. **b** Examples of flow cytometry histograms for two cases that are identical in EI but differ in E4P parameters

analysed in six species: *C. annuum*, *C. baccatum*, *C. chinense*, *N. rustica*, *N. sylvestris* and *N. tabacum*. Our data support the regular inclusion of *Solanaceae* on the list of 'polysomatic families'. The level of endopolyploidy appears to be moderate with a mean EI per organ always < 1.0.

This comprehensive study addressing endopolyploidy patterns and variation across several organ tissues of *Capsicum* and *Nicotiana* revealed previously unknown tissue-specific information. In the genus *Capsicum*, the lowest level of endopolyploidy in cells occurred in leaf lamina and corolla vegetative organs, while root tissues were identified as the vegetative organ with the highest EL.

Our results regarding EL in leaf tissue are consistent with previous studies wherein leaf lamina likely has minimal endopolyploid cells or a very low amount of 4C endopolyploid cells, while the leaf petiole is polysomatic (Lanteri et al. 1993, 1996; Barow and Meister 2003). We have recorded up to 8C nuclei in *C. baccatum*, and Barow and Meister (2003) reported a max EI of 0.13 in *C. frutescens*. In leaf petiole tissue, our results indicate a mean EI from 0.32 to 0.54 (depending on species), which is similar to previous reports (max EI = 0.51) for *C. frutescens* leaf tissue by Barow and Meister (2003).

With regard to species similarity, *C. annuum* and *C. chinense* showed nearly identical patterns, with the exception of sterile floral tissue. However, due to the low sample size in *C. chinense*, these results are not particularly conclusive. Notably, *C. baccatum* slightly deviated in EL from both *C. annuum* and *C. chinense*, but a significant differentiation in EL was only recorded in root tissue. These findings may stem from a difference in genetic relatedness (Carrizo García et al. 2016) and/or a larger genome size of *C. baccatum* (Fig. 1).

To date, EL reports for several organs have only been published for *N. tabacum* (Barow and Meister 2003), and these are highly congruent with our data. In this study, we have expanded these findings to include two novel *Nicotiana* species. In the genus *Nicotiana*, we found that the lowest EL occurred in leaf lamina and corolla tissue for all species, whereas the highest EL occurred in leaf petiole or root tissue depending on the species. In addition, Gilissen et al. (1996)

reported that the EL for stem tissue of *Nicotiana* (*N. tabacum*) was 2C – 8C cells, which is consistent with this study. Such congruence among studies highlights the precision of FCM determination of EL. Furthermore, it opens the possibility for future comparisons of data across several different studies.

In *N. tabacum*, we identified that the EI was significantly lower in the corolla limb than in the corolla tube tissue. Despite only being demonstrated in one of six investigated species, this could still be similar to some EL patterns in other polysomatic species of *Nicotiana*. Furthermore, the EI might be significantly lower as a result of corolla tube elongation, which is often a result not only of cell proliferation (increasing number of cells) but also of cell size expansion. This growth model has been elucidated in *Petunia* Juss. of the *Solanaceae* (Stuurman et al. 2004) and in *Lithospermum* L. of the related family *Boraginaceae* (Cohen et al. 2012). Generally, cell elongation accompanied by endopolyploidisation is an important component of petal development in polysomatic plants (e.g. Kudo and Kimura 2002; Agulló-Antón et al. 2013; Ho et al. 2016). A cytohistologically oriented study coupled with FCM screening of several taxa should be performed to test this assumption in *Nicotiana*.

Reproductive organs, e.g. pistils and stamens, contain much more diverse tissues than vegetative organs, e.g. leaves, calices or corollas. However, most of the nuclei of the pistil were released from style tissues in *Nicotiana* and were found to be 2C – 8C. Certainly, ~ 25 % of the pistil cells had undergone one endocycle. Conversely, stamens also contained 2C – 8C cells, which indicated that at least a few cells were endopolyploid. More than 60 % of all stamen cells were found to be 4C, which resulted in a high EI value (mean EI = 0.63). However, this finding should not be interpreted as the presence of a high amount of endopolyploid cells in the case of an active tissue, such as developing sporogenous tissue or microspores inside the anther of stamen. Some of 2C cells in the stamen were assumed to be generative cells of microspores (pollen grains), which are cells arrested in the G_2 phase of the cell cycle prior to post-pollination mitosis and formation of two sperm cells (D'Amato et al. 1965; Kron and Husband 2012). Thus, regarding EL determination, a lack of FCM differentiation between endopolyploid cells and cells arrested in the G_2 phase of the cell cycle requires the differentiation between mitotically active and inactive tissues and careful interpretation of FCM data (Rewers and Sliwinska 2014).

