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Measuring genome size of desert plants using dry seeds

Elwira Sliwinska, Ilona Pisarczyk, Andrzej Pawlik, and David W. Galbraith

Abstract: Use of seeds instead of leaves for the flow cytometric measurement of DNA content is of particular interest to botanists and plant ecologists, since it allows estimation of genome sizes for species having reduced leaves or that accumulate staining inhibitors within leaves, and also for species growing in regions where cytometers are not readily available. The seeds of 24 desert species, including wildflowers, cacti, shrubs, and trees were analyzed by flow cytometry. Nuclei were used from either total seeds or seed tissues, following dissection to determine the seed parts that were most suitable for genome size measurement. In addition, the mass of 100 seeds was established. The seeds of 14 species contained only cells occupying a mitotic cell cycle. For 10 other species, endoreplicated nuclei (up to 32C) were detected. Using entire seeds or their parts, it was possible to estimate genome sizes for all of the species, which ranged from 0.79 pg per 2C in *Parkinsonia aculeata* L. to 26.96 pg per 2C in *Agave parryi* Engelm., thus this kind of plant material can be used for the cytometric measuring of nuclear DNA content. However, a detailed understanding of seed biology is needed to interpret the results correctly. The relationships among genome size, seed mass, and desert growing conditions are also discussed.

Key words: cell cycle, endoreplication, flow cytometry, mean ploidy, nuclear DNA content, seed mass.

Résumé : L'utilisation de graines plutôt que de feuilles pour mesurer la teneur en ADN par cytométrie en flux présente un intérêt particulier pour les botanistes et les écologistes végétaux puisqu'elles permettent d'évaluer les dimensions des génomes chez des espèces à feuilles réduites ou qui accumulent des inhibiteurs de coloration dans leurs feuilles, et également pour des espèces qui poussent dans des régions où les cytomètres ne sont par directement disponibles. Les auteurs ont analysé les graines de 24 espèces du désert, incluant des fleurs sauvages, des cactus, des arbustes et des arbres, par cytométrie en flux. Ils ont utilisé des noyaux provenant de graines entières ou de tissus séminaux, suite à une dissection, afin de déterminer les parties des graines les plus commodes pour mesurer la dimension des génomes. Ils ont de plus établi la masse de cent graines. Les graines de 14 espèces ne contenaient que des cellules appartenant à un cycle mitotique cellulaire. Chez 10 autres espèces, on observe des noyaux endorépliqués (jusqu'à 32). En utilisant des graines entières ou leurs parties, il s'est avéré possible d'estimer la dimension des génomes chez toutes les espèces, lesquelles se situent entre 0,79 pg par 2C chez le *Parkinsonia aculeata* L. et 26,96 pg par 2C chez l'*Agave parryi* Englem. On constate donc que ce type de matériel végétal se prête bien à la mesure cytométrique de la teneur en ADN nucléique. Cependant, on doit obtenir une connaissance détaillée de la biologie des graines pour interpréter les résultats correctement. On discute les relations entre les dimensions des génomes, les masses séminales et les conditions de croissance désertiques.

Mots-clés : cycle cellulaire, endoréplication, cytométrie en flux, ploïdie moyenne, teneur en ADN nucléique, asse séminale.

[Traduit par la Rédaction]

Introduction

Knowledge of nuclear DNA content (C-value) is crucial for developing an understanding of an organism's genome in relation to its adaptation to specific environmental conditions. This information is also useful in the fields of plant ecology, phytogeography, systematics, and evolution, as well as in cell and molecular biology (Bennett 1987; Ohri 1998; Bennett and Leitch 2005*a*). However, C-values have been established, to date, for less than 5000 plant species, which includes only about 1.6% of the global angiosperm flora (Bennett and Leitch 2005*b*). Species growing in harsh environmental conditions have been particularly neglected; for example, the nuclear DNA content has been reported for only 20 of over 2000 species of the family Cactaceae, which grows under hot and dry conditions (Bennett and Leitch 2005*c*). One problem for establishing genome sizes of species within this family may relate to the fact that the standard procedure of sample preparation for flow cytometric measurement is inappropriate. Flow cytometry (FCM), currently the most widely-employed method for C-value estimation, as originally described (Galbraith et al. 1983) and in its typical implementation, involves leaves as source tissues for isolation of nuclei. However, the leaves of cacti have been reduced to the form of thorns that are not suitable for nuclear isolation. In addition, other parts of the plant, for

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E. Sliwinska,[1] I. Pisarczyk, and A. Pawlik. Laboratory of Molecular Biology and Cytometry, Department of Genetics and Plant Breeding, University of Technology and Life Sciences, Avenue Kaliskiego 7, 85-789 Bydgoszcz, Poland.
D.W. Galbraith. Department of Plant Sciences, University of Arizona, 303 Forbes Building, Tucson, AZ 85721, USA.

