

Retraction notice to ‘Detection aneuploidy in *Arabidopsis thaliana* using fluorescent in-situ hybridization (FISH) analysis’. [The South Pacific Journal of Natural and Applied Sciences 29 (2011), 12–16. doi:10.1071/SP11003]

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After due consideration, the editors of the journal and the authors of the paper agree that the paper be retracted from *The South Pacific Journal of Natural and Applied Sciences* because:

- (1) the findings of this paper were previously published in *The Plant Journal* (Henry *et al.* 2006) and were republished without proper cross-referencing, permission or justification;
- (2) the coauthors of the paper were included on the paper without their knowledge or consent by the first author; and
- (3) the first author (Anand P. Tyagi) was acknowledged in Henry *et al.* (2006) for his contribution to that work.

Reference

Henry, I. M., Dilkes, B. P., and Comai, L. (2006). Molecular karyotyping and aneuploidy detection in *Arabidopsis thaliana* using quantitative fluorescent polymerase chain reaction. *The Plant Journal* **48**, 307–319.

Detection aneuploidy in *Arabidopsis thaliana* using Fluorescent In-Situ Hybridization (FISH) analysis

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Abstract

Certain cellular processes are sensitive to changes in gene dosage. Aneuploidy is deleterious because of an imbalance of gene dosage on a chromosomal scale. Identification, classification and characterization of aneuploidy are therefore important for molecular, population and medical genetics and for a deeper understanding of the mechanisms underlying dosage sensitivity. Notwithstanding recent progress in genomic technologies, limited means are available for detecting and classifying changes in chromosome dose. The development of an inexpensive and scalable karyotyping method would allow rapid detection and characterization of both simple and complex aneuploid types. In addition to the problem of karyotyping, genomic and molecular genetic studies of aneuploids and polyploids are complicated by multiple heterozygous combinations possible at loci present in more than two copies. Finally, we screened the progeny of tetraploid individuals and found that more than 25% were aneuploid and that our artificially induced tetraploid strain produced fewer aneuploid individuals than a tetraploid strain isolated from nature.

Keywords: *Arabidopsis thaliana*, Karyotype, Aneuploidy, Polyploidy.

1 Introduction

Several methods for detection of aneuploidy have been developed, primarily for pre-natal diagnostics of aneuploidy in humans (Dudarewicz *et al.*, 2005). Some methods, such as chromosome banding and fluorescent in situ hybridization (FISH), result in complete karyotype information, the determination of both chromosome number and type. Other methods, such as quantitative fluorescent-polymerase chain reaction (QF-PCR) (Cilvie *et al.*, 2005), multiplex amplifiable probe hybridization, multiplex ligation-dependent probe hybridization (Schouten *et al.*, 2002), melting curve analysis of single-nucleotide polymorphisms and real-time quantitative PCR (Dudarewicz *et al.*, 2005), have been used to detect changes in gene dosage and to infer the dosage of specific chromosomes. Recently, comparative genomic DNA hybridization to microarrays has been used to determine gene copy number and whole chromosome aneuploidy in several organisms such as mouse (Bond *et al.*, 2004; Hughes *et al.*, 2000) and human (Sebat *et al.*, 2004). For example, this method was recently used to detect and characterize aneuploidy in cancerous cells (Lucito *et al.*, 2003). Similarly, the human single nucleotide polymorphism (SNP) arrays allow the identification of changes in copy numbers over short sequences (Rauch *et al.*, 2004) and could be used to detect variation in whole chromosome dosage.

Fluorescent in situ hybridization and flow cytometric analyses have been the methods of choice for detection and characterization of aneuploidy in plants (Burton and Husband, 2001; Galbraith, 2004; Henry *et al.*, 2005; Roux *et al.*, 2003). The use of flow cytometric measurement of nuclear DNA content cannot classify the type of aneuploidy and often cannot unequivocally distinguish individuals differing by a single chromosome because the difference in total nuclear DNA content is too small. As a result, the construction of a complete karyotype, i.e. the determination of the exact number of each chromosome

type, is not possible on the basis of DNA content alone. Fluorescent in situ hybridization can determine complete karyotype (Wang *et al.*, 2006), but is laborious and requires considerable expertise and sophisticated probes.