Overall, the EL pattern in *Nicotiana* reveals that *N. sylvestris* possesses a higher EL than *N. rustica* or *N. tabacum*. This relationship likely reflects the diploid chromosome constitution of *N. sylvestris*, whereas both other species are tetraploids. The phylogenetic relatedness does not likely play a role in this case since both tetraploids, *N. tabacum* and *N. rustica*, have different origins, and the diploid *N. sylvestris* is the originating maternal species for *N. tabacum* only (Clarkson et al. 2017).

It remains unclear why polyploids tend to lose EL compared to diploids. Overall, there has been little research addressing this topic. However, Nagl (1976) hypothesised that endopolyploidy may be an evolutionary novelty appearing at a tissue and cellular level, which compensates for a low whole organism ploidy level. This hypothesis appears to be supported by tens of well-documented diploids and their synthetic (isogenic) autotetraploids in the model species *Arabidopsis thaliana* (Pacey et al. 2020a). A similar compensatory effect for small organism genome size was also suggested for other plant groups, e.g. geophytes (Kolarčik et al. 2020). However, EL is genotype specific, and observing endopolyploidy diversification among populations is likely an evolutionary response to natural selection driven by environmental differentiation (Pacey et al. 2020b).

Comparative exploratory analysis of endopolyploid level parameters

Various equations to calculate a value characterising EL from data on frequencies of different ploidy classes discerned from FCM histograms have been proposed and are inconsistently applied in endopolyploidy research. This is an obstacle in any comparative study. To date, however, no attempts have been made to investigate the significance of these equations or to propose which of them should be recommended for continued use. Different FCM profiles of nuclei ploidy frequency of two samples may result in the calculation of the same value for some of the parameters that have been proposed to express EL, e.g. EI or MCV. Thus, comparative inspection of these parameters between each other, as well as to frequency of 2C, 4C, etc., should be performed. Some of the EL parameters capture different aspects of EL. For instance, EI is used to estimate proportion of endocycles, while E4P is used to evaluate mean ploidy level of only endopolyploid cells.

In this study, we have shown that a simple reliance on a single parameter as performed in some important studies (Barow and Meister 2003; Jovtchev et al. 2007; Bainard et al. 2012) may lead to overlooking some of the real differences between samples, which is consistent with the findings of Kobayashi (2019). Moreover, we have shown that a low correlation was found between the E4P parameter and other parameters (EI, MCV and ≥4C). Identical values of EI may result from a high proportion of endopolyploid cells with one endocycle compared to a proportion of basic 2C nuclei and from a lower proportion of nuclei with one (4C) or more (8C, 16C) endocycles (Fig. 4). Therefore, a comparison between EI and E4P parameters can be very informative and allows for the identification of causes for organ or species differences in EL, such as varying proportions of 2C and various endopolyploid 4C – 32C cells. Therefore, common parameters and standardised analyses of all existing EL parameters in any

study of endopolyploidy would be of great value and should be a goal in future studies.

Conclusions

Endopolyploidy plays a significant role in the development, differentiation and stress physiology of polysomatic plants. However, the current knowledge of endopolyploidy is lacking for most plant species, which is a considerable shortcoming in the interpretation of physiological or transcriptomic data. Surprisingly, ploidy surveys at cellular or tissue levels are rarely performed in such studies. For instance, Pirrello et al. (2018) suggested that a complete approach, which should be adopted, includes flow cytometry screening of endopolyploidy and nuclei ploidy classes sorting prior to transcriptome analysis. In this study, we provide foundational endopolyploidy information that will be instrumental in future robust transcriptomic analyses for solanaceous crops.

