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FLOWER: A Plant DNA Flow Cytometry Database

João Loureiro, Jan Suda, Jaroslav Doležel, and Conceição Santos

Overview

The ever-increasing number of articles on flow cytometric analysis of plant genomes highlights the need to collect the available information and make it accessible in one comprehensive database. This goal was materialized in the Plant DNA Flow Cytometry Database (FLOWER), a project aimed at gathering an exhaustive list of articles on flow cytometry of nuclear DNA content and providing a comprehensive overview of published data. DNA-based studies clearly dominate applications of flow cytometry in plant sciences, which often give a false impression of a well-established method devoid of pitfalls. However, many particulars of the methodology are still under discussion and quality standards have not yet been universally accepted. This chapter demonstrates the usefulness of the FLOWER database as a tool for providing unbiased and quantitative data on taxonomic representation, nuclear isolation buffers, standardization, including reference DNA standards, DNA fluorochromes and measures of result quality. In addition, issues related to the objective(s) of the studies, type of instrument(s) used, scientific journals, and countries of origin of the authors may also be assessed and quantified. The database is freely accessible for public use on the Internet (http://flower.web.ua.pt/) and users can undertake their own searches and analyses. The database is regularly updated by the authors who appreciate receiving newly published papers relevant to plant DNA flow cytometry.

18.1
Introduction

Flow cytometry (FCM) is a powerful approach for measuring optical properties (light scatter and fluorescence) of single particles (cells, protoplasts, nuclei, and chromosomes) in suspension. It has been increasingly applied in plant sciences since the late 1980s, with the estimation of DNA ploidy level and genome size be-

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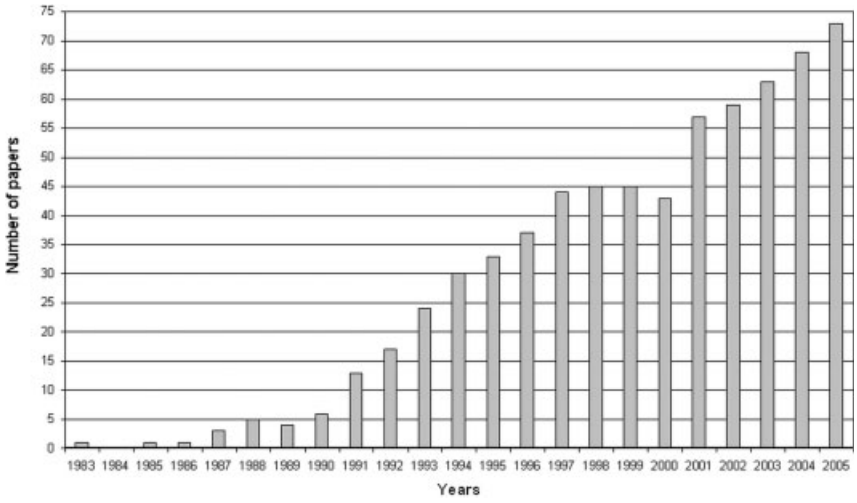

Fig. 18.1 Year distribution of the articles included in the Plant DNA Flow Cytometry (FLOWER) database.

ing the most frequent applications (Doležel and Bartoš 2005). Both uses rely on the determination of DNA amounts in cell nuclei.

Plant scientists are attracted by the numerous advantages of this technique (e.g. ease of sample preparation, rapidity of analysis, and no requirement for dividing cells) and, as expected, the number of articles has been continuously increasing over the years (Fig. 18.1). Nevertheless, there are also some weak points, which may hinder further, and perhaps more extensive use of FCM in plant sciences, such as the high cost of the instruments, difficulties in the analysis of some plant tissues/species and, in the case of estimation of genome size, occasionally contradicting results obtained in different laboratories.

The scientific community engaged in genome size analysis is conscious of such problems and has made several efforts to overcome them. A set of recommendations to achieve precise and highly comparable FCM results was thoroughly debated and finally approved at the Plant Genome Size Workshops held in the Royal Botanic Gardens, Kew in 1998 and 2003, and at the International Botanical Congress in Vienna in 2005. Among the "best practice" recommendations, a proper choice of calibration standard(s), fluorochrome(s) and buffer(s), and the awareness of potential negative effect of secondary compounds were discussed (see http://www.rbgkew.org.uk/cval/pgsm/; and Chapters 4, 7 and 12). Despite the experience of authorities participating at the Plant Genome Size Workshops, no quantitative data supporting the decisions were available and no large-scale survey of FCM literature, which could help to elucidate controversial topics and identify additional methodological issues, had been carried out. In addition, knowledge on how and to what extent the recommendations were followed is essential for the assessment of result credibility (crucial particularly for newcomers to the

FCM arena). Finally, an acquaintance with the ever-increasing number of plant FCM articles published in an ever-expanding list of journals is beyond the grasp of most FCM users and thus makes any exhaustive comparative study difficult.