In addition to improving the resolution of studies on polyploids, an inexpensive, scalable, high-throughput karyotyping method would allow larger-scale experimentation to elucidate the mechanism and consequences of dosage sensitivity in both population genetic and clinical settings. In addition to the problem of karyotyping, genomic and molecular genetic studies of aneuploids and polyploids are complicated by increased gene and allele copy numbers. Specifically, genotyping is complicated by the possibility of more than two alleles at each locus and the existence of different heterozygous states. For example, even with only two alleles, a trisomic heterozygous for A and a can exhibit allelic ratios of 1:2 (Aaa) and 2:1 (AAa). Similarly, in autotetraploids, AAAa (3:1), AAaa (1:1), and Aaaa (1:3) are all possible heterozygous combinations. Aneuploid individuals within polyploid populations further increase the number of heterozygous classes. For example, loss of one chromosomal copy in autotetraploids results in trisomy, adding two heterozygous combinations (1:2 and 2:1). Similarly, gain of one chromosomal copy adds two more heterozygous combinations associated with pentasomy. Correct identification of allelic dosage is required when the two alleles, A and a, have different additive values because the Aaaa genotype in a tetraploid would be phenotypically different from the AAAa genotype.

2 Materials and Methods

2.1 Plant Material: Lines, Growth Conditions and Crosses

Arabidopsis thaliana plants (ecotype lines) were grown on soil (Sunshine Professional Peat-Lite mix 4; SunGro Horticulture, Vancouver, BC, Canada) in a growth room lit by fluorescent lamps (model TL80; Phillips,

Sunnyvale, CA, USA) at $22 \pm 3^\circ\text{C}$ with a 16-h:8-h light:dark photoperiod or in a greenhouse at similar temperatures and light regimes, with supplementary light provided by sodium lamp illumination as required.

Tetraploid lines were generated as previously described (Henry *et al.*, 2005). Col-0 represents the diploid ecotype Columbia; 4x-Col represents tetraploidized Col-0 and Wa-1 represents the naturally occurring tetraploid ecotype Warschau-1 (CS6885). C and W refer to basic genomes or alleles of Col-0 and Wa-1, respectively. The CWW triploid was generated by crossing Col-0 as the seed parent to Wa-1. The CCWW and WWCC tetraploid plants were generated by the Wa-1 and 4x-Col tetraploid plants, with the seed parent genomic symbol listed first. Plant TC211 (Figure 1) and TC496, TC493, TC468 and TC473 (Figure 2) is one of the aneuploid plants produced by a crossing of a CWW triploid to Col-0.

2.2 Fluorescent In Situ Hybridization (FISH)

Developing flower buds were harvested and used for chromosome counts by FISH with centromeric probes as previously described (Comai *et al.*, 2003) except that the following steps were performed prior to chromosome denaturation: the slides were first incubated in $100 \mu\text{g ml}^{-1}$ ribonuclease A (catalogue no. R-4642; Sigma-Aldrich, Milwaukee, WI, USA) in 2x SSC for 30 min at 37°C . The slides were subsequently washed three times for 5 min each in 2x SSC and finally for 1 min in 10 mM HCl. The slides were incubated with $1 \mu\text{g ml}^{-1}$ pepsin (catalogue no. P6887; Sigma-Aldrich) in 10 mM HCl for 10 min at 37°C before being rinsed in water and washed three times for 5 min each in 2x SSC. The slides were then treated with 4% formaldehyde for 10 min at room temperature (instead of 65°C for 15 min in 70% formaldehyde) and washed three times for 5 min each in 2x SSC before being denatured in a series of ethanol dilutions (70%, 90% and 100%). After hybridization, the slides were stained with $0.25 \mu\text{g ml}^{-1}$ 4',6-diamidino-2-phenylindole (DAPI).

3 Results

3.1 Tetraploid *A. thaliana* Produces more than 25% Aneuploid Progeny

Quantitative genotyping was used to estimate the level of aneuploidy in a tetraploid population of *A. thaliana*. Tetraploid 4x-Col was crossed to the natural tetraploid Wa-1 in both directions (three independent crosses in each direction). F1 seeds were planted and 49 WWCC and 47 CCWW plants were genotyped at the 12 loci listed in Table 1. No homozygous individuals were detected, indicating that there was no contamination from accidental selfing. For each plant, a complete karyotype was inferred from the genotyping data, as described earlier. Nineteen out of 475 chromosomes (4%) were initially unresolved. These PCR reactions were re-run, allowing the resolution of all of them. Aneuploidy was indicated by a deviation in the %Wa-1 from the 1:1 ratio expected for CCWW and corresponded either to a missing or an additional chromosome.