[1]Corresponding author (e-mail: elwira@utp.edu.pl).

example the stem, frequently contain mucilages, making flow cytometric measurements difficult. Thus, for these species, it is imperative to identify other plant tissues that are suitable for nuclear isolation and C-value estimation using FCM.

Seeds of many species, where most of the embryo cells in the dry state are arrested in the G_0–G_1 phase of the cell cycle (having a 2C DNA content), are suitable for the estimation of DNA content using FCM (Sliwinska et al. 2005; Sliwinska 2006). For species that did not accumulate staining inhibitors within leaves, C-values based on FCM were identical for seeds and leaves. For species that accumulate DNA staining inhibitors in leaves, seeds remained suitable for FCM-based estimation of C-values. An additional advantage of using seeds instead of leaves for the estimation of DNA content is that they can be transported and stored dry with no distance or time limit, and analyzed when convenient. Consequently, seeds could be employed for C-value estimations for species growing in regions where cytometers are not readily available. Using seeds, which in nature are usually produced in excess, for FCM analyses, avoids endangering plants taken from conservation areas, examples being the cacti of the Sonoran Desert.

The reason that seeds, despite all these advantages, are not currently preferred as source tissues for genome size analysis relates to the requirement for a detailed understanding of the seed biology of each species of interest. Within the seeds of some species, additional peaks are seen within the FCM histograms of nuclear DNA content beyond those corresponding to 2C and 4C. These peaks originate from endosperm and (or) endoreplicated embryo tissues, and can greatly complicate interpretation of the results (Bino et al. 1993; Matzk et al. 2000, 2001; Sliwinska et al. 2005). In our previous paper (Sliwinska et al. 2005), we showed that it is possible to dissect tissues having nuclei predominantly arrested in the G_0–G_1 phase of the cell cycle, from seeds, prior to FCM analysis. However, that study investigated only five angiosperm herbaceous species; further studies with additional species containing different types of seeds would be needed to establish whether seeds can be generally employed for genome size estimation by flow cytometry, and to provide robust methods for users that are not familiar with seed biology. Since seed type is usually related to the taxonomy of the species, the protocols reported in the previous (Sliwinska et al. 2005) and present papers should be useful resources for these types of analysis in related species.

The objective of this study was to establish procedures for using seeds in flow cytometric estimations of genome sizes of selected desert species of different life habit (trees, shrubs, herbs), including those producing seeds that contain endosperm or endopolyploid embryo nuclei. For only one species, the gymnosperm *Cupressus arizonica* Greene, have seeds been previously studied by flow cytometry (Pichot and El Maataoui 1997). Of 24 species employed in the present experiment, nuclear DNA contents have been previously reported for only four (established using Feulgen densitometry); for the remaining 20, to the best of our knowledge, this is the first reported estimation of their C-values. These results also permitted a discussion of the possible impacts of the desert environment on genome size.

Materials and methods

Plant material

Seeds of 24 species found in the deserts of the southwest USA and Mexico, belonging to eight families (Table 1), were obtained either through collection from the Campus Arboretum of the University of Arizona, Tucson (Ariz., USA), or from commercial sources. Of the 23 angiosperms, two belonged to the monocotyledons and 21 to the dicotyledons. An additional species employed in this work, *C. arizonica*, is a gymnosperm.

For estimation of nuclear DNA content, the following internal standards were used: *Petunia hybrida* PxPc6 (2C = 2.85 pg; Marie and Brown 1993), *Pisum sativum* 'Set' (2C = 9.11 pg; Sliwinska et al. 2005), *Raphanus sativus* 'Saxa' (2C = 1.11 pg; Doležel et al. 1992), *Zea mays* CE-777 line (2C = 5.43 pg; Lysák and Doležel 1998). *Baileya multiradiata* Harv. & A. Gray ex Torr. and *Cassia artemisioides* (Gaud. ex DC.) Randell (2C = 14.64 pg and 1.89 pg, respectively; calibrated in this experiment) served as secondary internal standards.

Estimation of seed mass

For each species, seeds from different plants were bulked to form one sample. Four random subsamples, each containing 50 seeds, were then weighed using an analytical balance, The mean value was then recalculated to provide the mass of 100 seeds. These seed samples were also employed for measurement of cell cycle activities and genome size.