To cope with the above-mentioned issues, we built and released a comprehensive Plant DNA Flow Cytometry Database (with the acronym FLOWER). The database serves as a basic source of information for plant FCM users, providing bibliographic citations together with relevant data concerning methodology, material and instrumentation. The database aims to cover a full range of DNA FCM applications in plant sciences. Currently (July 2006), it harbours more than 700 entries and is regularly updated. To make data easily accessible to the public, the FLOWER database is available in a dynamic webpage format on the Internet (http://flower.web.ua.pt/). The basic structure (searchable and output fields) is presented in Table 18.1. The database allows researchers to undertake quantitative analyses of various parameters, to access insights into the use of FCM in plant sciences over the years and to assess the reliability of individual articles based on methodological details and observance of best practice recommendations. The aim of this chapter is to describe the FLOWER database and demonstrate its usefulness. For that purpose, database outputs for the most relevant FCM parameters are briefly presented and discussed below.

18.2 Taxonomic Representation in DNA Content Studies

As might be expected, angiosperms are the most frequently analyzed group of plants in FCM studies (92.4% of all publications). Gymnosperms account for 4.2% of the entries while the proportion of other major taxonomic groups is much smaller and does not exceed 2.0%. Indeed, there are only three papers dealing with lycophytes, nine with monilophytes (i.e. horsetails and ferns) and four with bryophytes. To some extent, the number of DNA FCM articles reflects the diversity of a particular taxonomic group and the number of recognized species. Nevertheless, the relative proportion of angiosperms investigated (with ~250 000 recognized species) is much lower than that of gymnosperms (with ~730 recognized species). Similarly, lycophytes (~900 recognized species), monilophytes (~9000 recognized species) and bryophytes (~18 000 recognized species) are also rather poorly represented.

Economically important (namely in agriculture and forestry) plant families dominate in FCM articles, with Poaceae, Fabaceae, Solanaceae and Brassicaceae together representing around 41.5% of the angiosperm database entries. In gymnosperms, the largest family, Pinaceae, prevails in FCM studies (77.8%) and the genus *Pinus* itself accounts for 42.4% of the entries.

More than three-quarters of the articles (76.8%) deal with herbaceous taxa. This is not surprising as they represent the highest number of recognized species, and most herbaceous species do not pose serious problems in FCM analyses. Woody species, which are generally considered more recalcitrant due to the presence

Table 18.1 Summary of searchable and output fields of the internet platform of the FLOWER database.

Searchable fields	Output fields	
Author	Author	
Year	Title	
Country	Year	
Nuclear isolation buffer	Country	
Fluorochrome	Nuclear isolation buffer	
Taxonomic fields	Buffer modification	
Main objective	Fluorochrome	
Standardization	Taxonomy	Plant group (bryophyte/lycophyte/monilophyte/gymnosperm/angiosperm)
Standard		
Flow cytometer		Family
Scientific journal		Species
		Growth type (herbaceous/woody/other)
	Main objective	
	Other objective	
	Standardization	Type (external/internal/pseudo-internal/no standardization/not applicable)
	Standard	Type (animal/plant)
		Species and cultivar
		2C nuclear DNA content
	Flow cytometer	Brand and model
	Scientific journal	
	Coefficient of variation	Given, range or not given
	DNA histograms	Shown or not shown
	Herbarium voucher	Available or not available

of secondary metabolites that may interfere with DNA staining (Loureiro et al. 2006a), were investigated in 20.1% of studies. Other recognized growth types (succulents, spore-bearing vascular plants and bryophytes) account for only 3.1% of the references.

18.3 Nuclear Isolation and Staining Buffers

Current methods to prepare nuclear suspensions for FCM analyses are mostly based on the breakthrough development of Galbraith et al. (1983). In their procedure, intact cell nuclei are released into the isolation or (isolation and staining) buffer simply by chopping a small amount of plant tissue with a razor blade. As reviewed in Chapter 4, the composition of the lysis buffer is crucial for obtaining precise, reliable, and high-resolution results. Given the diversity in tissue structure and chemical composition in the plant kingdom, it comes as no surprise that no single buffer works well with all species (as discussed by Doležel and Bartoš (2005) and experimentally confirmed by Loureiro et al. (2006b)). Nevertheless, the latter authors concluded that certain lysis buffers may consistently yield better results than others, at least when model species are analyzed.

Buffers undoubtedly represent one of the most important areas of the FLOWER database, offering both frequency analyses and assessment of various relationships and trends, such as which buffers have been used most frequently over the years, which buffers have been used by particular researchers and countries, and which buffers have been selected according to the type of plant material (herbaceous versus woody) under investigation.

