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The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome size in the lower limit of eukaryotes

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Abstract

The genome size, complexity, and ploidy of the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* was determined using flow cytometry, reassociation kinetics, and genomic reconstruction. Nuclei of *G. intraradices* from in vitro culture, were analyzed by flow cytometry. The estimated average length of DNA per nucleus was 14.07 ± 3.52 Mb. Reassociation kinetics on *G. intraradices* DNA indicated a haploid genome size of ~16.54 Mb, comprising 88.36% single copy DNA, 1.59% repetitive DNA, and 10.05% fold-back DNA. To determine ploidy, the DNA content per nucleus measured by flow cytometry was compared with the genome estimate of reassociation kinetics. *G. intraradices* was found to have a DNA index (DNA per nucleus per haploid genome size) of approximately 0.9, indicating that it is haploid. Genomic DNA of *G. intraradices* was also analyzed by genomic reconstruction using four genes (Malate synthase, RecA, Rad32, and Hsp88). Because we used flow cytometry and reassociation kinetics to reveal the genome size of *G. intraradices* and show that it is haploid, then a similar value for genome size should be found when using genomic reconstruction as long as the genes studied are single copy. The average genome size estimate was 15.74 ± 1.69 Mb indicating that these four genes are single copy per haploid genome and per nucleus of *G. intraradices*. Our results show that the genome size of *G. intraradices* is much smaller than estimates of other AMF and that the unusually high within-spore genetic variation that is seen in this fungus cannot be due to high ploidy.

Keywords: *Glomus intraradices*; Arbuscular mycorrhizal fungi; Genome size (*C* value); Flow cytometry; Genomic reconstruction; Reassociation kinetics; Ploidy

1. Introduction

In diploid or polyploid organisms, the genome size refers to the amount of DNA in the unreplicated haploid genome, such as that in the sperm nucleus. The genome size shows very low intraspecific variability and is fairly constant within any species (although there are exceptions among pathogenic fungi). Genome size is also called the "*C* value," where *C* stands for "constant" or "characteristic" (Graur and Li, 2000) or "Γ," where it describes the length of DNA in base pairs. In contrast, *C* values vary widely among species in both prokaryotes and eukaryotes. The genome size in prokaryotes varies over 23-fold, ranging from 0.58 Mb in protoplasma to 13.2 in several cyanobacteria species (Cavalier-Smith, 1985; Graur and Li, 2000). Genome size varies over 77,955-fold in eukaryotes ranging from 8 Mb in fungi to 686,000 Mb in amoebae (Cavalier-Smith, 1985; Graur and Li, 2000). Eukaryotes usually have much larger *C* values than prokaryotes, although there are exceptions especially in fungi. For example *Saccharomyces cerevisiae* (~13 Mb) or the basidiomycete *Bolbitius vitellinus* (~8 Mb; Latzelsberger and Krisai-Greilhuber, 2000) have similar genome size to many bacteria and smaller than that of many cyanobacteria.

To date there are very few reports on genome size and complexity in arbuscular mycorrhizal fungi (AMF) and their ploidy is still unknown. Nuclear DNA content was estimated for 11 AMF, using flow cytometry (Hosny et al., 1998) and Γ values varied over 8-fold from ~127.4 Mb in *Scutellospora pellucida* to ~1058.4 Mb in *S. gregaria*. Bianciotto and Bonfante (1992) also found