Furthermore, some studies have shown that endopolyploidy may be used in modern agriculture and plant breeding programmes for crop improvement. For instance, it is a reliable marker of seed development in many polysomatic species (Sliwinska 2009) and has also been shown to be indicative of crop quality (Kobayashi 2019). Endoreduplication also leads to doubling or multiplying of chromosomes in certain cell lines, which can be useful in the production of polyploid plants from tissue cultures or *in vitro* regeneration (Cheniclet et al. 2005; Chen et al. 2011). More RNA transcripts are produced through endoreduplication of DNA, and therefore, metabolite production increases (Lee et al. 2009; Scholes and Paige 2015). In this manner, it may be possible to either produce more bioactive metabolites in medicinal plants (Sliwinska 2018) or produce more metabolites for commercial use, such as increasing nicotine in *N. tabacum* or *N. rustica* or producing cytotoxic compounds and anti-herbivory toxins in plants that can be used as biopesticides (Leitch and Dodsworth 2017). In this study, we were able to identify tissues in *Nicotiana* with varying levels of endopolyploidy that, with further research, may be selected in the future to improve some of these desirable traits.

Acknowledgements The authors would like to thank Professor J. Doležel (Institute of Experimental Botany, Olomouc, Czech Republic), who provided seed material of the reference standard *Z. mays* 'CE-777' for flow cytometry. We would like to thank Enago (www.enago.com) for English language editing service. This study was partially funded by the granting agency VEGA, Slovak republic, grant no. 1/0669/19.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Abraham A, Ninan CA, Gopinath P (1965) Cytology of Development of Abnormal Endosperm in Philippine Makapuno Coconuts. Caryologia 18(3):395-408

Agulló-Antón M, Olmos E, Pérez-Pérez JM, Acosta M (2013) Evaluation of ploidy level and endoreduplication in carnation (*Dianthus* spp.). Plant Sci 201–202:1–11. https://doi.org/10.1016/j.plantsci.2012.11.006

Artlip TS, Madison JT, Setter TL (1995) Water deficit in developing endosperm of maize: Cell division and nuclear DNA endoreduplication. Plant Cell Environ 18:1034–1040. https://doi.org/10.1111/j.1365-3040.1995.tb00614.x

Bainard JD, Newmaster SG (2010) Endopolyploidy in bryophytes: widespread in mosses and absent in liverworts. J Bot 2010:1–7. https://doi.org/10.1155/2010/316356

Bainard JD, Henry TA, Bainard LD, Newmaster SG (2011a) DNA content variation in monilophytes and lycophytes: large genomes that are not endopolyploid. Chromosome Res 19:763–775. https://doi.org/10.1007/s10577-011-9228-1

Bainard LD, Bainard JD, Newmaster SG, Klironomos JN (2011b) Mycorrhizal symbiosis stimulates endoreduplication in angiosperms. Plant Cell Environ 34:1577–1585. https://doi.org/10.1111/j.1365-3040.2011.02354.x

Bainard JD, Bainard LD, Henry TA, Fazekas AJ, Newmaster SG (2012) A multivariate analysis of variation in genome size and endoreduplication in angiosperms reveals strong phylogenetic signal and association with phenotypic traits. New Phytol 196:1240–1250. https://doi.org/10.1111/j.1469-8137.2012.04370.x

Barkla BJ, Rhodes T, Tran K-N, Wijesinghege C, Larkin JC, Dassanayake M (2018) Making epidermal bladder cells bigger: Developmental- and salinity-induced endopolyploidy in a model halophyte. Plant Physiol 177:615–632. https://doi.org/10.1104/pp.18.00033

Barow M, Meister A (2003) Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. Plant Cell Environ 26:571–584. https://doi.org/10.1046/j.1365-3040.2003.00988.x

Bartoli G, Felici C, Castiglione MR (2017) Female gametophyte and embryo development in *Helleborus bocconei* Ten (Ranunculaceae). Protoplasma 254:491–504. https://doi.org/10.1007/s00709-016-0969-8