Estimation of the proportion of nuclei of different DNA contents in entire seeds

Flow cytometric analyses were performed at the Laboratory of Molecular Biology and Cytometry, UTLS Bydgoszcz (Poland). Single seeds were chopped with a sharp razor blade in a plastic Petri dish containing 1 mL Galbraith's buffer (45 mmol·L^{-1} $MgCl_2$, 30 mmol·L^{-1} sodium citrate, 20 mmol·L^{-1} 3-[*N*-morpholino] propanesulphonic acid, 0.1% *v*/*v* Triton X-100, pH 7.0; Galbraith et al. 1983), supplemented with 4′,6-diamidino-2-phenylindole (DAPI; 2 μg·mL^{-1}). After chopping, the suspension was passed through a 50 μm mesh nylon filter and incubated for 15 min. For each sample, 5000–10 000 nuclei were analysed using a Partec CCA (Münster, Germany) flow cytometer and a mercury HBO lamp as the light source. Analyses were performed on 15 replicates (each originating from a different seed(s) of the original bulked sample), using a logarithmic scale. Histograms were analysed using the DPAC version 2.2 computer programme (Partec GmbH, Münster, Germany), and the percentages of nuclei having particular DNA contents were determined. The mean C-value (mean ploidy) of a sample was calculated as described by Lemontey et al. (2000). In this work, only embryo nuclei having a DNA content of at least 8C were considered to be endopolyploid, since it is not possible to distinguish, by FCM analysis, 4C nuclei of cells having just entered endoreplication (i.e., being at the G_1 phase of the first endocycle) from those of cells within the G_2 phase of the mitotic cycle.

Estimation of the nuclear DNA content

For species having seeds that contain a considerable proportion of endoreplicated nuclei (Table 2), the seeds were

Table 1. List of the 24 desert species included in study.

Family	Species	Common name	Monocot (M) or dicot (D)	Life habit[a]	Life cycle type[b]
Agavaceae	*Agave deserti* Engelm. var. *deserti*	Desert agave	M	SS, SH, FB	P
	Agave parryi Engelm. var. *huachucensis* (Baker) Little in L.D. Benson	Ft. Huachuca agave	M	SS, SH, FB	P
Asteraceae	*Ambrosia deltoidea* (Torr.) Payne	Triangle-leaf bursage	D	SS, SH	P
	Ambrosia dumosa (A. Gray) Payne	White bursage	D	SS, SH	P
	Baileya multiradiata Harv. & A. Gray ex Torr.	Desert marigold	D	FB	A, B, P
	Encelia farinosa A. Gray ex Torr.	Brittle bush	D	SS, SH	P
Cactaceae	*Astrophytum ornatum* Britt. & Rose	Monk's hood	D	SH	
	Carnegiea gigantea (Engelm.) Britt. & Rose	Giant saguaro	D	TR	
	Echinocactus grusonii Hildm.	Golden barrel	D	SH	P
	Ferocactus wislizenii (Engelm.) Britt. & Rose	Fishhook barrel	D	TR, SH	P
	Neobuxbaumia polylopha (DC.) Backeb.	Cone bactus	D	TR	P
	Opuntia violacea var. *santa-rita* (Griffiths & Hare) L.D. Benson in L.D. Benson	Purple prickly pear	D	TR, SH	P
	Stenocereus thurberi (Engelm.) Buxb.	Organ pipe cactus	D	TR, SH	P
Chenopodiaceae	*Atriplex polycarpa* (Torr.) Wats.	Desert saltbush	D	SH	P
Cupressaceae	*Cupressus arizonica* Greene	Arizona cypress	n/a	TR	P
Fabaceae	*Acacia constricta* Benth.	White-thorn acacia	D	TR, SH	P
	Acacia smallii Isely	Sweet acacia	D	TR, SH	P
	Cassia artemisioides (Gaud. ex DC.) Randell	Feathery cassia	D	SH	P
	Olneya tesota A. Gray	Desert ironwood	D	TR, SH	P
	Parkinsonia aculeata L.	Mexican palo verde	D	TR, SH	P
	Prosopis velutina Woot.	Arizona mesquite	D	TR, SH	P
Oleaceae	*Fraxinus velutina* Torr.	Arizona ash	D	TR	P
Zygophyllaceae	*Kallstroemia grandiflora* Torr. ex A. Gray	Arizona poppy	D	FB	A
	Larrea tridentata Coville	Creosote bush	D	SH	P

[a]FB, forb–herb; SH, shrub; SS, subshrub; TR, tree; according to NRCS Plants Database, plants.usda.gov.
[b]A, annual; B, biannual; P, perennial.

Table 2. Characteristics of the seeds of 24 desert species.

