

IDENTIFICATION OF PLOIDY LEVEL ON VARIETIES AND HYBRIDS OF KIWIFRUIT (*ACTINIDIA SP.*)

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Abstract

The genus *Actinidia* includes over 66 species and over 118 taxon, that range in ploidy level from diploid to octoploid. Although there is basic information on ploidy levels of various species, sampling has been limited and little information on specific cultivars and hybrids is available. The objective of this research was to determine ploidy levels among a diverse collection of species, hybrids, and cultivars using flow cytometry. Nuclei were extracted, stained with propidium iodide (PI), and analyzed using a flow cytometer. Among the selections tested we found *Actinidia arguta* as diploid ($2n = 2x$) and tetraploid ($2n = 4x$), *Actinidia deliciosa* 'Hayward' as hexaploid ($2n = 6x$), *Actinidia chinensis* 'Soreli' tetraploid ($2n = 4x$) and the interspecific hybrid *A. arguta* x *A. deliciosa* as diploid ($2n = 2x$). This information provides further insight into reproductive biology, substantiation of numerous hybrids and induced polyploids that will help facilitate the development of improved hybrids in the future.

Key words: chromosomes, flow cytometry, interspecific hybrid, polyploidy, plant breeding.

INTRODUCTION

Commercial kiwifruit production is based on two *Actinidia* species, *A. deliciosa* C.F. Ling et A.R. Ferguson and *A. chinensis* Planch. Nevertheless, at present, *A. arguta* is commercially cultivated in Oregon in US, Chile and New Zealand, and small-scale production for local consumption is conducted in many regions under a relatively cool climate (Ferguson and Huang, 2001). The genus *Actinidia* Lindl. comprises 66 species and about 118 taxa with remarkably wide natural range extending from the tropics (latitude 0°) to cold temperate regions (50° N). Since the first seed introduction of *A. deliciosa* was made from China to New Zealand in 1904, the economic potential of kiwifruit has been extensively exploited (Ferguson and Bollard 1990).

There is considerable genetic diversity amongst the species within the genus *Actinidia*, particularly in fruit skin type, skin colour, flesh colour and flavour (Ferguson and Huang, 2007).

This diversity provides many opportunities for developing new types of kiwifruit. Dioecy, long generation cycles, high heterozygosity and

variation in ploidy make hybridization between *Actinidia* species difficult. Better knowledge and understanding of polyploidy of cultivars and hybrids could facilitate kiwifruit improvement.

Polyploidy has been an important process in the evolution of plants and is an important factor in plant breeding because it can influence reproductive compatibility, fertility, and phenotypic traits (Chen and Ni, 2006; Jones and Ranney, 2009; Ranney, 2006; Soltis et al., 2004). Hence, accurate and specific knowledge of ploidy levels of species and cultivars is important information for kiwi breeders.

The base chromosome number for *Actinidia* is $1n = 1x = 29$. However, different subgenera contain species with a variety of ploidy levels ranging from $2n = 2x = 58$ to $2n = 6x = 174$; respectively $2n = 8x = 232$. The range in ploidy levels within this genus also provides an opportunity to indirectly substantiate hybridity when parents differ in ploidy levels.

Because many *Actinidia* species are polyploids with high chromosome numbers, traditional cytology based on light microscopic examination is a difficult and time-consuming process.

Flow cytometry has proved to be an efficient means of estimating genome size and associated ploidy level (Dolež'el et al., 2007; Jones et al., 2007). Therefore, the objective of this study is to identify the ploidy levels of a diverse collection of species, hybrids, and cultivars of *Actinidia* developing a database for use by kiwi breeders.

MATERIALS AND METHODS

The plant material was constituted by a collection of plants from *Actinidia arguta*, *Actinidia deliciosa*, *Actinidia chinensis* and one interspecific crosses of *Actinidia*, total of 130 plants were kept in a green house.

Flow cytometric analysis was performed for each of the *Actinidia* accessions by collecting leaf samples (3-5 per each), young fully expanded leaf collected from the shoot tips; to maintain an adequate moisture and ensure a correct conservation tissues, the leaves were placed in plastic bags in a refrigerator at 4°C.

Using the method of Galbraith et al. (1983), 50 mg *Actinidia* leaf tissue without midribs was placed in plastic Petri dishes chopped with a razor blade adding 0.5 ml of ice-cooled homogenization (Otto I buffer) to homogenize the tissues and release the nuclei, and kept on ice for 5 min.

