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# Reference standards for determination of DNA content of plant nuclei[1]

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Flow cytometry was used to compare 14 potential reference standards for plant DNA content determination. Both chicken and plant internal standards were used, as were propidium iodide (PI) and 4'-6-diamidino-2-phenylindole (DAPI) as fluorochromes. Means and standard errors of the means are presented for the 14 potential reference standards, and the means are compared to those obtained by Feulgen densitometry. Five species are recommended as an initial set of international standards for future plant DNA content determinations: *Sorghum bicolor* cv. Pioneer 8695 (2C = 1.74 pg), *Pisum sativum* cv. Minerva Maple (2C = 9.56 pg), *Hordeum vulgare* cv. Sultan (2C = 11.12 pg), *Vicia faba* (2C = 26.66 pg), and *Allium cepa* cv. Ailsa Craig (2C = 33.55 pg). It is recommended that the reference standard of choice be one with 2C and 4C nuclear DNA content peaks similar to, but not overlapping, the 2C and 4C peaks of the target species. We recommend PI as the fluorochrome of choice for flow cytometric determination of plant DNA content. DAPI should be used only if the estimated DNA value is corroborated by using a second stain that has no bias for AT- or GC-rich sequences within genomes.

**Key words:** DAPI; DNA content; flow cytometry; propidium iodide; reference standards.

Feulgen microspectrophotometry and flow cytometry are commonly used procedures for determining the DNA content of plant nuclei. The importance of reference standards and their proper use have long been emphasized (Bennett and Smith, 1976; Dhillon, Berlyn, and Miksche, 1977; Price et al., 1980). However, a significant source of error affecting estimates of DNA amount in plant nuclei continues to be the lack of proper calibration of target species against reliable standards of known DNA amount. Most of the published DNA values for plants have been calibrated directly or indirectly (Bennett and Smith, 1976, 1991; Bennett and Leitch, 1995) to *Allium cepa* (2C DNA content = 33.55 pg; Van't Hof, 1965), but some have been calibrated against chicken erythrocytes or other animal nuclear standards (e.g., Dhillon, Berlyn, and Miksche, 1977; Arumuganathan and Earle, 1991; Costich et al., 1993; Kankanpaa, Mannonen, and Schulman, 1996; and Keeler, 1992). Many such animal DNA contents directly or indirectly go back to the chemically determined value of 2.52 pg per erythrocyte nucleus for the chicken (Rasch, Barr, and Rasch, 1971). In some reports, e.g., Arumuganathan and Earle (1991), Kankanpaa, Mannonen, and Schulman (1996), and Keeler (1992), the DNA content of the chicken used was unknown and an assumed DNA content was used. This may result in significant error in estimated DNA contents, as there is considerable reported variation in DNA content among chicken lines (Bennett and Leitch, 1995). Although animal nuclei are the standards of choice in animal studies, Price et al. (1980) suggested that for technical reasons a plant standard is better for estimating the DNA content of plants. To help resolve the problem of calibration of DNA values, Bennett and colleagues (Bennett and Smith, 1976, 1991; Bennett and Leitch, 1995) calibrated a series of plants against *Allium cepa* cv. Ailsa Craig (33.5 pg) and recommended these as DNA content standards.

Price and Johnston (1996) considered the choice of a standard as critical for flow cytometric determination of DNA contents, and recommended that the standard(s) used should have DNA values close to, but not overlapping the 2C and 4C peaks of the target species. They further recommended that species from the list of Bennett and Leitch (1995) undergo further scrutiny to see which are suitable as standards for both flow and Feulgen cytometry. In this paper, we tested the suitability of a set of species, including several from the list of Bennett and Leitch (1995), as standards for flow cytometry of plant nuclei. We also recommend the use of propidium iodide as the fluorochrome of choice for flow cytometric determination of plant nuclear DNA contents.