Family	Species	100 seeds mass (g) (n = 4)	Mean C-value (C±SE, n = 15)	Percentage of the nuclei with DNA content (n = 15)							
				1C	2C	3C	4C	6C	8C	16C	32C
Agavaceae	*A. deserti*	1.005	2.47±0.02		58.5	39.8		1.7			
	A. parryi	0.974	2.47±0.02		60.6	37.0		2.4			
Asteraceae	*A. deltoidea*	1.965	2.10±0.00		92.8	4.7	2.5				
	A. dumosa	0.509	2.12±0.00		91.0	5.8	3.2				
	B. multiradiata	0.075	2.14±0.01		93.0		7.0				
	E. farinosa	0.101	2.12±0.01		90.4	7.2	2.4				
Cactaceae	*A. ornatum*	0.192	3.97±0.05		39.8	5.7	36.1	1.2	15.8	1.4	
	C. gigantea	0.161	3.44±0.02		34.2	6.1	54.4	1.3	4.0		
	E. grusonii	0.060	3.24±0.03		43.8	10.4	39.3	2.2	4.3		
	F. wislizenii	0.206	3.16±0.04		46.1	7.1	41.8	2.4	2.6		
	N. polylopha	0.375	6.28±0.09		22.5		37.8		26.9	12.1	0.7
	O. violacea	1.511	2.50±0.02		73.8	4.0	21.6	0.6			
	S. thurberi	0.164	3.36±0.03		38.9	6.8	48.4	1.3	4.6		
Chenopodiaceae	*A. polycarpa*	0.086	3.02±0.03		59.7	4.6	27.0	4.3	4.4		
Cupressaceae	*C. arizonica*	0.758	1.47±0.03	52.8	47.2						
Fabaceae	*A. constricta*	1.569	3.80±0.11		47.3		38.6		11.8	2.3	
	A. smallii	7.032	5.55±0.12		29.4		39.0		22.7	7.9	1.0
	C. artemisioides	1.430	2.22±0.01		89.4	2.9	5.8	1.9			
	O. tesota	19.610	2.08±0.01		96.2		3.8				
	P. aculeata	12.720	2.10±0.01		94.8		5.2				
	P. velutina	3.908	3.25±0.03		50.9		42.4		6.7		
Oleaceae	*F. velutina*	2.642	2.39±0.02		60.8	39.2					
Zygophyllaceae	*K. grandiflora*	0.384	2.38±0.03		81.0		19.0				
	L. tridentata	0.444	2.44±0.02		74.1	16.2	5.7	4.0			

Note: n, refers to the number of replications (see Materials and methods: Estimation of seed mass).

dissected, and the tissues with the greatest proportion of embryo nuclei arrested in the G_0–G_1 phase of the cell cycle were selected for DNA content estimation (Table 3). For the remaining species, entire seeds were used. If they were characterized by low mass, up to eight seeds were amalgamated to form a single sample, to obtain a sufficient number of nuclei. For all of the internal standards, with the exception of *P. hybrida*, radicles were used as the source material. Because of the small size of petunia seed, a few entire seeds were included in an individual sample. Before genome size estimation, the seeds of all species were tested for the presence of staining inhibitors (Price et al. 2000). An entire seed(s) or seed part(s) was chopped simultaneously with the seed (radicle) of an internal standard in 1 mL Galbraith's buffer (Galbraith et al. 1983), supplemented with propidium iodide (PI; 50 $\mu g \cdot mL^{-1}$; although this fluorochrome gives lower resolution histograms than DAPI, the latter fluorochrome is not appropriate for genome size estimation, because it binds preferentially to A-T base pairs) and ribonuclease A (50 $\mu g \cdot mL^{-1}$), and then prepared as described above, except that the time of incubation for *Carnegiea gigantea* (Engelm.) Britt. & Rose was reduced to 5 min, and the samples of *Parkinsonia aculeata* L., *Prosopis velutina* Woot., and *Fraxinus velutina* Torr. were analyzed immediately after preparation (the optimal time of sample incubation had been established for each species in preliminary experiments). Instead of an HBO lamp, an argon laser was used as the source of illumination, and a linear scale was employed. For each species, 15 samples (each originating from different seed (seeds) in the original bulked sample) were analyzed. Nuclear DNA contents were calculated by comparing the positions of the 2C peaks of the test species and the internal standard within the histograms of fluorescence intensities (Galbraith et al. 1983). DNA contents (pg) were converted to megabase pairs of nucleotides using the relationship 1 pg = 978 Mbp (Doležel et al. 2003). Finally, the Pearson's correlation coefficient was calculated to quantify the relationship between genome size and seed mass for the angiosperm species.

Results

Seed masses, and proportions of nuclei having different DNA contents

The values for the masses of 100 seeds varied considerably across species, from about 0.06 g for the cactus *Echinocactus grusonii* Hildm., to almost 20 g for *Olneya tesota* A. Gray (Fabaceae; a 327-fold difference; Table 2). The species having the second and the third largest seeds (*P. aculeata*, 13 g, and *Acacia smallii* Isely, 7 g, respectively) also belonged to the Fabaceae. The mass values for 100 seeds of 14 species was below 1 g, including three of only about 0.1 g.

Only the seeds of the four species, *Agave deserti* Engelm., *Agave parryi* Engelm., *C. arizonica*, and *F. velutina* can be considered as endospermic; for these species, the nuclei of the embryo (2C and 4C) comprised only about 50%–60% of the total number of nuclei within the seed (Table 2;

Table 3. Nuclear DNA content in 24 desert species.