Twenty-six different nuclear isolation and staining buffers were found in the literature excerpted. The chemical composition of the top 10 non-commercial buffers is presented in Chapter 4. The relative use of individual buffers is shown in Fig. 18.2. The six most popular buffers (Galbraith's, commercial buffers, $MgSO_4$, LB01, Otto's and Tris.$MgCl_2$ – arranged in descending order) collectively account for 72.6% of the references while the next group of five buffers and the remaining 15 buffers account for only 17.4 and 10.0% of references, respectively.

Analysis of temporal variation (over 5-year periods) in the use of the six most popular buffers (Fig. 18.3) shows that the relative contribution of the pioneering Galbraith's buffer has been decreasing over time. The same applies to LB01 and $MgSO_4$ buffers, which, after a period of frequent use in the 1990s, experienced a decline over the last 6 years. In contrast, the number of articles using commercial buffers is escalating and since 2001, these buffers represent the most popular choice. Such success is plausibly related to the fact that they are provided as ready-to-use kits. As the commercial buffers do not yield better results, we hypothesize that novices in plant DNA flow cytometry, who are unaware of the ease of preparation of other nuclear isolation buffers, are the main users of commercial products. Tris.$MgCl_2$ and Otto's buffers are also increasingly being used. While the former was the worst performing buffer in a comparative experimental

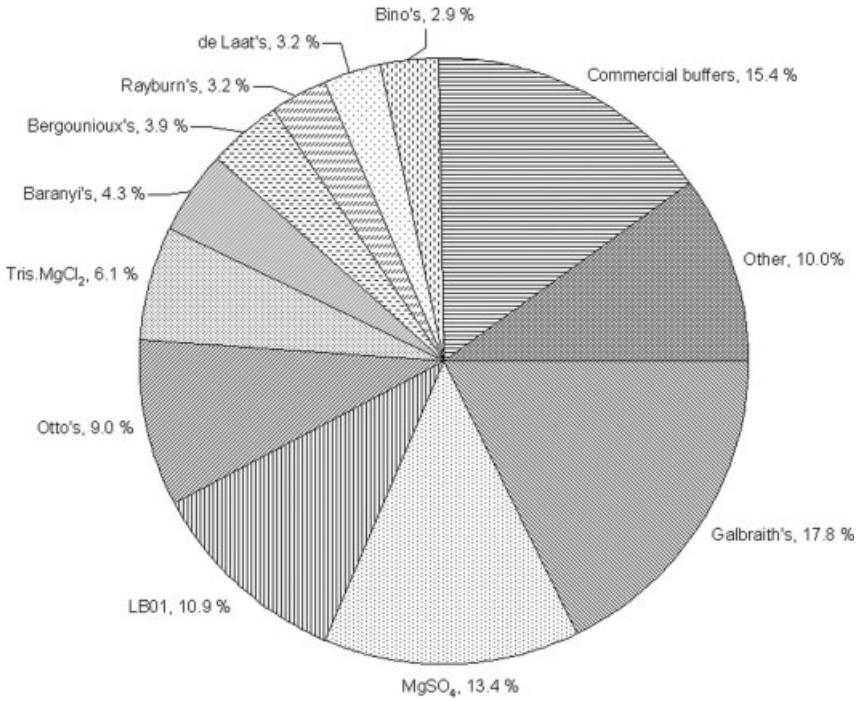

Fig. 18.2 The use of nuclear isolation buffers in plant DNA flow cytometry. The composition of each buffer is given in Chapter 4. Original references: Galbraith's buffer (Galbraith et al. 1983); $MgSO_4$ (Arumuganathan and Earle 1991); LB01 (Doležel et al. 1989); Otto's (Doležel and Göhde 1995; Otto 1981); Tris.$MgCl_2$ (Pfosser et al. 1995); Baranyi's (Baranyi and Greilhuber 1995); Bergounioux's (Bergounioux et al. 1986); Rayburn's (Rayburn et al. 1989); de Laat's (de Laat and Blaas 1984); Bino's (Bino et al. 1993).

study (Loureiro et al. 2006b), the latter is known for yielding DNA histograms with unsurpassed resolution in many plant species (Doležel and Bartoš 2005; Loureiro et al. 2006b). Oddly enough, it took about two decades for Otto's methodology to become widely adopted in plant sciences, considering that the buffer composition was first published in 1981 (Otto). In contemporary plant FCM, Otto's buffer became the third favorite just behind Galbraith's buffer, although still lagging behind commercial solutions.