## MATERIALS AND METHODS

***Plant material***—A set of plants was chosen that has been reported to possess 2C DNA contents ranging from ~1 to 34 pg. This included several from the list of plants recommended as standards by Bennett and Leitch (1995). A complete list of plants and their sources are listed in Table 1. All plants were grown from seed in growth chambers adjusted to a 16/8 h, 28°/20°C day/night cycle.

***Flow cytometry using propidium iodide (PI)***—Healthy young leaves from seedlings were chopped with a razor blade in ice-cold buffer consisting of, per litre, 4.26 g $MgCl_2$, 8.84 g sodium citrate, 4.2 g 3-[N-morpholino]propane sulfonic acid, 1 mL Triton X-100, 1 mg boiled ribonuclease A, pH 7.0–7.2, as described by Galbraith et al. (1983) and

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TABLE 1. A list of plant species analyzed by propidium iodide based flow cytometry and/or DAPI-based flow cytometry.

| Species | Source |
|---|---|
| *Allium cepa* cv. Ailsa Craig | M. D. Bennett, Royal Botanic Garden (RBG) Kew |
| *Hordeum vulgare* cv. Sultan | M. D. Bennett, RBG, Kew |
| *Lactuca sativa* cv. Grand Rapids | Northrup King |
| *Nicotiana tabacum* cv. Xanthi | V. Sisson, USDA Tobacco Res. Lab. |
| *Oryza sativa* cv. IR36 | W. Park, Texas A&M University (TAMU) |
| *Pisum sativum* cv. Minerva Maple | M. D. Bennett, RBG, Kew |
| *Secale cereale* cv. Petkus Spring | M. D. Bennett, RBG, Kew |
| *Secale cereale* inbred line JIC3030010 | John Innes Centre, Norwich |
| *Sorghum bicolor* Pioneer 8695 | A. L. Rayburn, University of Illinois |
| *Triticum aestivum* cv. Chinese Spring | M. D. Bennett, RBG, Kew |
| *Triticum aestivum* cv. Chinese Spring | N. Tuleen, TAMU |
| *Triticum durum* cv. Langdon | N. Tuleen, TAMU |
| *Vicia faba* cv. GS011, Lot K475 | BWI, Texarkana, Texas |
| *Vigna radiata* cv. Berken | M. D. Bennett, RBG, Kew |
| *Zea mays* VA35 | J. D. Smith, TAMU |

Price and Johnston (1996). The chopped leaves were filtered through a 53-μm mesh nylon filter or 60-μm and 15-μm mesh nylon filters, and centrifuged at 1000 × *g* for 3–5 min at 4°C. The pellet was resuspended in 500 μL stain (50 ppm PI in chopping buffer). The stain solution was replaced at 5 min, after centrifugation, by drawing off the original stain solution and adding 300 μL of new stain solution. The standard (*Hordeum vulgare* cv. Sultan, and/or chicken erythrocytes) and experimental samples were mixed at this point, stored in a dark refrigerator, and analyzed after 1–2 h by flow cytometry.

The flow cytometers were Coulter Elite (Hialeah, FL) models operated at 514 nm and an output of 500 mW (Texas A&M), and at 488 nm and an output of 20 mW (Arizona). Fluorescence emission was detected using a photomultiplier screened by a long pass filter permitting passage of light of wavelength above 615 nm.