After filtration through 42-µm nylon mesh the nuclear suspension were treated with 2.5 ml of a staining solution containing 10 ml Tris, 50 mM sodium citrate, 2 mM MgCl₂, 1% (w/v) PVP 30, 0.1% (v/v) Triton X-100 and 1 mg/ml propidium iodide (PI), pH 7.5, letting the samples stand 5 min prior to flow cytometric analysis.

Samples were run on a Coulter Epics XL-MCL flow cytometer EXPO 32 ADC; for each accession was gated between 5000-10000 events of a sample. Relative fluorescence intensity was assessed using FL2, green light source detector (excitation 488 nm).

Data were interpreted using WinMDI 2.9 software. As an internal standard was used *Pisum sativum* cv. *Ctirad*. The measurements were triplicated by using three leaf samples collected from each plant.

RESULTS AND DISCUSSIONS

We have evaluate for ploidy level six selections of *Actinidia arguta*, two of *A. deliciosa* 'Hayward', one of *A. chinensis* 'Soreli' and one interspecific hybrid, *A. arguta* x *A. deliciosa*, using flow cytometry (Table 1).

Table 1. Selection of species, hybrids of kiwi (*Actinidia* sp.)

Species	Genotype	No. plants
<i>Actinidia arguta</i>	R8P23	10
<i>Actinidia arguta</i>	R10P10	10
<i>Actinidia arguta</i>	R9P18	10
<i>Actinidia arguta</i>	R8P1	10
<i>Actinidia arguta</i>	R9P16	10
<i>Actinidia arguta</i> x <i>deliciosa</i>	P1	10
<i>Actinidia deliciosa</i> 'Hayward'	Hk	10
<i>Actinidia deliciosa</i> 'Hayward'	H8	30
<i>Actinidia chinensis</i> 'Soreli'	S	30
Total		130

The ploidy levels of *Actinidia* plants were clearly distinguished by flow cytometry. Among *Actinidia* sp. tested, we found: *Actinidia arguta* (2n=2x) R9P18, R8P23 as diploid plants, R10P10, R8P1, R9P16 as tetraploid *Actinidia arguta* (2n=4x), plants; for *Actinidia deliciosa* 'Hayward' (2n=6x) HK, H8 as hexaploid; for *Actinidia chinensis* 'Soreli' (2n=4x) tetraploid; for interspecific crosses *A. arguta* x *A. deliciosa* (2n=2x) P1 as diploid (Table 2).

Table 2. Estimation of ploidy level of kiwi genotypes (*Actinidia* sp.)

Species	Genotype	Ploidy level	No. chromosomes
<i>Actinidia arguta</i>	R8P23	2x	58
<i>Actinidia arguta</i>	R10P10	4x	116
<i>Actinidia arguta</i>	R9P18	2x	58
<i>Actinidia arguta</i>	R8P1	4x	116
<i>Actinidia arguta</i>	R9P16	4x	116
<i>Actinidia arguta</i> x <i>deliciosa</i>	P1	2x	58
<i>Actinidia deliciosa</i> 'Hayward'	Hk	6x	174
<i>Actinidia deliciosa</i> 'Hayward'	H8	6x	174
<i>Actinidia chinensis</i> 'Soreli'	S	4x	116

The results of an analysis are described as a mono-parametric (fluorescence intensity/object) histograms peaks, each of them

representing the fluorescence intensity of a population of nuclei. Their intensity (position

of the histogram) is proportional to the amount of nuclear DNA (Figure 1).