Bennici A, Caceres ME, Cionini G, Cionini PG (2008) A DNA cytophotometric study of salt adaptation in *Allium cepa* and *Nicotiana bigelovii*. Caryologia 61:176–181. https://doi.org/10.1080/00087114.2008.10589627

Bhosale R, Boudolf V, Cuevas F, Lu R, Eekhout T, Hu ZB, Van Isterdael G, Lambert GM, Xu F, Nowack MK, Smith RS, Vercauteren I, De Rycke R, Storme V, Beeckman T, Larkin JC, Kremer A, Höfte H, Galbraith DW, Kumpf RP, Maere S, De Veylder L (2018) A spatiotemporal DNA endoploidy map of the *Arabidopsis* root reveals roles for the endocycle in root development and stress adaptation. Plant Cell 30:2330–2351. https://doi.org/10.1105/tpc.17.00983

Bourdon M, Pirrello J, Cheniclet C, Coriton O, Bourge M, Brown S, Moïse A, Peypelut M, Rouyère V, Renaudin JP, Chevalier C, Frangne N (2012) Evidence for karyoplasmic homeostasis during endoreduplication and a ploidy-dependent increase in gene transcription during tomato fruit growth. Development 139:3817–3826. https://doi.org/10.1242/dev.084053

Carrizo García C, Barfuss MH, Sehr EM, Barboza GE, Samuel R, Moscone EA, Ehrendorfer F (2016) Phylogenetic relationships, diversification and expansion of chili peppers (Capsicum, *Solanaceae*). Ann Bot 118:35–51. https://doi.org/10.1093/aob/mcw079

Ceccarelli M, Santantonio E, Marmottini F, Amzallag GN, Cionini PG (2006) Chromosome endoreduplication as a factor of salt adaptation in *Sorghum bicolor*. Protoplasma 227:113–118. https://doi.org/10.1007/s00709-005-0144-0

Chen W-H, Kao Y-L, Tang C-Y, Jean G-T (2011) Endopolyploidy in *Phalaenopsis* orchids and its application in polyploid breeding. In: Chen W-H, Chen H-H (eds) Orchid Biotechnology II. World Scientific Publishing Co Ltd, Singapore, pp 25–48

Cheniclet C, Rong WY, Causse M, Frangne N, Bolling L, Carde JP, Renaudin JP (2005) Cell expansion and endoreduplication show a large genetic variability in pericarp and contribute strongly to tomato fruit growth. Plant Physiol 139:1984–1994. https://doi.org/10.1104/pp.105.068767

Chevalier C, Bourdon M, Pirrello J, Cheniclet C, Gévaudant F, Frangne N (2014) Endoreduplication and fruit growth in tomato: evidence in favour of the karyoplasmic ratio theory. J Exp Bot 65:2731–2746. https://doi.org/10.1093/jxb/ert366

Clarkson JJ, Dodsworth S, Chase MW (2017) Time-calibrated phylogenetic trees establish a lag between polyploidisation and diversification in *Nicotiana* (*Solanaceae*). Plant Sys Evol 303:1001–1012. https://doi.org/10.1007/s00606-017-1416-9

Cohen JI, Litt A, Davis JI (2012) Comparative floral development in *Lithospermum* (*Boraginaceae*) and implications for the evolution and development of heterostyly. Am J Bot 99:797–805. https://doi.org/10.3732/ajb.1100329

Cookson SJ, Radziejwoski A, Granier C (2006) Cell and leaf size plasticity in *Arabidopsis*: what is the role of endoreduplication? Plant Cell Environ 29:1273–1283. https://doi.org/10.1111/j.1365-3040.2006.01506.x

D'Amato F (1964) Endopolyploidy as factor in plant tissue development. Caryologia 17:41–52. https://doi.org/10.1080/00087114.1964.10796115

D'Amato F (1984) Role of polyploidy in reproductive organs and tissues. In: Johri BM (ed) Embryology of Angiosperms. Springer, Berlin, pp 519–566

D'Amato F (1989) Polyploidy in cell differentiation. Caryologia 42:183–211. https://doi.org/10.1080/00087114.1989.10796966