***Flow cytometry using 4′-6-diamidino-2-phenylindole (DAPI)***—Nuclei were isolated from seedlings following procedures of Rayburn et al. (1989, 1993). *Sorghum bicolor* cv. Pioneer 8695, calibrated against *Hordeum vulgare* cv. Sultan, is routinely used as a standard by ALR and, therefore, was used as the standard for the DAPI-based flow cytometry of this study. Seedlings of the target species and *S. bicolor* were ground together in a small homogenizer for 30 s in 10 mL of extraction buffer consisting of 1.0 mol/m$^3$ hexelene glycol, 10 mol/m$^3$ Tris (pH = 8.0), 10 mol/m$^3$ $Mg_2Cl_2$, and 0.5% Triton X-100. The homogenized tissues were filtered through 250 μm, 53 μm, and 20 μm mesh and centrifuged at 500 × *g* for 15 min. The pellet was resuspended in 70% ethanol for 10 min, centrifuged at 500 × *g* for 10 min, and resuspended in 250 μL extraction buffer. DAPI (4 μg) was added and the samples were kept in a dark refrigerator for 1 h prior to analysis. Samples of nuclei were analyzed with a Coulter EPICS 751 flow cytometer cell sorter system. The excitation beam was provided by a 5-W argon-ion laser tuned to a wavelength of 351 nm and operating at 250 mW. Fluorescence emission was detected using a photomultiplier screened by a band pass filter permitting passage of light of wavelength 480 ± 20 nm.

***Comparisons of chicken erythrocytes***—Chicken (*Gallus domesticus* L.) red blood cells (CRBCs) were compared from three sources: (1) a White Leghorn from the University of Arizona collected in heparinized tubes and stored several years at −80°C prior to use; (2) an inbred pathogen-free White Leghorn line from the College of Veterinary Medicine, Texas A&M University (TAMU), and (3) a Rhode Island Red line (Biosure CEN Cytometry Control, Product Number 1006, Reese Enterprises, Inc., Grass Valley, California) prepared within 1 wk of use and held at −20°C until preparation. One sample of CRBCs from a TAMU White Leghorn had been stored in heparinized tubes for several years at −80°C, and a second sample from a recent collection from the same populations was kept at −20°C for <1 wk prior to use in these studies. CRBCs from these sources were prepared for flow cytometric analysis individually and in paired combinations (six total), following the detergent and proteolytic enzyme-based technique of Vindelov, Christensen, and Nisson (1983).

TABLE 2. DNA content of species determined by flow cytometry of propidium-iodide-stained nuclei using *Hordium vulgare* cv. Sultan as a standard.

| Species | Mean 2C DNA content (pg) | Number of plants | SE |
|---|---|---|---|
| *Oryza sativa* IR36 | 1.08 | 17 | 0.01 |
| *Vigna radiata* cv. Berken | 1.40 | 21 | 0.01 |
| *Lactuca sativa* cv. Grand Rapids | 5.95 | 10 | 0.02 |
| *Zea mays* VA35 | 5.73 | 36 | 0.01 |
| *Pisum sativum* cv. Minerva Maple | 9.39 | 14 | 0.01 |
| *Nicotiana tabacum* cv. Xanthi | 10.04 | 6 | 0.03 |
| *Secale cereale* cv. Petkus Spring | 16.65 | 24 | 0.02 |
| *Secale cereale* inbred line JIC3030010 | 15.39 | 10 | 0.09 |
| *Sorghum bicolor,* Pioneer 8595 | 1.74 | 10 | 0.01 |
| *Triticum durum* cv. Langdon | 21.50 | 16 | 0.37 |
| *Vicia faba* cv. GS011 | 26.66 | 11 | 0.05 |
| *Triticum aestivum* cv. Chinese Spring (Bennett) | 31.90 | 10 | 0.16 |
| *Triticum aestivum* cv. Chinese Spring (Tuleen) | 33.22 | 20 | 0.08 |
| *Allium cepa* cv. Ailsa Craig | 32.97 | 17 | 0.04 |