Geographical survey of the use of a particular isolation buffer suggests that the choice is primarily correlated with a researcher's personal history and/or the laboratory's practice rather than with the buffer quality and/or species and tissue adequacy. The two prevailing buffers (Galbraith's and commercial buffers) also have the largest geographical coverage, being used in no less than 23 different countries. Nevertheless, there is a marked disproportion among the relative contribution of individual countries; nearly two-thirds of Galbraith's buffer hits come from the USA (28.5%), France (19.2%) and New Zealand (15.4%); while Japan

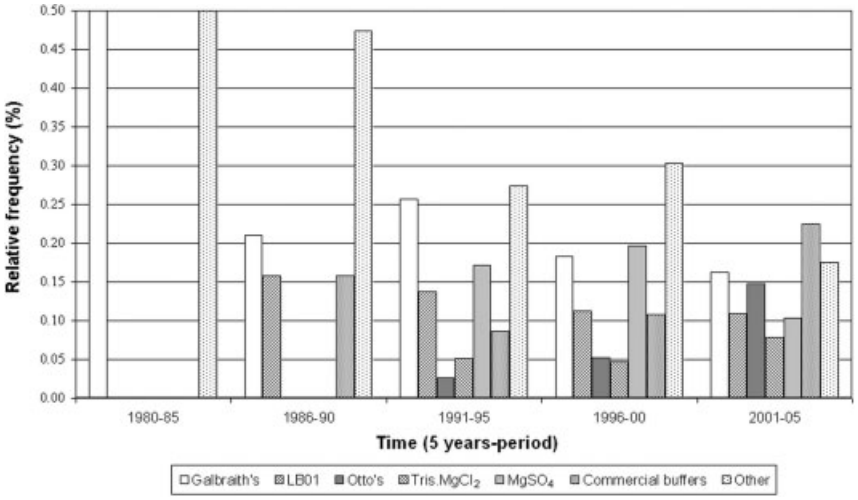

Fig. 18.3 The use of nuclear isolation buffers in plant DNA flow cytometry through the years. Data were grouped into 5-year periods.

(27.7%), Germany (15.2%) and USA (9.9%) account for more than half of the commercial buffers hits. This tendency is even more obvious for other buffers. The majority of LB01 users resides in the Czech Republic (34.2%), the country where the buffer originated, and in France (21.5%); $MgSO_4$ is most widely used in the USA (64.0%), where it was also developed; Otto's buffer is preferred in the Czech Republic, Slovenia and Belgium, with 46.1, 13.8 and 10.8% of database entries, respectively; and Tris.$MgCl_2$ buffers are mostly used in Japan (27.9%), Poland (27.9%) and the USA (16.3%). The most conspicuous example of a locally restricted use is Rayburn's buffer with 23 occurrences, but all from the same country (USA) and 91.3% of them even from the same research group. Once again, these data indicate that researchers generally use only one or two buffers throughout their publication history. When multiple buffers are employed, there is usually a favorite, which accounts for a substantial percentage of the references. However, such strict adherence to a particular methodology may have important consequences for the quality of the data obtained as no ideal buffer exists and testing several different alternatives prior to routine FCM investigation is always advisable.

Assessment of buffer selection according to the plant growth type did not show any clear preferences. Galbraith's and LB01 buffers were more often used for investigation of woody plants, while commercial buffers, $MgSO_4$, Otto's and Tris.$MgCl_2$ buffers predominated in the research on herbaceous species. However, no explanation for a particular choice was provided in the publications and it seems that it was merely standard laboratory practice that guided the selection of a buffer. As expected, minor modifications to buffer composition (e.g. addition of antioxidants) were often made when recalcitrant woody plants were analyzed by flow cytometry.

18.4 Standardization and Standards

The estimation of nuclear DNA content requires the use of a reference standard with known nuclear DNA content. C/Cx-value or DNA ploidy level of the plant to be analyzed is then inferred by comparing sample and standard peak positions. There are two basic types of standardization: external and internal. While in the former procedure, nuclei of the sample and standard are processed separately, the latter involves simultaneous isolation, staining and analysis. Although no extensive comparative study has been performed, internal standardization is generally recommended as the most reliable option (see also Chapter 4). Nevertheless, demands on standardization are usually less strict in DNA ploidy studies, at least when the aim of the study is to detect differences in the scale of whole chromosome sets (see Chapter 5).

Quantitative analysis of the type of standardization in ploidy-based studies revealed the following figures: internal 46.5%, external 7.8%, and no standardization 44.9%. In genome size studies, the proportion of internal standardization was much higher (91.8%) while external standardization was adopted in only 6.1% of articles; 2.1% of investigations were carried out with both approaches. Merging ploidy and genome size datasets indicates that 7.1% of publications use external standardization, which implies the successful adoption of the preferred internal standardization practice by most researchers.