		2C DNA content (n = 15)					
Family (2C range; pg)[a]	Species	pg ±SE	Mbp	Genome size category[b]	Plant material used for analysis	Standard species[c]	CV[d]
Agavaceae (5.10–25.05)	*A. deserti*	17.71±0.09	17320	Intermediate	Embryo	B	3.29
	A. parryi	26.96±0.10	26367	Intermediate	Embryo	B	3.43
Asteraceae (4.20–52.30)	*A. deltoidea*	4.94±0.03	4831	Small	Entire seed	P	3.45
	A. dumosa	7.92±0.07	7746	Intermediate	Entire seed	Z	3.46
	B. multiradiata	14.64±0.05	14318	Intermediate	Entire seed	P	2.98
	E. farinosa	3.13±0.04	3061	Small	Entire seed	Z	4.30
Cactaceae (3.05–14.20)	*A. ornatum*	3.66±0.02	3579	Small	Radicle and part of hypocotyl	P	5.12
	C. gigantea	2.87±0.04	2807	Small	Entire seed	R	3.42
	E. grusonii	2.85±0.03	2787	Small	Entire seed	R	4.61
	F. wislizenii	2.80±0.03	2738	Very small	Entire seed	R	3.54
	N. polylopha	3.20±0.02	3130	Small	Entire seed	P	4.98
	O. violacea	4.07±0.01	3980	Small	Entire seed	Z	3.88
	S. thurberi	3.44±0.04	3364	Small	Entire seed	Z	4.74
Chenopodiaceae (0.85–4.30)	*A. polycarpa*	3.04±0.01	2973	Small	Entire seed	Z	4.02
Cupressaceae (16.50–40.00)	*C. arizonica*	25.34±0.22	24783	Intermediate	Entire seed	P	2.69
Fabaceae (0.61–54.80)	*A. constricta*	1.45±0.01	1418	Very small	Radicle	C	5.45
	A. smallii	1.72±0.00	1682	Very small	Radicle	Ph	4.66
	C. artemisioides	1.89±0.01	1848	Very small	Radicle	R	4.12
	O. tesota	1.96±0.02	1917	Very small	Radicle	Ph	4.51
	P. aculeata	0.79±0.01	773	Very small	Radicle	R	6.17
	P. velutina	0.86±0.01	841	Very small	Radicle	R	5.35
Oleaceae (1.95–4.14)	*F. velutina*	1.74±0.02	1702	Very small	Radicle	R	3.95
Zygophyllaceae (0.70–5.45)	*K. grandiflora*	1.39±0.02	1359	Very small	Radicle	C	4.19
	L. tridentata	3.46±0.02	3384	Small	Entire seed	P	3.42

[a]According to Bennett and Leitch, 2005*c*.

[b]According to Soltis et al. 2003.

[c]B, *B. multiradiata*; C, *C. artemisioides*; P, *P. sativum*; Ph, *P. hybrida*; R, *R. sativus*; Z, *Z. mays*.

[d]Mean coefficient of variation for the peak of the species in question.

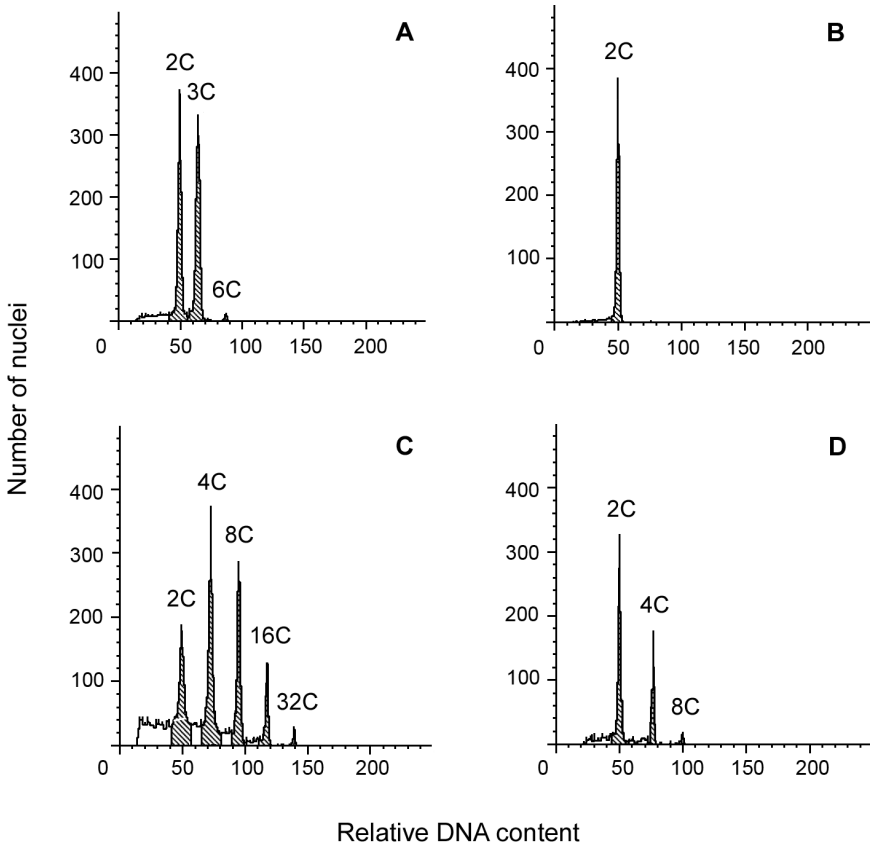
Fig. 1A). Endosperm nuclei were not found in the seeds of 8 species, and in the remaining 12 species, 2%–20% of endosperm nuclei were detected. Endoreplication did not occur in the endosperm of any species, but endopolyploid nuclei were found in the embryos of 10 species. Endoreplication was the highest, up to 32C, in the seeds of *Neobuxbaumia polylopha* (DC.) Backeb. and *A. smallii* (Fig. 1C), and consequently the mean C-value was the highest in those seeds (about 6; Table 2). The mean C-value was approximately four in the seed of two species with endoreplication up to 16C, *Astrophytum ornatum* Britt. & Rose and *Acacia constricta* Benth., about three in those in which no more than two endoreplication cycles occurred, and slightly over two in the seeds containing only 2C and 4C nuclei. It was below two only for *C. arizonica*, the gymnosperm species, which possessed a 1C endosperm.