## RESULTS

***Analysis of genome sizes using propidium iodide (PI)***—Table 2 presents DNA values determined at TAMU for the plants using PI-stained nuclei and *Hordeum vulgare* cv. Sultan (2C DNA content = 11.12) as a standard. *Hordeum* was chosen as a standard based upon our extensive previous use at TAMU, which indicates stability in DNA content and ease of preparation (Price and Johnston, 1996). Although most of the plants possessed little variation in DNA content among repeated runs, the coefficient of variation of the G1 peak was high for some of the cereals, i.e., *Oryza sativa, Secale cereale* cv. Petkus spring, *Triticum turgicum,* and *T. aestivum.* Table 3 presents a similar set of plants for which the DNA contents were determined at the University of Arizona by flow cytometry of PI-stained nuclei using male White Leghorn CRBCs as the internal standard. The plant genome values determined by flow cytometry at the two locations are in close agreement (see Table 6), if a value for the chicken standard at the University of Arizona is taken to be 3.01 pg. This value was obtained following transport of heparinized samples on ice from the University of Arizona to TAMU and involved calibration against recently collected CRBCs from a White Leghorn rooster (2.54 pg) and *Tetraodes* sp. (1.0 pg) (Table 4). We also compared these to CRBCs from a rooster of the same TAMU population, which had been stored in heparinized tubes at −80°C for several years and to CRBCs from a recent commercial preparation from a Rhode Is-

TABLE 3. DNA content of plant species determined by flow cytometry of propidium-iodide-stained nuclei using chicken erythrocytes as a standard.

| Species | Mean 2C DNA content (pg)[a] | Number of plants | SE |
|---|---|---|---|
| *Oryza sativa* IR36 | 1.16 | 2 | 0.05 |
| *Vigna radiata* cv. Berken | 1.42 | 5 | 0.09 |
| *Pisum sativum* cv. Minerva Maple | 9.21 | 6 | 0.09 |
| *Hordeum vulgare* cv. Sultan | 11.13 | 7 | 0.13 |
| *Nicotiana tabacum* cv. Xanthi | 10.17 | 7 | 0.14 |
| *Secale cereale* cv. Petkus Spring | 17.12 | 6 | 0.19 |
| *Triticum durum* cv. Langdon | 25.93 | 6 | 0.53 |
| *Triticum aestivum* cv. Chinese Spring (Bennett) | 36.34 | 6 | 0.86 |
| *Triticum aestivum* cv. Chinese Spring (Tuleen) | 37.01 | 6 | 1.57 |

[a] Chicken red blood cells standardized against *Tetraodes* sp. and another chicken had fluorescence equivalent to a 2C DNA content of 3.01 pg (see Table 4).

land Red hen. These had mean fluorescence equivalent to that expected for DNA contents of 2.98 pg and 2.48 pg, respectively (data not shown).

***Analysis of genome sizes using DAPI***—The DNA estimates of the plants using DAPI as a fluorochrome are presented in Table 5. These were calibrated using a *Sorghum bicolor* line whose DNA amount was estimated in this study using Sultan Barley (11.12 pg) as a standard. The DNA values obtained after DAPI staining agreed well with those obtained using PI with three exceptions; the DNA contents of rice, sorghum, and *A. cepa* are 28, 34, and 74% higher relative to those determined using PI as the fluorochrome.

***Correlation of flow cytometric and microspectrophotometric measurements***—The results acquired using PI and DAPI are compared to each other and to DNA values estimated using Feulgen microspectrophotometry in Table 6. The values obtained with PI using barley or chicken as standards agree well with microspectrophotometric determinations of Feulgen-stained nuclei in our laboratories. The correlation statistics for Feulgen microspectrophotometric DNA contents vs. PI (*H. vulgare* standard) are $Y = 0.368 + 0.949X$ ($R^2 = 0.996$). A similar high correlation was obtained between the Feulgen and PI values (chicken standard) $Y = -0.193 + 1.04X$ ($R^2 = 0.998$). A weaker correlation was observed between the Feulgen data and that obtained using DAPI ($Y = -1.554 + 1.344X$, $R^2 = 0.837$). However, the weak correlation using DAPI was almost entirely due to the discrepancy between the Feulgen and DAPI values for *A. cepa.* When the remaining values are compared, the correlation of Feulgen and DAPI values is $Y = 0.542 + 0.966X$ ($R^2 = 1.00$). The disparate DAPI-based value for *A. cepa* was confirmed at TAMU on a Partec flow cytometer using *H. vulgare* cv. Sultan as the standard (J. Burson and J. Actkinson, unpublished data).