The origin of the aneuploid gametes was inferred from the genotype of the aneuploid individual. For example, the CCW genotype in an individual derived from a 4x-Col x Wa-1 cross indicated lack of a Wa-1 chromosomal copy in

the pollen. Similarly, a CCCWW genotype from the same cross indicated an additional copy of a Col-0 chromosome in the ovule. Pooling of the results obtained in the different crosses allowed us to compare the rate of production of aneuploid gametes by 4x Col and Wa-1 as well as by the female and male gametophytes.

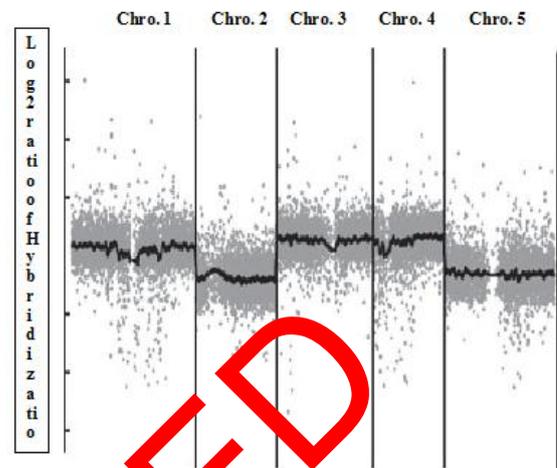


Figure 1. Whole genome karyotyping by comparative competitive genome hybridization on DNA microarrays and quantitative fluorescence polymerase chain reaction (QF-PCR) returned identical results. Hybridization ratios are expressed as the \log_2 (TC211/Col-0) for all features represented on the microarrays and ordered along the five chromosomes of *Arabidopsis thaliana*. Each grey dot represents a single feature present on the microarray. The bold black curve was obtained by calculating the average values over a sliding window of 101 features. The black vertical lines represent the boundaries between chromosomes.

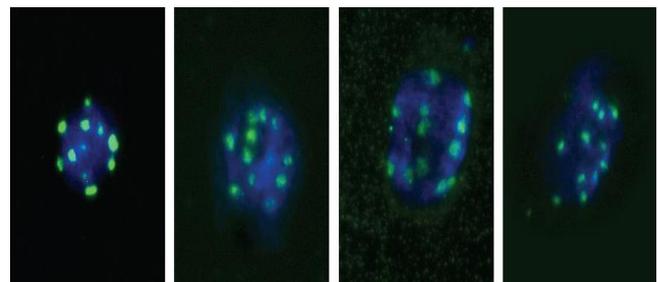


Figure 2: Chromosome counts in *A. thaliana* aneuploid individuals using fluorescent in situ hybridization (FISH). Cells were stained with DAPI (4',6-diamidino-2-phenylindole; blue) and hybridized with a FITC-labelled *A. thaliana* centromere-specific probe (green). For each of four aneuploid individuals, one somatic cell is shown. The number of chromosome and karyotype, as inferred from the quantitative fluorescence-PCR results, are as follows: (a) TC 496, 11 chromosomes ($2x + \text{Chr. 5}$), (b) TC 493, 12 chromosomes ($2x + \text{Chr. 2} + \text{Chr. 5}$), (c) TC 468, 12 chromosomes ($2x + \text{Chr. 3} + \text{Chr. 4}$), (d) TC473, 11 chromosomes ($2x + \text{Chr. 2}$).

3.2 Chromosome Counts Using FISH Confirm the Inferred Karyotypes

From the plants that were karyotyped, anthers were harvested from eight aneuploid individuals and used to perform chromosome counts using FISH. Although the

exact karyotype of each plant could not be obtained using this technique, the number of chromosomes could be determined by counting the number of brightly staining spots after hybridization with a FITC-labelled centromeric DNA probe. For each of these eight individuals, the number of chromosomes observed in somatic cells using FISH was in agreement with the karyotypes previously determined using our karyotyping method. Hybridization results for four of these individuals are shown in Figure 2.

Table 1. 12 loci and their physical location in five chromosomes.