Several requirements imposed on proper DNA reference standards, such as a close but non-overlapping genome size in relation to that of a target species (Bagwell et al. 1989; see also Chapter 4), led to the employment of many different standards and have fueled a discussion about the selection of a universal set of reference materials. As a comprehensive survey of reference standards has not yet been carried out, the FLOWER database can provide the first insights into the type of standards and the frequency of their use, and contribute to the identification of potential sources of variation.

Plant and animal reference standards were employed in 73.0 and 27.0% of articles, respectively. However, the use of animal standards such as chicken red blood cells (CRBCs), which is the main type of animal standard used with a 68.2% incidence, was not recommended by the 1997 Genome Size Workshop and further warnings were issued 6 years later. The plant FCM community responded positively to this recommendation and the contribution of CRBCs clearly decreased over time (Fig. 18.4). The use of CRBCs as a reference standard has been questioned mainly because there has been no general agreement regarding the size and stability of the chicken genome (see Chapter 4). The FLOWER database supports this contention and shows that published 2C-values vary from 1.88 pg (Chen et al. 2002) to 2.50 pg (Iannelli et al. 1998), with the most common 2C DNA value being 2.33 pg (87.3% of references).

Nevertheless, the problem of a non-identical genome size may persist even when plant reference standards are employed. Table 18.2 lists the most common plant standards with a range of 2C-values assigned by different authors. *Pisum*

Fig. 18.4 The use of chicken red blood cells (CRBCs) as a reference standard in plant genome size estimations using flow cytometry over the years. Data were grouped into 5-year periods.

sativum (Fabaceae; 15.0%), *Hordeum vulgare* (Poaceae; 12.7%), *Petunia hybrida* (Solanaceae; 11.1%) and *Zea mays* (Poaceae; 8.6%) were the most popular standards, being used in 47.5% of the genome size estimation studies. DNA amounts in these plants vary from 2.85 pg/2C in *P. hybrida* to 11.26 pg/2C in *H. vulgare*. It may therefore be expected that a great many of the available nuclear DNA content estimates will lie within a corresponding range. However, a careful analysis of the plant DNA C-values database (Bennett and Leitch 2005) revealed that this was not the case and that many estimates actually fall into the lower end of such a DNA range. Figure 18.5 illustrates this, and reveals that the frequency of reference standards used for genome size estimations in the lower end of DNA range is appropriate. Moreover it is clear that reference standards covering the 5.0–15.0 pg/2C DNA range are overused. This may suggest that in some cases, the best standard for a given species was not chosen. Our data also highlights the necessity of reducing the number of reference species currently used for genome size estimations. For example, the two most frequently used standards, *Pisum sativum* and *Hordeum vulgare*, cover nearly identical DNA ranges, although the former species is a preferred primary reference standard (Doležel and Bartoš 2005; Loureiro et al. 2006b).

Also, the lack of agreement on which cultivars should be used in several popular reference species (e.g. *H. vulgare* and *P. sativum*) may potentially contribute to the heterogeneity of FCM estimates. On the other hand, different genome sizes

Table 18.2 The most popular plant DNA reference standards (without cultivar distinction) used for FCM estimation of genome size.

Plant DNA reference standards	Range of assigned 2C DNA contents		No. of papers	Frequency of use	
	Min–Max (pg)	Variation (%)		%	| = 1%
Arabidopsis thaliana (L.) Heynh.	0.14–0.32	128.6	4	1.3%	|
Oryza sativa L.	0.89–1.20	34.8	12	3.8%	||||
Vigna radiata (L.) R. Wilczek	1.06	–	7	2.2%	||
Raphanus sativus L.	1.11	–	5	1.6%	||
Lycopersicon esculentum Mill.	1.96–2.01	2.6	20	6.4%	||||||
Trifolium repens L.	2.07	–	6	1.9%	||
Glycine max Merr.	2.27–2.70	18.9	19	6.1%	||||||
Petunia hybrida Vilm.	2.85–3.35	17.5	35	11.1%	|||||||||||
Zea mays L.	5.00–5.47	9.4	27	8.6%	||||||||
Pisum sativum L.	8.11–9.73	20.0	47	15.0%	||||||||||||||
Hordeum vulgare L.	9.81–11.26	14.8	40	12.7%	||||||||||||
Secale cereale L.	15.58–16.80	7.8	5	1.6%	||
Agave americana L.	15.90	–	7	2.2%	||
Vicia faba L.	25.95–26.90	3.7	8	2.5%	|||
Triticum aestivum L.	30.90–34.85	12.8	19	6.1%	||||||
Allium cepa L.	33.50–34.89	4.1	13	4.1%	||||
Other species	–	–	40	12.7%	||||||||||||

may be assigned to the same reference cultivar. An illustrative example is *Pisum sativum* cv. Minerva Maple, with the following DNA values: 2C = 8.22 pg (three references; first cited in Joyner et al. 2001), 2C = 9.56 pg (six references; Price et al. 1998), 2C = 9.64 pg (one reference; Johnston et al. 1999), and 2C = 9.73 pg (seven references; Leitch et al. 2001). The difference in input values, amounting to 18.4%, may well be an underlying cause of the artifactual variation in genome size data among different research groups.