TABLE 4. Calibration of two different chickens using *Tetraodes* sp. (2C DNA content = 1.0 pg) as an internal standard.

| Sample number | *Tetraodes* sp. Mean channel number | Texas A&M chicken Mean channel number | Texas A&M chicken Mean DNA (pg) content | Arizona chicken Mean channel number | Arizona chicken Mean DNA (pg) content |
|---|---|---|---|---|---|
| 1 | 228.5 | 561.1 | 2.46 | — | — |
| 2 | 285.1 | 709.6 | 2.49 | — | — |
| 3 | 215.9 | 536.6 | 2.48 | — | — |
| 4 | 216.5 | 537.6 | 2.48 | — | — |
| 5 | 213.6 | — | — | 644.6 | 3.02 |
| 6 | 201.6 | — | — | 617.2 | 3.06 |
| 7 | 218.7 | — | — | 661.8 | 3.03 |
| 8 | 271.9 | 681.0 | 2.50 | 810.7 | 2.98 |
| 9 | 272.1 | 678.1 | 2.49 | 808.0 | 2.97 |
| 10 | 272.1 | 683.9 | 2.51 | 810.5 | 2.98 |
| | | | $\bar{X}$ = 2.49 | | $\bar{X}$ = 3.01 |

TABLE 5. DNA content of species determined by flow cytometry of DAPI-stained nuclei.

| Species | Mean 2C DNA content (pg) | Number of plants | SE |
|---|---|---|---|
| *Oryza sativa* IR36 | 1.39 | 13 | 0.04 |
| *Zea mays* VA35 | 5.64 | 13 | 0.10 |
| *Secale cereale* cv. Petkus Spring | 16.58 | 13 | 0.18 |
| *Triticum durum* cv. Langdon | 24.77 | 13 | 0.35 |
| *Triticum aestivum* cv. Chinese Spring (Bennett) | 34.00 | 13 | 0.39 |
| *Triticum aestivum* cv. Chinese Spring (Tuleen) | 34.29 | 13 | 0.79 |
| *Allium cepa* cv. Ailsa Craig | 57.48 | 12 | 0.94 |

*Note:* The standard was *Sorghum bicolor* cv. Pioneer 8695 (2C DNA content = 2.34 pg) calibrated against *H. vulgare* cv. Sultan.

## DISCUSSION

This study was undertaken first to evaluate the accuracy of measurement of plant nuclear DNA contents us-

TABLE 6. Comparison of DNA values determined by flow cytometry and Feulgen microspectrophotometry.

| Species | Mean 2C DNA content (pg) Flow cytometry PI[a] | PI[b] | DAPI | Feulgen microspectrophotometry |
|---|---|---|---|---|
| *Oryza sativa* IR36 | 1.08 | 1.16 | 1.39 | 1.01[c] |
| *Vigna radiata* cv. Berken | 1.40 | 1.42 | | 1.06[c] |
| *Lactuca sativa* cv. Grand Rapids | 5.95 | | | 5.61[d] |
| *Zea mays* VA35 | 5.73 | | 5.64 | 5.16[e] |
| *Pisum sativum* cv. Minerva Maple | 9.39 | 9.21 | | 9.73[c] |
| *Nicotiana tabacum* cv. Xanthi | 10.04 | 10.17 | | |
| *Hordeum vulgare* cv. Sultan | 11.12 | 11.13 | 11.12 | 11.12[c] |
| *Secale cereale* cv. Petkus Spring | 16.65 | 17.12 | 16.58 | 16.57[c] |
| *Secale cereale* inbred JIC3030010 | 15.39 | | | 16.92[f] |
| *Sorghum bicolor,* Pioneer 8695 | 1.74 | | 2.34 | 1.74[g] |
| *Triticum durum* cv. Langdon | 21.50 | 25.93 | 24.77 | |
| *Vicia faba* cv. GS011 | 26.66 | | | 26.66[c] |
| *Triticum aestivum* cv. Chinese Spring | | | | |
| (Bennett) | 31.90 | 36.34 | 34.00 | 34.64[c] |
| (Tuleen) | 33.22 | 37.01 | 34.29 | |
| *Allium cepa* cv. Ailsa Craig | 32.97 | | 57.48 | 33.55[c,h] |