D'Amato F, Devreux M, Scarascia Mugnozza GT (1965) The DNA content of the nuclei of the pollen grains in tobacco and barley. Caryologia 18:377–382. https://doi.org/10.1080/00087114.1965.10796180

de Almeida Engler JD, Gheysen G (2013) Nematode-induced endoreduplication in plant host cells: why and how? Mol Plant Microbe In 26:17–24. https://doi.org/10.1094/mpmi-05-12-0128-cr

Dilkes BP, Dante RA, Coelho C, Larkins BA (2002) Genetic analyses of endoreduplication in *Zea mays* endosperm: Evidence of sporophytic and zygotic maternal control. Genetics 160:1163–1177

Doležel J, Bartoš J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot 95:99–110. https://doi.org/10.1093/aob/mci005

Doležel J, Göhde W (1995) Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high-resolution flow cytometry. Cytometry 19:103–106. https://doi.org/10.1002/cyto.990190203

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:2233–2244. https://doi.org/10.1038/nprot.2007.310

Frawley LE, Orr-Weaver TL (2015) Polyploidy. Curr Biol 25:R353–R358. https://doi.org/10.1016/j.cub.2015.03.037

Galbraith DW (2014) Endoreduplicative standards for calibration of flow cytometric C-Value measurements. Cytom Part A 85:368–374. https://doi.org/10.1002/cyto.a.22440

Gegas VC, Wargent JJ, Pesquet E, Granqvist E, Paul ND, Doonan JH (2014) Endopolyploidy as a potential alternative adaptive strategy for *Arabidopsis* leaf size variation in response to UV-B. J Exp Bot 65:2757–2766. https://doi.org/10.1093/jxb/ert473

Gilissen LJW, van Staveren MJ, Hakkert JC, Smulders MJM (1996) Competence for regeneration during tobacco internodal development - Involvement of plant age, cell elongation stage, and degree of polysomaty. Plant Physiol 111:1243–1250. https://doi.org/10.1104/pp.111.4.1243

Greilhuber J, Obermayer R (1997) Genome size and maturity group in *Glycine max* (soybean). Heredity 78:547–551. https://doi.org/10.1038/hdy.1997.85

Hasinoff BB, Abram ME, Chee GL, Huebner E, Byard EH, Barnabé N, Ferrans VJ, Yu ZX, Yalowich JC (2000) The catalytic DNA topoisomerase II inhibitor dexrazoxane (ICRF-187) induces endopolyploidy in Chinese hamster ovary cells. J Pharmacol Exp Ther 295:474–483

Ho T-T, Kwon A-R, Yoon Y-J, Paek K-Y, Park S-Y (2016) Endoreduplication level affects flower size and development by increasing cell size in *Phalaenopsis* and *Doritaenopsis*. Acta Physiol Plant 38:190. https://doi.org/10.1007/s11738-016-2222-7

Husband BC, Baldwin SJ, Suda J (2013) The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. In: Greilhuber J, Doležel J, Wendel JF (eds) Plant Genome Diversity. Springer, Vienna, pp 255–276

Joubès J, Chevalier C (2000) Endoreduplication in higher plants. Plant Mol Biol 43:735–745. https://doi.org/10.1023/a:1006446417196

Jovtchev G, Barow M, Meister A, Schubert I (2007) Impact of environmental and endogenous factors on endopolyploidization in angiosperms. Environ Exp Bot 60:404–411. https://doi.org/10.1016/j.envexpbot.2006.12.007

Kladnik A (2015) Relationship of nuclear genome size, cell volume and nuclei volume in endosperm of *Sorghum bicolor*. Acta Biol Slov 58:3–11

Kladnik A, Chourey PS, Pring DR, Dermastia M (2006) Development of the endosperm of *Sorghum bicolor* during the endoreduplication-associated growth phase. J Cereal Sci 43:209–215. https://doi.org/10.1016/j.jcs.2005.09.004

Kobayashi H (2019) Variations of endoreduplication and its potential contribution to endosperm development in rice (*Oryza sativa* L.). Plant Production Science 22:227–241. https://doi.org/10.1080/1343943x.2019.1570281