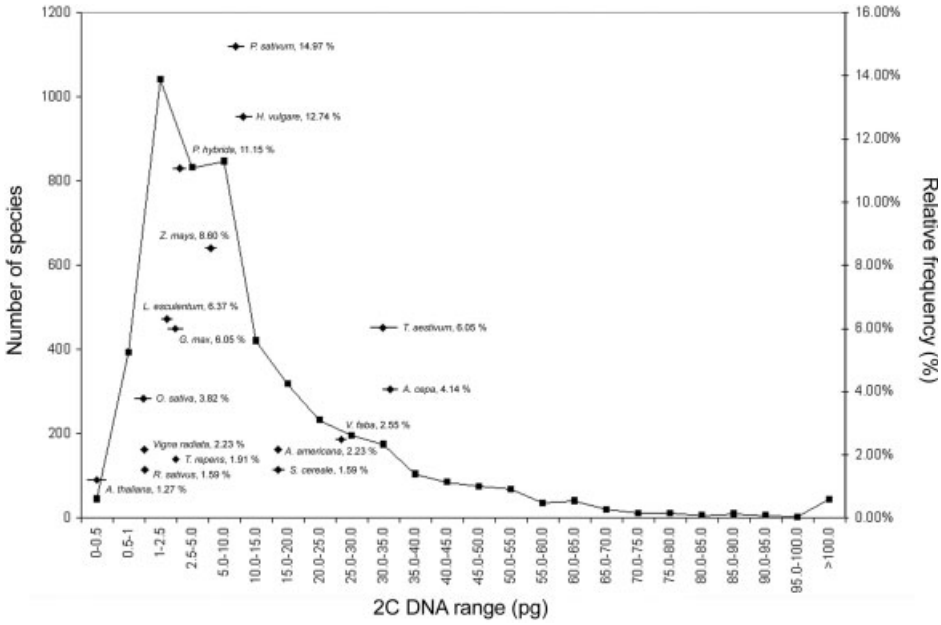

Fig. 18.5 Distribution of 2C-values for 5015 plant species (primary *y* axis) and the use of the most popular DNA reference standards in plant genome size estimations using flow cytometry (secondary *y* axis). For each reference standard, the frequency of use, its mean 2C DNA amount and a range of assigned 2C values is shown. Data on DNA amounts were taken from the Plant DNA C-values database (Bennett and Leitch 2005).

18.5 Fluorochromes

A range of DNA-specific fluorochromes has been used to study plant genomes. They are mostly grouped according to their binding properties: intercalation into double-stranded DNA (ethidium bromide, EB; propidium iodide, PI), preference for AT-rich regions (DAPI, Hoechst dyes) or preference for GC-rich regions of DNA (chromomycin, mithramycin and olivomycin). While the binding mode is of little importance in DNA ploidy studies, precise genome size estimates require intercalating dyes (as shown for the first time by Doležel et al. (1992)).

The analysis of fluorochrome data from the FLOWER database revealed that PI is the most frequently used fluorescent dye, with a 45.3% incidence. DAPI was employed in 38.2% of FCM studies while the frequency of any other fluorochrome did not exceed a 6% threshold. The obvious preference of DAPI among other base-specific fluorochromes such as chromomycin A_3, results from its lower toxicity, the likelihood of obtaining high-resolution histograms of DNA content and the fact that it can be used in cheaper, lamp-based instruments. Most