[a] Barley Standard.
[b] Chicken standard.
[c] Bennett and Smith (1976).
[d] Michaelson et al. (1991).
[e] Laurie and Bennett (1985).
[f] Current study.
[g] Michaelson et al. (1991), cv. Hegari White.
[h] Van't Hof (1965), chemically determined.

ing two commonly used fluorochromes (PI and DAPI) and flow cytometry. Second, we wanted to examine the appropriateness of using plant and/or chicken nuclei as internal standards for these measurements. Third, we wished to examine the degree to which flow cytometric results correlated with results from Feulgen microspectrophotometry. From this study, we make recommendations on the use of fluorochromes and internal standards, and provide an initial set of plant standards that appear well suited for both flow cytometry and Feulgen microspectrophotometry.

***Choice of standards***—Animal nuclei have been used by some investigators for standards in estimating plant DNA by Feulgen microspectrophotometry (Dhillon, Berlyn, and Miksche, 1977; Berlyn, Berlyn, and Beck, 1986) and by flow cytometry (Galbraith et al., 1983; Arumuganathan and Earle, 1991; also see Bennett and Leitch, 1995). An advantage of a chicken standard is that a single chicken can supply an easily extracted source of cells for numerous experiments. Dhillon, Berlyn, and Miksche (1977) and Berlyn, Berlyn, and Beck (1986) were strong proponents of chicken erythrocytes as standards for plant DNA contents determined by Feulgen microspectrophotometry. However, Price et al. (1980) recommended plant nuclei for internal standards. This was based in part on differences in the hydrolysis rates of chicken and plant nuclei. Since the peak in the maximum absorbancy of chicken erythrocytes following acid hydrolysis occurs at a much shorter time than that of most plants, chicken and plant nuclei must be hydrolyzed separately and combined on a common slide after hydrolysis (Dhillon, Berlyn, and Miksche, 1977). Therefore, the chicken does not provide a true internal standard for Feulgen microspectrophotometry. A further disadvantage to using chicken as a standard in both microspectrophotometry and flow cytometry is its low nuclear DNA content relative to many plant species. Since DNA contents are more accurate when the standard and sample have nuclear DNA contents of similar size, the chicken is not preferable for use as a standard for plant species of high DNA content. This problem is exemplified in Table 6, which indicates a divergence in the estimated DNA contents of plants with larger genomes when comparing those determined with chicken (3.01 pg) and *H. vulgare* (11.12 pg) standards to Feulgen-derived values. Another potential problem with a chicken standard is that mean fluorescence of samples may not be the same among different preparations, even though the DNA content is apparently the same (Table 4)

The reason for the different mean fluorescence values among CRBC samples is not known, but the values at 3 pg have in common that they were collected in heparinized tubes and stored at −80°C for several years prior to use in this study. Heparin can induce swelling of nuclei and depletion of histones from interphase chromatin of CRBCs (Adolph, Cheng, and Laemmli, 1977) and hence may be expected to increase accessibility of PI. Therefore, the observed differences in fluorescence likely represent differential PI intercalation by CRBCs rather than real differences in DNA content.