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that the nuclear DNA length of *Glomus versiforme* was ~250 Mb and *Gigaspora margarita* was ~740 Mb. No published studies have measured ploidy in AMF although the reassociation kinetics performed by Hosny (1997) indicated that *Scutellospora castanea* was haploid. AMF form symbioses with approximately 60% of vascular plant species. They are important because they improve phosphorus and nitrogen acquisition by plants (Hodge et al., 2001; Smith and Read, 1997) and play a positive role in plant diversity and ecosystem function (van der Heijden et al., 1998). There are considerable efforts from many research groups on the identification of genes involved in this important symbiosis, although to date, only a few have been identified from the fungal side of the partnership (Harrison and van Buuren, 1995; Requena et al., 2002). Although this is an important group of fungi, the genetics of AMF is still poorly understood (Sanders, 2002). Unusually high genetic diversity has been seen within single isolates of several AMF species and even within-spore polymorphism has been observed (Clapp et al., 2001; Hijri et al., 1999; Kuhn et al., 2001; Lloyd-MacGilp et al., 1996; Sanders et al., 1995). AMF have also been shown to harbor genetically different nuclei (Kuhn et al., 2001). It would, therefore, clearly be of great value to AMF research to sequence the genome(s) of an AMF. The choice of a candidate AMF species to sequence is difficult given that there are few reports on AMF genome size, complexity and ploidy. The fact that AMF could contain more than one genome per isolate means that the effective amount of sequencing performed to cover all the genomes may be much greater than that predicted by genome size estimates alone. Therefore, it would be desirable to find an AMF with a small genome size for any sequencing program. In addition, information on the genome size, complexity, and ploidy level in an AMF will be extremely helpful in resolving open issues regarding the extent of genetic polymorphisms within AMF isolates and how it is arranged (within or among nuclei).

In this investigation, we report the genome size estimate of the AMF *Glomus intraradices*. We chose this species because it is increasingly used in molecular AMF studies since it can be successfully produced in a large amounts in an axenic culture with transformed carrot roots (Bécard and Fortin, 1988). This also avoids contamination by other fungi which has previously been reported for some pot cultured AMF (Hijri et al., 2002). Furthermore, high DNA polymorphism has been seen in a single isolates of this species in genes that are thought to be single copy (Kuhn et al., 2001). An investigation into ploidy would also help to elucidate whether variation seen in AMF genes is distributed among nuclei or, because of high ploidy, within nuclei. We combined three different methods to obtain independent estimates of genome size and DNA content per nucleus. Flow cytometry allows an estimate of DNA content per nucleus, but without knowledge about ploidy, it does not give the haploid genome size. Reassociation kinetics methods give an estimate of the haploid genome size. Combining these can give the genome size and ploidy level of each nucleus. Genomic reconstruction also gives an estimate of genome size using a gene of known copy number as a hybridization probe. However, if the ploidy and genome size are already known, by the two above-mentioned techniques, then this method can be used to show the copy number of a given gene. Our genome size estimates of *G. intraradices*, using these independent methods showed very small and consistent Γ values at the a lower limit of eukaryotes. This result is important because it indicates that *G. intraradices* is an ideal candidate for genome sequencing project and the results will contribute to studies of genome evolution in AMF and understanding the arrangement of genetic variation in these fungi.

2. Material and methods

2.1. Strains, media, and growth conditions

The *G. intraradices* strain DAOM 181 602 was co-cultured with Ri T-DNA-transformed carrot (*Daucus carota* L.) roots that were kindly provided by Guillaume Bécard (University of Toulouse, France). *G. intraradices* and transformed carrot roots were cultured and maintained on a minimal medium (Bécard and Fortin, 1988), and solidified by 0.4 w/v gellan gum (St-Arnaud et al., 1995), at 25 °C. The *S. cerevisiae* haploid and diploid strains were a kind gift from Claudio De Virgilio (University of Geneva, Switzerland). A tetraploid strain of *S. cerevisiae* (ATCC 204713) was obtained from the Yeast Genetic Stock Center (LGC Promochem, France). Yeast cells were grown in YPD medium at 25 °C. *Escherichia coli* (JM 109) was grown in Luria–Bertani (LB) medium at 37 °C.