Genome size

None of the seeds studied contained staining inhibitors. For 13 species, entire seeds could be used for the estimation of DNA content (Table 3). In the remaining cases, isolation of the embryo or radicle was necessary (Table 3, Fig. 1). For each species, the appropriate internal standard was chosen not only according to the closeness of distance between the G_0–G_1 peak of the studied and reference species, but also according to the presence of additional peaks (of the endosperm or the embryo endoreplicated nuclei) that might also overlap the standard peaks. After the selection of the appropriate plant material, histograms of acceptable quality were obtained for all species; the CVs of the G_0–G_1 peaks were 3%–5% (Table 3, Fig. 2).

The DNA content ranged from 0.79 pg per 2C in *P. aculeata* to 26.96 pg per 2C in *A. parryi* (a 34-fold difference; Table 3). Applying the categorization proposed by Soltis et al. (2003), 9 species can be considered as having very small genomes (C-value < 1.4 pg), 10 as having small genomes (> 1.4 to < 3.5 pg), and 5 as having genomes of intermediate size (> 3.5 pg to < 14 pg). Intermediate genome sizes were seen only within the Agavaceae, Asteraceae, and Cupressaceae. No significant correlation at $P = 0.05$ (two-tailed probability) was found between genome size and seed mass ($r = –0.220$; $P = 0.313$) when the calculation included all angiosperm species. The three species with the largest genomes produced rather small seeds, the mass of 100 seeds of the two *Agave* species was about 1 g, and that of seeds of *B. multiradiata* was below 0.1 g (Tables 2 and 3). The two species producing the largest seeds, *O. tesota* and *P. aculeate*, possessed very small genomes. The correlations were also not significant when calculated only for dicots ($r = –0.300$; $P = 0.186$), or separately for Asteraceae ($r = –0.380$; $P = 0.620$), Cactaceae ($r = 0.752$; $P = 0.051$), and Fabaceae ($r = 0.062$; $P = 0.907$).

Fig. 1. Histograms of relative DAPI fluorescence (logarithmic amplification; twofold increase in fluorescence appears as a 22 channels displacement of the peak position) in nuclei of the seeds of *Agave deserti* (A and B), and *Acacia smallii* (C and D). (A and C) entire seed, (B) embryo, and (D) radicle.