Name of locus	Chr	Physical location (bp)*	Name of locus	Chr	Physical location (bp)*
MN 1.5	1	898627	nga162	3	4608284
MN 1.6	1	11362256	MN3.3	3	15770898
MN 1.7	1	13634035	MN4.2	4	4197691
MN1.2	1	21949869	MST 4.18	4	10931201
Nga 1145	2	683000	MN5.1	5	7843029
Nga 1126	2	9000000	MSAT 5.19	5	25911521

Table 2. Number of aneuploid individuals in F1 populations of *Arabidopsis thaliana* derived from tetraploid parents.

	CCWW			WWCC			n
	A1*	A2	A3*	B1	B2	B3	
Chr. 1							
Pollen	0	0	0	1+	0	1-	
Ovule	2+	1+	5+	0	2+	1-	
Chr. 2							
Pollen	0	0	1+	0	1+	0	
Ovule	0	1+	0	1+	0	0	
Chr. 3							
Pollen	0	0	0	0	0	1+	
Ovule	0	0	0	0	0	0	
Chr. 4							
Pollen	0	0	0	0	1-, 1+	0	
Ovule	0	1-, 1+	0	0	0	0	
Chr. 4							
Pollen	8+	0	0	0	0	1+	
Ovule	2+	1+	0	1-, 1+	0	0	
n	20	10	16	13	22	13	

Numbers in bold indicate loss or gain of a Wa-1 chromosome (Chr.) while numbers in normal font indicate loss or gain of a Col-0 chromosome. The '+' sign indicates gain of a chromosome and the '-' sign indicates loss of a chromosome. A1 to A3 and B1 to B3 represent independent crosses. Asterisks indicate crosses that were excluded from further analysis because of potential aneuploidy in one parent; n indicates the number of individuals assayed from each cross.

A total of 36 instances of aneuploidy were detected (Table 2). Comparison of the rates of aneuploidy obtained in the progeny of replicate crosses (4x-Col x Wa-1 A1 to

A3 as well as Wa-1 x 4x-Col B1 to B3) suggested that two of the six crosses probably involved an aneuploid parent. Specifically, eight of the 20 plants analysed from cross A1 contained an extra copy of chromosome 5 from Col-0, suggesting that the Col-0 seed parent used in that cross was pentasomic for chromosome 5. Similarly, five of the 16 plants analysed from cross A3 contained an extra copy of chromosome 1 from Wa-1, suggesting that the Wa-1 pollen parent used in that cross was pentasomic for chromosome 1. (Henry *et al.*, 2005). Therefore, data from crosses A1 and A3 were omitted from further analyses. With these aneuploids excluded, 16 of the 56 remaining F1 plants (27.6%) were determined to be aneuploid.

Table 3. Number of chromosomes in the F1s from tetraploid parents

Chromosome no	No of individuals	% Individuals
19	4	6.9
20	42	72.4
21	10	17.2
22	2	3.4
Total	58	100

Table 4. Origin of aneuploidy in F1s from tetraploid parents

	Col-0 pollen	Col-0 ovule	Wa-1 pollen	Wa-1 ovule	Extra chr.	Missing chr	All
Chr. 1	3	0	1	2	5	1	6
Chr. 2	1	0	1	1	3	0	3
Chr. 3	0	0	0	1	1	0	1
Chr. 4	0	0	2	2	2	2	4
Chr. 5	2	0	1	1	3	1	4
Total	6/	0/	5/	7/	14/	4/	18/
	48	10	10	48	58	58	58

The number of gametes aneuploid for chromosomes (Chr.) 1 to 5 depending on parental genotype (Col-0 or Wa-1 parent), gamete type (pollen grain or ovules) and gamete karyotype (hypoploid or hyperploid).

As described in Table 3, most instances of aneuploidy were associated with an additional copy of a chromosome type (hyperploidy) as opposed to the lack of a chromosome type (hypoploidy). Specifically, only four of the aneuploid genotypes corresponded to the lack of a chromosome while the remaining 14 aneuploid genotypes corresponded to one or two additional chromosomes (4/18 versus 14/18, χ^2 P-value < 0.0001).

Our data suggest that the natural autotetraploid Wa-1 produced more aneuploid gametes than the synthetically induced tetraploid of Col-0 (12/18 versus 6/18, χ^2 P-value $\frac{1}{4}$ 0.0027). This was true both for pollen grains and ovules (Table 4). The frequency of aneuploidy in the functional pollen grains was higher than in the functional ovules but the difference was not significant (7/18 versus 11/18, χ^2 P-value $\frac{1}{4}$ 0.053). Finally, the frequency of altered number of chromosome copies depended on the chromosome type. Specifically, six of the aneuploid individuals were aneuploid for chromosome 1 while only one individual was aneuploid for chromosome 3.