Kocová V, Kolarčik V, Straková N, Mártonfi P (2014) Endopolyploidy patterns in organs of *Trifolium* species (*Fabaceae*). Acta Biol Cracov Bot 56:111–120. https://doi.org/10.2478/abcsb-2014-0011

Kocová V, Bubanová D, Rákai A, Kolarčik V, Mártonfi P (2017) Salinity has no effect on polysomatic pattern in seedlings of *Trifolium pratense* and *T. repens*. Acta Biol Cracov Bot 59:55–65. https://doi.org/10.1515/abcsb-2017-0005

Kolarčik V, Fráková V, Kocová V, Koprivý L, Mártonfi P (2020) Endopolyploidy pattern in Corydalis early spring geophytes. Flora 270:151651

Kron P, Husband BC (2012) Using flow cytometry to estimate pollen DNA content: improved methodology and applications. Ann Bot 110:1067–1078. https://doi.org/10.1093/aob/mcs167

Kudo N, Kimura Y (2001) Flow cytometric evidence for endopolyploidy in seedlings of some *Brassica* species. Theor Appl Genet 102:104–110. https://doi.org/10.1007/s001220051624

Kudo N, Kimura Y (2002) Nuclear DNA endoreduplication during petal development in cabbage: relationship between ploidy levels and cell size. J Exp Bot 53:1017–1023. https://doi.org/10.1093/jexbot/53.371.1017

Laimbeer FPE, Holt SH, Makris M, Hardigan MA, Buell CR, Veilleux RE (2017) Protoplast isolation prior to flow cytometry reveals clear patterns of endoreduplication in potato tubers, related species, and some starchy root crops. Plant Methods 13:27. https://doi.org/10.1186/s13007-017-0177-3

Lanteri S, Kraak L, De Vos CHR, Bino RJ (1993) Effects of osmotic preconditioning on nuclear replication activity in seeds of pepper

(*Capsicum annuum* L.). Physiol Plant 89:433–440. https://doi.org/10.1111/j.1399-3054.1993.tb05195.x

Lanteri S, Saracco F, Kraak HL, Bino RJ (1994) The effects of priming on nuclear replication activity and germination of pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) seeds. Seed Sci Res 4:81–87. https://doi.org/10.1017/S0960258500002051

Lanteri S, Nada E, Belletti P, Quagliotti L, Bino RJ (1996) Effects of controlled deterioration and osmoconditioning on germination and nuclear replication in seeds of pepper (*Capsicum annuum* L.). Ann Bot 77:591–597. https://doi.org/10.1093/aob/77.6.591

Larkins BA, Dilkes BP, Dante RA, Coelho CM, Woo YM, Liu Y (2001) Investigating the hows and whys of DNA endoreduplication. J Exp Bot 52:183–192. https://doi.org/10.1093/jexbot/52.355.183

Lee HO, Davidson JM, Duronio RJ (2009) Endoreplication: polyploidy with purpose. Gene Dev 23:2461–2477. https://doi.org/10.1101/gad.1829209

Leitch IJ, Dodsworth S (2017) Endopolyploidy in Plants. In: Hetherington AM (ed) Encyclopedia of life sciences. Wiley, Chichester, pp 1–10. https://doi.org/10.1002/9780470015902.a0020097.pub2

Lemontey C, Mousset-Déclas C, Munier-Jolain N, Boutin JP (2000) Maternal genotype influences pea seed size by controlling both mitotic activity during early embryogenesis and final endoreduplication level/cotyledon cell size in mature seed. J Exp Bot 51:167–175. https://doi.org/10.1093/jexbot/51.343.167

Li S, Liu L, Li T, Lan T, Wang Y, Zhang Z, Liu J, Xu S, Zhang X, Zhu J, Xue J, Guo D (2019) The distribution pattern of endopolyploidy in maize. Theor Appl Genet 132:1487–1503. https://doi.org/10.1007/s00122-019-03294-4

Lingua G, Fusconi A, Berta G (2001) The nucleus of differentiated root plant cells: modifications induced by arbuscular mycorrhizal fungi. Eur J Histochem 45:9–20. https://doi.org/10.4081/1609