Discussion

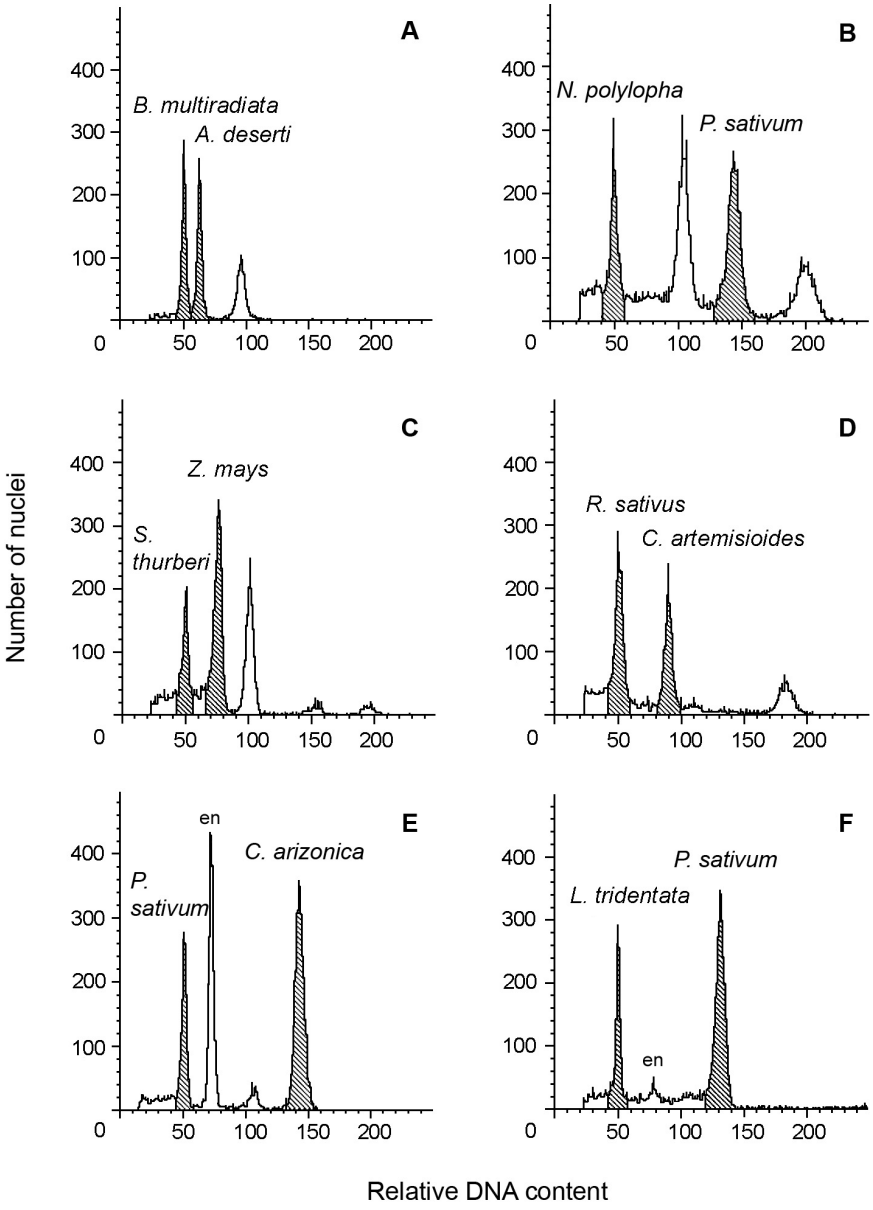
Selecting seed tissues suitable for estimation of DNA content by flow cytometry

At present, mostly young leaves are used for flow cytometric estimations of nuclear DNA contents in plants (Arumuganathan and Earle 1991; Galbraith et al. 1998; Doležel and Bartoš 2005). They are usually easily available, and contain a predominance of cells within the G_0–G_1 phase of the cell cycle, having a 2C DNA content. However, leaves should be analyzed fresh, and therefore, transportation over a long distance and (or) storage can become problematic. In addition, the leaves of some species contain staining inhibitors that systematically bias DNA content estimation (Noirot et al. 2000; Price et al. 2000; Sliwinska et al. 2005; Bennett et al. 2008). In old leaves, endoreplication is frequently observed, which can confuse FCM genome size estimation (De Rocher et al. 1990; Galbraith et al. 1991; Lukaszewska and Sliwinska 2007). Thus, in some cases, an alternative plant source tissue would be highly desirable.

The seeds of desiccation-tolerant species are also suitable for flow cytometric analysis. When dry, they are quiescent with most, if not all, of the embryo cells arrested in the G_0–G_1 phase of the cell cycle (Bewley and Black 1994). In the work reported here, such seeds contained >80% of cells with 2C DNA contents, and were found in all species belonging to the family Asteraceae, three species of the Fabaceae, *C. artemisioides*, *O. tesota*, *P. aculeata*, and *Kallstroemia grandiflora* Torr. ex A. Gray (Zygophyllaceae). However, the seeds of some species can contain a considerable proportion of endosperm (usually haploid in gymnosperms, e.g., in *C. arizonica*, and triploid in angiosperms, e.g., in *Agave* species) that can comprise regularly divided, as well as endoreplicated cells. Embryos can also contain endopolyploid cells (e.g., up to 32C in *A. smallii* and *N. polylopha*). Thus, on the flow cytometric histogram obtained from a seed, additional peaks, corresponding to the nuclei of different (higher than in the G_0–G_1 embryo nuclei) DNA content may appear, which can confuse interpretation of the results (Sliwinska 2006).

All these possibilities were taken into consideration when developing procedures for the measurement of nuclear DNA content as described in the present experiment. Therefore, the proportions of nuclei having different DNA contents in the seed of each species were first determined to identify the seed parts most appropriate for sample preparation. Only four species, *A. deserti*, *A. parryi*, *C. arizonica*, and *F. velutina* produced endospermic seeds, and they required the isolation of the embryo or radicle to obtain histograms having only a 2C peak (Figs. 1A and 1B). For *C. arizonica*, which has small seeds, isolation of the embryo was difficult, requiring use of a microscope and an experienced technician. Fortunately, dissection of the seeds was not required for this species when *P. sativum* was used as a standard (Fig. 2E), because the G_0–G_1 peaks of both species included in a sample were well separated.