***Choice of fluorochromes (PI vs. DAPI)***—The choice of fluorochromes is primarily determined by the excitation source available. PI is excited by visible light with an absorbancy maximum at 490 nm, while DAPI is excited by UV light at 350 nm. However, the two dyes have quite different stain reactions. PI intercalates between base pairs of double-stranded DNA and RNA with little or no base specificity (Properi, Giangare, and Bottiroli, 1991), while DAPI is a nonintercalating stain that binds preferentially and in a complex manner to A-T base regions (Godelle et al., 1993). We show here that PI-based flow cytometry produces results very consistent with those based on Feulgen microspectrophotometry. Moreover, the results were consistent for different laboratories using different excitation wavelengths, different excitation energy, and different internal standards.

With few exceptions, DAPI-based flow cytometry gave values that agreed well with Feulgen and PI results. The largest exception involved DAPI staining of *A. cepa* nuclei, where the estimated value was almost twice that found for measurements using Feulgen or PI. This major discrepancy was detected using different standards in different laboratories and indicates that DAPI should not be used as the sole basis for DNA content comparisons. Dolezel, Sgorbati, and Lucretti (1992) detected highly significant differences in plant nuclear DNA contents obtained using DAPI and PI and questioned the reliability of base preference fluorochromes. Therefore any initial genome size report based upon DAPI should be verified using a base ratio independent stain. For UV excitation, ethidium bromide, which is excited at 350 nm, has been successfully used to measure genome size (Baranyi, Greilhuber, and Swiecicki, 1996).

***Recommended plant standards***—For flow cytometry it is preferable to select an internal standard with 2C and 4C peaks close to those for the target species, so that large errors are not introduced by relatively small random errors in the estimated mean of the standard itself. Also, differences in nuclear size and amounts of heterochromatin influence light scattering properties of the measured object and therefore the measurement itself. If the standard and target nuclei have similar chromatin/DNA properties, this source of error is minimized. At the same time, the peaks of the standard should not overlap those of the target species. Therefore, a set of standards should be available for use with plants of different C values. After considering agreement between Feulgen and PI determined values, ease of preparation, clean peaks with low coefficients of variation, and representation of a wide range of nuclear DNA contents, the following plants are recommended as excellent DNA content standards for flow cytometry using PI and Feulgen microspectrophotometry: *Sorghum bicolor* cv. Pioneer 8695 (2C = 1.74 pg); *Pisum sativum* cv. Minerva Maple (2C = 9.56 pg); *Hordeum vulgare* cv. Sultan (2C = 11.12 pg); *Vicia faba* cv. GS011 (2C = 26.66 pg); and *Allium cepa* cv. Ailsa Craig (2C = 33.55 pg). The above values were obtained by averaging the DNA contents calculated by flow cytometry (PI, barley standard) and Feulgen microspectrophotometry, with the exception of *Allium cepa,* which is a well-established plant standard, determined by chemical means (Van't Hof, 1965).

We have found *P. sativum* cv. Minerva Maple to be an extremely good standard in terms of availability of

plant material, ease of preparation, and stability within and among runs. Greilhuber and Ebert (1994) observed from a sample of 25 acquisitions, including Minerva Maple, representing a large geographic area that nuclear DNA content was essentially identical among the reported *P. sativum* genotypes. The same conclusion was drawn from subsequent studies involving flow cytometric determination of genome size using ethidium bromide as the fluorochrome (Baranyi and Greilhuber, 1995, 1996). However, the 2C DNA content of 8.84 pg for *P. sativum* reported by Greilhuber and Ebert (1994) is lower than the 9.73 pg estimated using Feulgen cytometry by Bennett and Smith (1976) and the 9.39 pg estimated by PI-based flow cytometry reported herein. Murray, Cuellar, and Thompson (1978), using the very different technique of reassociation kinetics, estimated the 2C DNA content of *P. sativum* to be 9.2 pg. Although there are some laboratory to laboratory incongruities, it is very important that a common set of plant calibration standards be agreed upon and used throughout the world. Further internationally coordinated research should lead to the regularization of differences in assumed nuclear DNA amounts of the standards and allow the subsequent adjustments in DNA amounts of target species. It should also lead to the addition of other species to the list of recommended standards.

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