4 Discussion

Aneuploidy Detection is Required in Polyploid Populations

In addition to the problem of polysomic inheritance of alleles, mapping in polyploid populations can be complicated by the presence of aneuploid individuals. Although aneuploidy is rare post-birth in humans, it is common in other species. For example, systematic aneuploidy detection using comparative microarray hybridization demonstrated whole chromosome or segmental aneuploidy in 8% of 300 deletion mutant strains of *Saccharomyces cerevisiae* (Hughes *et al.*, 2000). Using the same technique, several instances of aneuploidy were detected in the genome of two lager yeast strains (Bond *et al.*, 2004).

In plants, aneuploid individuals appear spontaneously within diploid populations at low frequency (Khush, 1973). In most species, these are easily detectable because they are phenotypically distinct from the diploid individuals and sometimes exhibit reduced fertility (Khush, 1973). In polyploid populations, on the other hand, the frequency of aneuploid individuals can increase drastically. We found that more than 25% of the progeny of tetraploid *A. thaliana* were aneuploid (Table 2) and that most of the aneuploid plants observed in our study carried an extra copy of a chromosome while only a few were lacking a chromosome copy (Table 3). Depending on the species, the percentage of aneuploid individuals detected within tetraploid populations is variable: 50% in maize (Randolph, 1935); 44% in barley (Rommel, 1961); 25% in *Datura* (Bell and Blakeslee, 1924); 15–23% in rye (Hagberg and Ellerstrom, 1959; Morrison, 1956; Muntzig, 1951); 20% in lettuce (Einset, 1947) and 3–6% in alfalfa (Bingham, 1968). This suggests that different species might produce aneuploid gametes at different rates or exhibit varying levels of tolerance of aneuploidy. Consistent with our data, the majority of the aneuploids in these studies were also found to be hyperploid. These data are consistent with the idea that extra chromosomal copies are less deleterious than the lack of a chromosomal copy (Birchler *et al.*, 2001). Contrary to triploid individuals, which exhibit obvious phenotypes in *Arabidopsis* (Koorneef and Van der Veen, 1983; Lee-Chen and Steinitz-Sears, 1967; Steinitz-Sears, 1962; Steinitz-Sears and Lee-Chen, 1970), most of the aneuploid plants with near-tetraploid genome contents were not readily identifiable by casual phenotypic inspection, probably because of a reduced relative chromosome imbalance in polyploid backgrounds. If this fraction of the population is undetected, their phenotypes can distort the analysis of many traits. For example, the presence of aneuploids within tetraploid populations of many crops has been shown to result in reduced seed set (Einset, 1944, 1947; Hagberg and Ellerstrom, 1959; Morrison, 1956; Rommel, 1961; Shaver, 1962). Quantitative genotyping makes it possible to tackle this problem by allowing the rapid scoring of a previously unexplored quantitative trait: aneuploid production.

Our experimental design allows the aneuploidy to be traced to a specific parent and gametophyte (Table 4). Of the two tetraploids used in this study, Wa-1 is a natural autotetraploid and 4x-Col originated from a colchicines induced line. Surprisingly, the rate of aneuploid gametes

produced was higher in Wa-1 than in 4x-Col arguing against a stabilization of tetraploid meiosis in Wa-1. It is also possible that the higher incidence in aneuploidy originates from an increased tolerance to aneuploidy in Wa-1 compared to Col-0. Seed and pollen parents produced aneuploid gametes at similar rates but aneuploidy was more often attributed to certain chromosome types than others. Specifically, chromosome 1 was most often found to be unbalanced with respect to the other chromosome types.

As presented in this work and in others earlier (Bond *et al.*, 2004; Hughes *et al.*, 2000; Sebat *et al.*, 2004), comparative genomic hybridization can be used to measure chromosomal dosage relative to a control sample. In our study, the karyotype of one of the aneuploid plants was confirmed using comparative genome hybridization to microarray slides (Figure 1). The use of microarrays for comparative genomic hybridization has the advantage of simultaneously determining gene dosage over small genomic regions and spanning the whole genome. Quantitative fluorescent-PCR (data not shown) on the other hand, allows the processing of a large number of individuals and is significantly less expensive than microarrays. Finally, the aneuploidy was confirmed using FISH analysis which complements with the results obtained using microarrays.

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