2.2. Flow cytometry

Spores and hyphae of *G. intraradices* were freshly collected from plates by dissolving the gellan gum in a solution containing 0.0083 N sodium citrate and 0.0017 N citric acid, washed in phosphate-buffered saline (PBS), (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) and fixed with 4% formaldehyde in PBS for 2 h at room temperature. Nuclei were released by crushing spores and hyphae with a micropestle in a 1.5 Eppendorf tube in 0.2% Triton X-100 in BPS. The fixed-nuclear suspension was filtered twice through a nylon membrane with a pore size of 30 and 5 μm to remove debris. Nuclei were prepared and stained with propidium iodide (PI) using CycleTest Plus DNA reagent kit (Becton–Dickinson, Switzerland) according to the

manufacturers recommendations. Yeast cells were collected by centrifugation at 300*g* for 10 min, washed with PBS, and then fixed with 4% formaldehyde in PBS for 2 h at room temperature. The cell wall of yeast cells was lysed enzymatically with 200 U lyticase (Sigma, Switzerland) in 600 ml of 1 M sorbitol, 100 mM EDTA and 14 mM β-mercaptoethanol at 30 °C for 30 min. Spheroplasts were pelleted by centrifugation for 10 min at 300*g* and the cell membrane was dissolved by 0.2% Triton X-100 in PBS buffer. Chicken erythrocyte nuclei (CEN) were prepared according to Hosny et al. (1998). Yeast nuclei, CEN, and *G. intraradices* nuclei were stained simultaneously as described above. PI stained nuclei were analyzed using a FACScan flow cytometer (Becton–Dickinson, Switzerland) equipped with a 488 nm argon laser. PI red emission fluorescence was measured through a 625 nm band pass filter. Linear (FL2-High) and logarithmic (FL2) scales were registered. The forward scatter (FSC) and side scatter (SSC) of particles were measured simultaneously and the data were recorded on Macintosh computer using CellQuest software (Becton–Dickinson). Nuclear suspensions of *G. intraradices* were analyzed by flow cytometry to determine the average amount of DNA per nucleus. Channel number represents the relative fluorescence intensity, which is directly proportional to the amount of DNA contained in each nucleus. The important feature of flow cytometry analysis is that measurements are made separately on each nucleus within the suspension in turn, and not just as average values for the whole population. Given the difference in GC content between *G. intraradices* (30.5%, unpublished data), CEN (42.7%, Galbraith et al., 1983), and *S. cerevisiae* (40.3%, Vaughan-Martini and Martini, 1993) we used the intercalating fluorophore PI that binds to DNA, irrespectively of GC content. First, CEN were analyzed by flow cytometry (Fig. 1A) and used as a standard to evaluate the length of DNA per nucleus of *G. intraradices*, using the following formula: $\Gamma_{\text{sample}} = \text{FL}_{\text{sample}}/\text{FL}_{\text{standard}}) \times \Gamma_{\text{standard}}$, where Γ is the length of DNA (bp) per nucleus and FL is fluorescence intensity (arbitrary units). Haploid, diploid, and tetraploid strains of *S. cerevisiae* were also analyzed by flow cytometry (Figs. 1B, C, and D) and used to construct a standard curve (Fig. 1E), where fluorescence intensity of peaks of haploid, diploid and tertapoid strains of *S. cerevisiae* were plotted against the length of DNA of each strain.

2.3. DNA extraction and quantitation for reassociation kinetics and genomic reconstruction

Freshly harvested spores and hyphae of *G. intraradices* were ground in FastDNA 2 ml tubes (Bio 101) containing a lysing matrix combination (sphere, garnet, and cylinder). Tubes were placed in a FastPrep Instrument (Q-BIOgene, France) an agitation speed of 4.5 for