Loureiro J, Rodriguez E, Doležel J, Santos C (2007) Two new nuclear isolation buffers for plant DNA flow cytometry: A test with 37 species. Ann Bot 100:875–888. https://doi.org/10.1093/aob/mcm152

Lysák MA, Doležel J (1998) Estimation of nuclear DNA content in *Sesleria* (*Poaceae*). Caryologia 51:123–132. https://doi.org/10.1080/00087114.1998.10589127

Melaragno JE, Mehrotra B, Coleman AW (1993) Relationship between endopolyploidy and cell size in epidermal tissue of *Arabidopsis*. Plant Cell 5:1661–1668. https://doi.org/10.1105/tpc.5.11.1661

Mishiba K, Mii M (2000) Polysomaty analysis in diploid and tetraploid *Portulaca grandiflora*. Plant Sci 156:213–219. https://doi.org/10.1016/s0168-9452(00)00257-0

Musseau C, Jorly J, Gadin S, Sørensen I, Deborde C, Bernillon S, Mauxion J-P, Atienza I, Moing A, Lemaire-Chamley M, Rose JKC, Chevalier C, Rothan C, Fernandez-Lochu L, Gévaudant F (2020) The tomato guanylate-binding protein SlGBP1 enables fruit tissue differentiation by maintaining endopolyploid cells in a non-proliferative state. Plant Cell 32:3188–3205. https://doi.org/10.1105/tpc.20.00245

Nagl W (1976) DNA endoreduplication and polyteny understood as evolutionary strategies. Nature 261:614–615. https://doi.org/10.1038/261614a0

Ogawa D, Ishikawa K, Nunomura O, Mii M (2010) Correlation between fruit characters and degree of polysomaty in fruit tissues of *Capsicum*. J Jpn Soc Hortic Sci 79:168–173. https://doi.org/10.2503/jjshs1.79.168

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan

Pacey EK, Maherali H, Husband BC (2020a) The influence of experimentally induced polyploidy on the relationships between endopolyploidy and plant function in *Arabidopsis thaliana*. Ecol Evol 10:198–216. https://doi.org/10.1002/ece3.5886

Pacey EK, Maherali H, Husband BC (2020b) Endopolyploidy is associated with leaf functional traits and climate variation in *Arabidopsis thaliana*. Am J Bot 107:993–1003. https://doi.org/10.1002/ajb2.1508

Peterson BG, Carl P (2019) Performance analytics: Econometric tools for performance and risk analysis. R package version 1.5.3. https://CRAN.R-project.org/package=PerformanceAnalytics

Pirrello J, Deluche C, Frangne N, Gévaudant F, Maza E, Djari A, Bourge M, Renaudin JP, Brown S, Bowler C, Zouine M, Chevalier C, Gonzalez N (2018) Transcriptome profiling of sorted endoreduplicated nuclei from tomato fruits: how the global shift in expression ascribed to DNA ploidy influences RNA-Seq data normalization and interpretation. Plant J 93:387–398. https://doi.org/10.1111/tpj.13783

Portis E, Marzachì C, Quagliotti L, Lanteri S (1999) Molecular and physiological markers during seed development of peppers (*Capsicum annuum* L.): DNA replication and β-tubulin synthesis. Seed Sci Res 9:85–90. https://doi.org/10.1017/S0960258599000094

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 12 Nov 2019

Rewers M, Sliwinska E (2014) Endoreduplication in the germinating embryo and young seedling is related to the type of seedling establishment but is not coupled with superoxide radical accumulation. J Exp Bot 65:4385–4396. https://doi.org/10.1093/jxb/eru210

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytol 206:19–26

Robinson DO, Coate JE, Singh A, Hong LL, Bush M, Doyle JJ, Roeder AHK (2018) Ploidy and size at multiple scales in the *Arabidopsis* sepal. Plant Cell 30:2308–2329. https://doi.org/10.1105/tpc.18.00344

Sabelli PA, Liu Y, Dante RA, Lizarraga LE, Nguyen HN, Brown SW, Klingler JP, Yu JJ, LaBrant E, Layton TM, Feldman M, Larkins BA (2013) Control of cell proliferation, endoreduplication, cell size, and cell death by the retinoblastoma-related pathway in maize endosperm. Proc Natl Acad Sci USA 110:E1827–E1836. https://doi.org/10.1073/pnas.1304903110