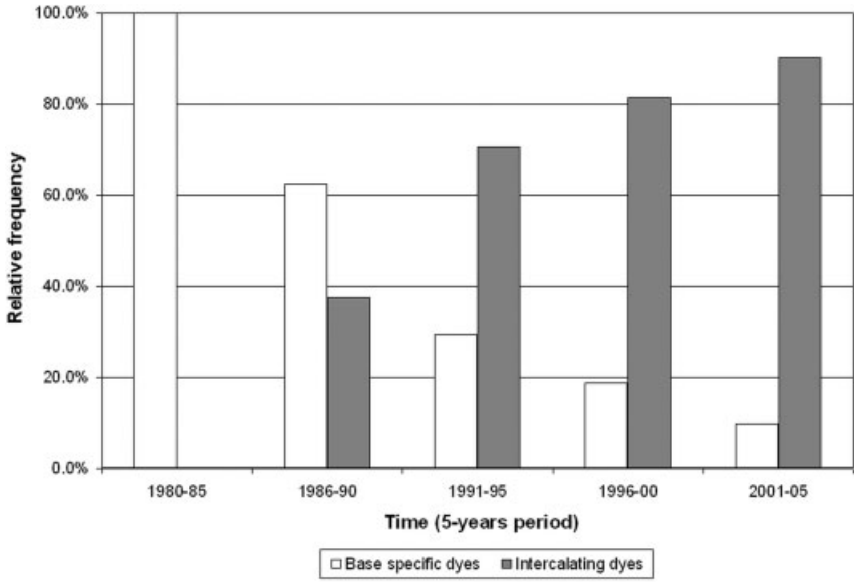

Fig. 18.6 The use of the two basic types of DNA fluorochromes (base-specific and intercalating dyes) in genome size estimations using flow cytometry over the years. Data were grouped into 5-year periods.

DAPI measurements refer to DNA ploidy estimations or base composition studies. In essays focused on absolute genome size estimations, PI reported in 71.1% of articles clearly surpasses the other intercalating dye, EB, which is mentioned in only 11.1% of reports. This disproportion may be related to the belief that PI produces histograms with lower coefficients of variation. Despite the known base preference, DAPI and other base-specific dyes were used in the remaining 17.8% of studies.

An assessment of temporal variation in the use of base-specific versus intercalating fluorochromes (Fig. 18.6) reveals that the former were preferred until the 1990s. Actually, early researchers paid little attention to the mode of binding and used any fluorochrome for a wide range of applications. Since the 1990s, a shift toward intercalating dyes is evident, plausibly triggered by the results of a comparative study of three fluorochromes performed by Doležel et al. (1992).

18.6 Quality Measures of Nuclear DNA Content Analyses

Coefficient of variation (CV) and the distribution of relative nuclear DNA content (DNA histogram) are the main tools for assessing the quality of FCM analyses

and should therefore be presented in every publication. A literature survey, however, shows that the situation is not satisfactory, and CV values and DNA histograms were included in only 31.2 and 66.3% of articles, respectively. The corresponding figures change to 45.6% (CV) and 58.8% (histogram) when only genome size studies are evaluated, and to 21.9% (CV) and 69.8% (histogram) in ploidy-based studies alone. This difference may be driven by distinct requirements in the quality and design of both types of studies. While low CV is a crucial prerequisite for high-standard genome size work, FCM estimation of DNA ploidy level is generally less demanding. On the other hand, an FCM histogram represents the most straightforward proof of ploidy differentiation.

The FLOWER database also provides information on the range of CV values for DNA peaks. In 33.2% of the articles, the CV values were below 3.0%, in 39.6% they ranged from 3.0 to 5.0%, in 22.6% they ranged from 5.0 to 10.0%, and CV values above 10.0% were obtained in only 4.6% of the references. This analysis reveals that in published works, CV values mostly fall within the recommended range (i.e. below 5.0%; see Chapter 4 for further information on quality control and data presentation).

18.7 The Uses of DNA Flow Cytometry in Plants

The major applications of DNA flow cytometry are ploidy level and genome size estimations, and cell cycle analysis. Indeed, a survey of the literature stored in the FLOWER database revealed that a substantial proportion of plant FCM work dealt with DNA ploidy level (50.2%) and genome size (36.9%). The remaining uses cover cell cycle analysis (6.1%) and estimations of DNA base composition (4.1%). Other applications, which include sex determination in dioecious plants and technical and standardization experiments, account for only 2.8% of the studies. The low number of cell cycle studies is quite surprising considering the extensive use of FCM in human and animal cell cycle research.

18.8 Instrumentation

FCM users may also seek information regarding the contribution of particular brands and models of flow cytometers. Based on the number of articles, the leading brand used in plant sciences is Partec® which was mentioned in 44.1% of publications (the most successful model appears to be the Cell Analyser II), followed by Beckman-Coulter® (30.8% of the studies; most successful model, the EPICS V), and Becton-Dickinson® (19.2% of the reports; most successful model, the FACScan). Instruments from other manufacturers, which include the discontinued models from Leitz, Ortho Instruments and Phywe, and more recent offerings from Bio-Rad (now acquired by Apogee flow systems) and Dako, were used

in only 5.7% of the studies. The prominent position of Partec may be related either to suitability for analysis of plant materials and/or to the relatively low price of their products. As project budgets in plant sciences are generally smaller than those in other fields where FCM is routinely employed (e.g. clinical studies), price is undoubtedly a significant criterion in instrument purchase.