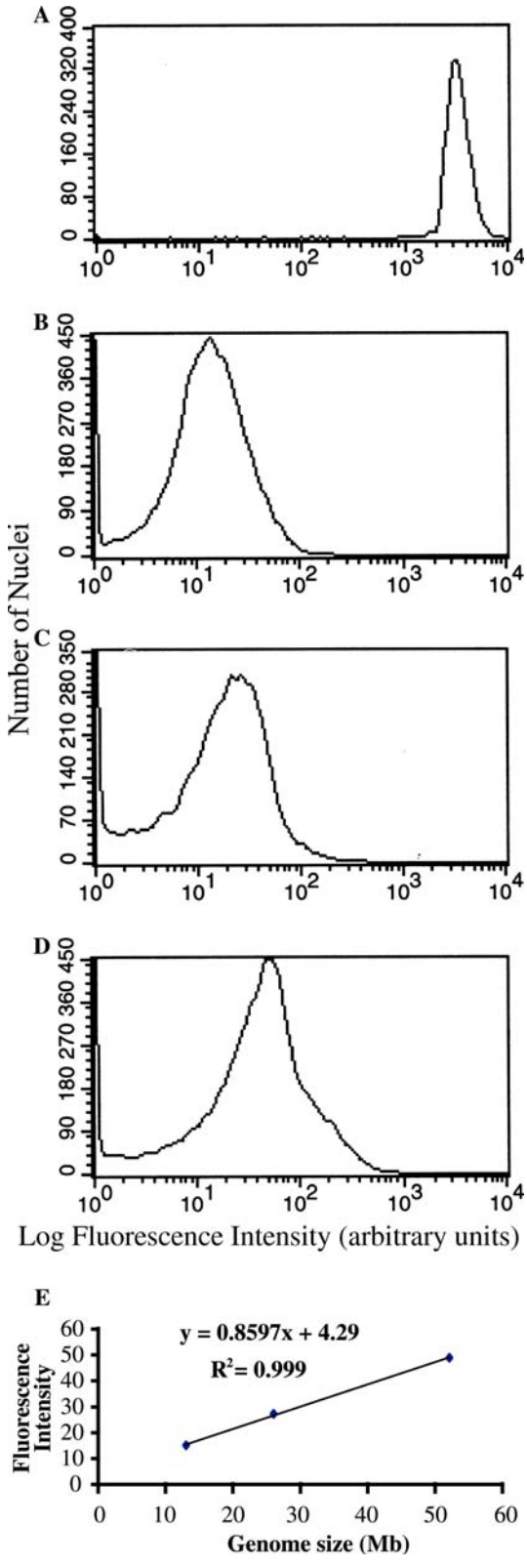

Fig. 1. Histograms showing fluorescence intensities obtained by flow cytometry with propidium iodide-stained chicken erythrocyte nuclei (A), haploid (B), diploid (C), and tetraploid (D) *S. cerevisiae*. Panel (E) shows a standard curve relating fluorescence intensity to DNA content per cell of haploid, diploid, and tetraploid *S. cerevisiae*.

30 s (three times). DNA was then extracted with Gen Elute Plant Genomic DNA kit (Sigma, Switzerland) according to the manufacturers instructions. DNA was quantitated by fluorescence spectrophotometry using PicoGreen dsDNA Quantitation kit (Molecular Probes, Switzerland) according to the manufacturers instructions. AMF exibit high AT contents (Hosny et al., 1997; Corradi et al., in press). PicoGreen dye is not selective in whether it binds to AT-or CG-, thus it is very accurate for quantitation of AMF DNA. Fluorescence was measured in 96-well microplates, in a volume of 200 µl in a Perkin–Elmer Instruments LS55 luminescence spectrometer. Samples were excited at 480 nm and the fluorescence emission intensity was measured at 520 nm.