Fig. 2. Selected histograms of nuclear DNA content (linear amplification) obtained after flow cytometric analysis of the PI-stained nuclei isolated from the seeds of the appropriate internal standard and: (A) *Agave deserti*, (B) *Neobuxbaumia polylopha*, (C) *Stenocereus thurberi*, (D) *Cassia artemisioides*, (E) *Cupressus arizonica*, and (F) *Larrea tridentata*. Peaks with hatch marks indicate 2C nuclei of internal standards and species in question; en, endosperm nuclei of species in question. Note that the endosperm of *C. arizonica* contains 1C DNA.



The radicle is generally considered the most reliable part of the seed for genome size estimations, especially for the seeds whose cotyledons and (or) hypocotyls contain endopolyploid cells (Sliwinska et al. 2005). For our work, if the isolation of the radicle was easy and rapid, we employed this part of the seed as the source material. However, as we found here, the entire seed is suitable as the source material for many species if 2C nuclei constitute a considerable majority of those measured.

Estimation of genome size of selected desert trees, shrubs, and herbs

Genome sizes have been established previously for four of the 24 species studied: *A. deserti*, *C. arizonica*, *P. aculeata*, and *Larrea tridentata* Coville. All but one of the 2C values estimated in this study fall within the ranges reported for the respective genera and (or) families in the Plant DNA C-values Database (Bennett and Leitch 2005*c*; Table 3). The genome size determined for *A. parryi* (26.96 pg) slightly exceeded the maximum 2C value previously reported within the genus *Agave* (*A. asperrima*, 25.05 pg). The DNA content of *A. deserti* var. *deserti* estimated in this research, 17.7 pg, is about twice that published by Bennett and Smith (1991; 9.6 pg; subspecies not provided). Since *A. deserti* comprises three subspecies, *deserti*, *simplex*, and *pringlei* (Turner et al. 1995), which possibly can differ in DNA content, and since polyploidy seems to be quite common within the genus *Agave* (Palomino et al. 2003; Bennett and Leitch 2005*c*), it can be assumed that the form studied here was tetraploid. Our

estimate for the genome size of *C. arizonica* (25.3 pg per 2C) was similar to that obtained by Ohri and Khoshoo (1986) using Feulgen densitometry and *Allium cepa* as a standard (23.6 pg per 2C). In contrast, there are inconsistencies between the 2C values of *P. aculeata* established here (0.79 pg) and previously (1.45 pg; Ohri 2002). This nearly twofold difference cannot be explained by the difference in methods, and again suggests polyploidy may need to be considered. For the fourth species, *L. tridentata*, for which the diploid, tetraploid, and hexaploid forms are reported to have 2C values of 1.5, 2.55, and 3.2 pg, respectively (Poggio et al. 1989), the form studied here, possessing 3.5 pg per 2C within the seed, most probably was a hexaploid.

Relationship between genome size and desert conditions and seed mass

One of the final objectives of this study was to determine whether any relationship might exist between the desert environment and the genome sizes of species adapted to these growing conditions. Previous reports have been conflicting, and do not provide clear evidence for the evolution of DNA content in plants growing in different environmental conditions (Knight et al. 2005 and references therein). Our results confirm the suggestion that species having large genomes are excluded from the extremes of climatic ranges (Knight and Ackerly 2002; Knight et al. 2005). The C-values in the desert species varied over 30-fold, but the majority comprised small and very small genomes. This supports a correlation of genome size with temperature and precipitation, observed previously, for example in North American *Pinus* subsp. (Wakamiya et al. 1993). Conversely, the woody angiosperms, which predominated in our study, are believed to posses a small and relatively uniform genome size, probably due to the physical constraints on nuclear size by the small cambial cells forming wood fibres (Ohri 1998 and references therein).

A positive correlation between genome size and seed mass, as previously reported (Wakamiya et al. 1993; Knight and Ackerly 2002; Knight et al. 2005; Beaulieu et al. 2007) was not confirmed in the present study, perhaps because of the limited number of species that were included. Unexpectedly, all the species with the highest DNA contents (intermediate genome size) produced rather small seeds, and those which produced the largest seeds possessed very small genomes.

In conclusion, the present work has shown that seeds are suitable source materials for the estimation of genome size by flow cytometry. They can be used as a whole, or after isolation of the embryo or radicle. The isolation of seed parts containing a majority of the embryo nuclei arrested in the G_0–G_1 phase of the cell cycle is particularly recommended for endospermic seeds and for those having high amounts of endopolyploid nuclei. However, such isolation can be often avoided when an appropriate internal standard is selected. Using seeds instead of leaves, allows one to perform the DNA content analyses with no time (season) and location limitations.

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