18.9 Where Are the Results Published?

The FLOWER database also offers quantitative analyses of scientific journals in which plant DNA FCM studies were published. The top 10 journals are listed in Table 18.3. This synopsis may help authors to select the most appropriate periodical for their work. The year-trend overview of the top six scientific journals reveals that, with the exception of *Theoretical and Applied Genetics* (TAG), the number of published articles concerning plant DNA flow cytometry has been increasing over time (Fig. 18.7). *Plant Cell Reports* (PCR) and *Plant Cell, Tissue and Organ Culture* (PCTOC) experienced the highest increase in recent years. The former, together with *Annals of Botany* (AoB), has been the preferred journal for publication of plant FCM studies over the last 6 years, and the latter is placed third, after more than a 300% increase in the number of articles being published.

The spectrum of FCM applications covered by particular journals also deserves attention. While most papers in AoB concern genome size estimations, DNA ploidy level studies, particularly those related to *in vitro* cultures and transformation experiments, prevail in TAG, PCR and PCTOC. *Euphytica* is devoted to plant breeding and most of the FCM papers also fall into this category while *Plant*

Table 18.3 The 10 most popular scientific journals in plant DNA flow cytometry.

Scientific journal	No. of papers
Annals of Botany	57 (8.2%)
Theoretical and Applied Genetics	52 (7.5%)
Plant Cell Reports	49 (7.0%)
Plant Science	40 (5.7%)
Plant Cell, Tissue and Organ Culture	37 (5.5%)
Euphytica	36 (5.2%)
Plant Systematics and Evolution	25 (3.6%)
Crop Science	19 (2.7%)
Genome	18 (2.6%)
American Journal of Botany	16 (2.3%)
Other	342 (497%)

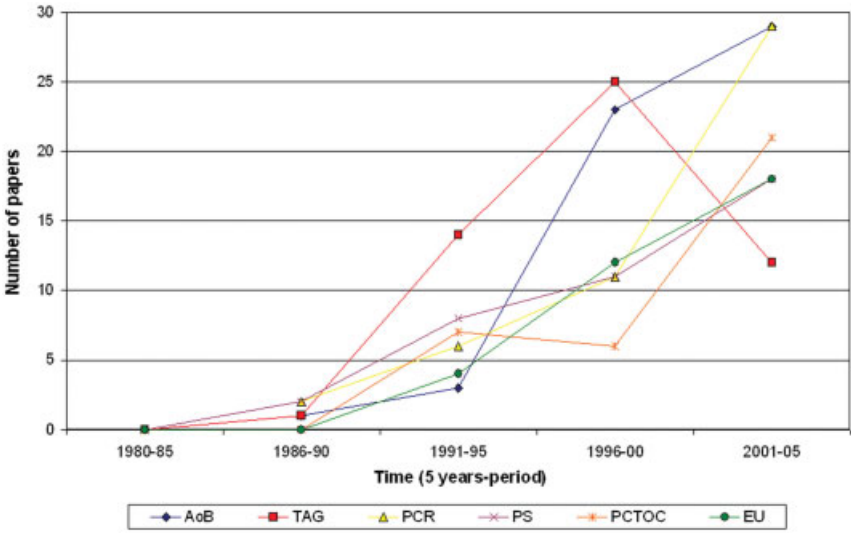

Fig. 18.7 Number of papers related to plant DNA flow cytometry published in the six most popular scientific journals, over the years. Data were grouped into 5-year periods. AoB, *Annals of Botany*; TAG, *Theoretical and Applied Genetics*; PCR, *Plant Cell Reports*; PS, *Plant Science*; PCTOC, *Plant Cell, Tissue and Organ Culture*; EU, *Euphytica*.

Science has a more general scope and publishes results both on genome size and DNA ploidy.

18.10 Conclusion

This chapter introduces a database of scientific publications which use DNA flow cytometry to study plant materials. The database with the acronym FLOWER is intended as a comprehensive, easily accessible and user-friendly source of information on plant FCM articles (search tool) as well as a platform for carrying out quantitative analyses of selected aspects important in FCM practice (survey tool). Excerpted methodology- and instrumentation-related data (such as types of nuclear isolation buffer, standards, and fluorochromes) form a basis for unbiased and statistically well-founded assessments of historical applications and approaches, methodological trends, developments, and the current state of affairs in plant FCM. Keyword filters offer rapid tracking of relevant information useful for both newcomers and experts. Evaluation of the reliability of results and close inspection of how the best practice recommendations were met can also be easily carried out. We believe that this ready-to-hand set of FCM articles will stimulate further use of DNA flow cytometry in plant sciences, contribute to the discussion

on the best methodology, support the formulation of recommendations, and help to identify other hot topics. Availability of the interactive FLOWER database on the internet (http://flower.web.ua.pt/) guarantees accessibility to plant FCM-users worldwide and regular data updating.

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