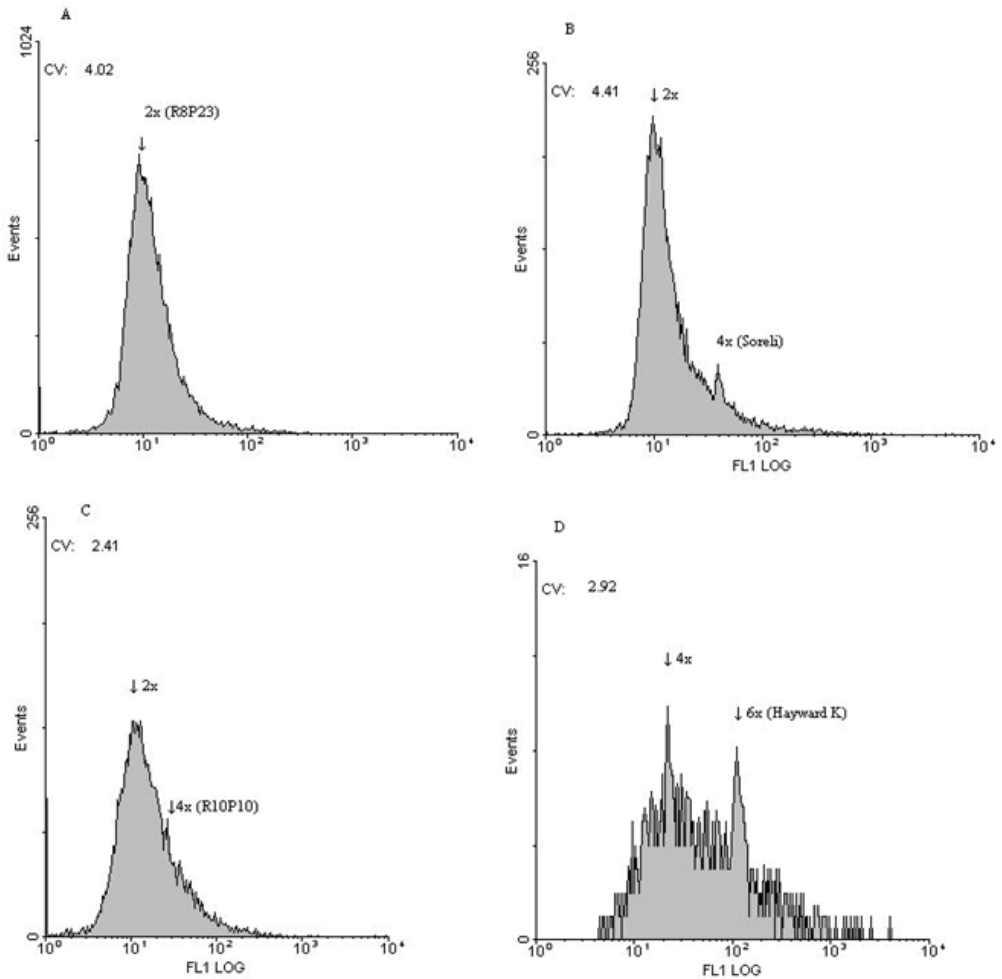


Figure 1. Frequency distribution histograms of fluorescence intensity stained with propidium iodide (PI) (A, *A. arguta* R8P23; B, *A. chinensis* 'Soreli'; C, *A. arguta* 108P10, D, *A. deliciosa* var. *deliciosa* cv. Hayward K; Log values of fluorescence intensity% CV for nuclei are given on each histogram).

The comparison between the position of the sample's peak and the internal standard's peak give a ratio of relative intensities. The ploidy levels are calculated by comparing experimental values with the value obtained from *Pisum sativum* sample having a known ploidy level.

As a result, ploidy analysis in which the DNA contents of *Arguta sp.* collection, nuclei were compared to those from a specie having known ploidy (*Pisum sativum* $2n=2x$).

This comparison can be made either between two analyses performed under identical conditions, or, in our case, through mixing the two samples for simultaneous measurement.

Counting chromosomes in *Actinidia* species and their hybrids is technically difficult due to the high numbers of chromosomes involved and their small size. Chromosome numbers have been reported for only a few species but the data support a polyploid sequence $2n = 2x$, $2n = 4x$, $2n = 6x$ with $x = 29$. To determine ploidy, the number of basic chromosome sets in

cell nuclei, using chromosome counting in dividing cells is an unambiguous way and is time consuming, that is way a high-throughput solution is to use flow cytometry, making possible a rapid and reliable ploidy estimation.

CONCLUSIONS

Flow cytometry (FCM) was originally developed as a rapid technique to analyse blood samples for medical diagnosis and is nowadays used for countless medical applications by the analyses of individual cells at high speed. In plant sciences, and breeding in particular, flow cytometry is mainly known as a tool for ploidy analysis. The first ploidy applications focused on crop plants (De Laat et al. 1987) and plant breeding still dominates this field.

The method is fast, accurate and simple and replaced microscopic chromosome counts since Galbraith et al. (1983) introduced chopping with a razor-blade as an easy standard protocol for plant analysis.

At different stages in plant breeding flow cytometry generates valuable information. As well for the selection of parent plants for hybridisation as for the evaluation of hybrids, ploidy determination or genome size analyses are useful. Flow cytometry is an indispensable tool in polyploidisation to screen plants treated with mitotic inhibitors.

Such conclusions clearly highlight the indispensability of cytotype determination (most effectively realized by FCM) in any experimental study that may involve heteroploid plant samples.

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