Scholes DR, Paige KN (2015) Plasticity in ploidy: a generalized response to stress. Trends Plant Sci 20:165–175. https://doi.org/10.1016/j.tplants.2014.11.007

Skaptsov MV, Lomonosova MN, Kutsev MG, Smirnov SV, Shmakov AI (2017) The phenomenon of endopolyploidy in some species of the Chenopodioideae (*Amaranthaceae*). Bot Lett 164:47–53. https://doi.org/10.1080/23818107.2016.1276475

Sliwinska E (2009) Nuclear DNA replication and seed quality. Seed Sci Res 19:15–25. https://doi.org/10.1017/s0960258508186275

Sliwinska E (2018) Flow cytometry - a modern method for exploring genome size and nuclear DNA synthesis in horticultural and medicinal plant species. Folia Hortic 30:103–128. https://doi.org/10.2478/fhort-2018-0011

Smant G, Helder J, Goverse A (2018) Parallel adaptations and common host cell responses enabling feeding of obligate and facultative plant parasitic nematodes. Plant J 93:686–702. https://doi.org/10.1111/tpj.13811

Smulders MJM, Rus-Kortekaas W, Gilissen LJW (1994) Development of polysomaty during differentiation in diploid and tetraploid tomato (*Lycopersicon esculentum*) plants. Plant Sci 97:53–60. https://doi.org/10.1016/0168-9452(94)90107-4

Straková N, Kocová V, Kolarčik V, Mártonfi P (2014) Endopolyploidy in organs of *Trifolium pratense* L. in different ontogenetic stages. Caryologia 67:116–123. https://doi.org/10.1080/00087114.2014.931632

Stuurman J, Hoballah ME, Broger L, Moore J, Basten C, Kuhlemeier C (2004) Dissection of floral pollination syndromes in *Petunia*. Genetics 168:1585–1599. https://doi.org/10.1534/genetics.104.031138

Temsch EM, Greilhuber J, Krisai R (2010) Genome size in liverworts. Preslia 82:63–80

Trávníček P, Ponert J, Urfus T, Jersáková J, Vrána J, Hřibová E, Doležel J, Suda J (2015) Challenges of flow-cytometric estimation of nuclear genome size in orchids, a plant group with both whole-genome and progressively partial endoreplication. Cytom Part A 87:958–966. https://doi.org/10.1002/cyto.a.22681

Van de Peer Y, Mizrachi E, Marchal K (2017) The evolutionary significance of polyploidy. Nat Rev Genet 18:411–424. https://doi.org/10.1038/nrg.2017.26

Von Stetina JR, Frawley LE, Unhavaithaya Y, Orr-Weaver TL (2018) Variant cell cycles regulated by Notch signaling control cell size and ensure a functional blood-brain barrier. Development 145: dev157115. https://doi.org/10.1242/dev.157115

Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WHA, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B (2019) gplots: Various R Programming Tools for Plotting Data. R package version 3.0.1.1. https://CRAN.R-project.org/package=gplots

Weber J, Georgiev V, Pavlov A, Bley T (2008) Flow cytometric investigations of diploid and tetraploid plants and in vitro cultures of *Datura stramonium* and *Hyoscyamus niger*. Cytom Part A 73: 931–939. https://doi.org/10.1002/cyto.a.20628

Weiss H, Maluszynska J (2001) Molecular cytogenetic analysis of polyploidization in the anther tapetum of diploid and autotetraploid *Arabidopsis thaliana* plants. Ann Bot 87:729–735. https://doi.org/10.1006/anbo.2001.1402

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. 1-212https://doi.org/10.1007/978-0-387-98141-3Wickham H (2009) ggplot2: Elegant graphics for data analysis. 1-212. https://doi.org/10.1007/978-0-387-98141-3

Zedek F, Veselý P, Horová L, Bureš P (2016) Flow cytometry may allow microscope-independent detection of holocentric chromosomes in plants. Sci Rep 6:27161. https://doi.org/10.1038/srep27161