2.4. Reassociation kinetics

Reassociation kinetics experiments were performed according to the protocol of Carr and Shearer (1998) and Le Quéré et al. (2002). Approximately 50 µg of purified DNA from *G. intraradices* and *E. coli* was sonicated to yield fragments between 200 and 2000 bp in length. The DNA was ethanol precipitated and re-dissolved in 2× SSC 1 mM EDTA (pH 7.0) to a concentration of 100 ng/µl. Aliquots of 10 µl were overlayed with sterile mineral oil, denatured by boiling, and placed at a reassociation temperature of 72 °C. Three independent reassociation experiments were performed with *G. intraradices* DNA, each with approximately 15 EC_0t values ranging from 0.01 to 1000. EC_0t represents the equivalent C_0t corrected for non-standard salt concentration (Britten et al., 1974), where C_0t is the product of the total DNA concentration [C_0] multiplied by the time in seconds [t] during renaturation. Single-stranded DNA (ssDNA) was digested with S1 nuclease (Fermentas, Switzerland) at 37 °C for 45 min. The renatured DNA was quantitated by fluorescence spectrophotometry as described above. *E. coli* DNA was used simultaneously as a standard for each experiment. The combined data from *G. intraradices* were analyzed by the nonlinear least-squares computer program provided by Pearson et al. (1977). The best-fit curve for *G. intraradices* had a root mean squares [RMS] of 0.03. $C_0t_{1/2}$ values, complexity, and fractions for *G. intraradices* and *E. coli* were determined from the computer model on the basis of the best-fit curve. Comparison of these values, using the *E. coli* standard genome size of 4.7 Mb, was used to estimate the haploid genome size of *G. intraradices*.

2.5. Sequencing of putative single-copy genes for use in genomic reconstruction

Four partial sequences of putative single-copy genes were selected as probes for genomic reconstruction experiments. These were malate synthase (1112 bp, Accession No. BE603747), heat shock protein Hsp88 (514 bp, Accession No. AU082852), Rad32 (868 bp), and RecA (919 bp). Rad32 and RecA genes were cloned and sequenced in our laboratory (N. Corradi, personal communication).

2.6. Genomic reconstruction

Genomic reconstruction experiments were performed using four homologous probes (malate synthase, Rad 32, RecA, and Hsp88) according to methods described by Francis et al. (1990), Carr and Shearer (1998), and Le Quéré et al. (2002). Three replicates of serial two-fold dilutions were prepared from *G. intraradices* genomic DNA (ranging from 600 to 4.68 ng, except for the Hsp88 probe which ranged from 800 to 6.25 ng). Three replicates of PCR products corresponding to each of the four genes, were prepared (ranging between 100 and 0.78 pg). Herring sperm DNA was used as a carrier to yield 800 ng of total DNA per sample in a total volume of 200 µl. Samples were deposited on a nylon membrane using a Hybri-Dot manifold (BRL). Each membrane was hybridized to its corresponding probe. Probes had previously been labeled with [α-^{32}P]dATP using the random primer method with a Nanoprimer labeling kit (Q-BIOgene, France) according to the manufacturers instructions. Membranes were then exposed to a phosphor imager (GS-250 Molecular Imager, Bio-Rad, Switzerland) on a BI imaging screen. Image capture and analysis were performed using Molecular Analyst software (Bio-Rad) on a Macintosh computer. Hybridization signals obtained using the appropriate probe were compared between genomic DNA and the probe itself. A standard curve was calculated to correlate hybridization intensity with the amount of each of the four genes. Genome size (Γ) could then be calculated using the formula: $\Gamma_{\text{sample}} = W_{\text{gDNA}}/W_{\text{probe}} \times L_{\text{probe}}$, where W_{gDNA} represents a given weight of genomic DNA (ng), W_{probe} represents the weight of genomic DNA (ng) to which the probe hybridized (this value was taken from the standard curve) and L_{probe} represents the length of the probe (bp). Estimation of genome size using this equation assumes that the copy number of the gene is known. If the copy number is not known then the equation cannot be used alone to estimate genome size. However, because in the equation estimated genome size is equal to actual genome size when the copy number is 1, therefore, the equation can be used to reveal copy number of a gene if the genome size has already been elucidated using another method.

3. Results

3.1. Flow cytometry

Seven independent samples of a nuclear suspension of *G. intraradices* were measured using CEN as a standard

(Fig. 2A). Because there is an initial peak of fluorescence for *G. intraradices* nuclei, we performed a second test with unlabeled nuclei (Fig. 2B) to check for autofluorescence by non-DNA molecules in the suspensions. This control showed that the initial peak is not due to DNA fluorescence and, therefore, should not be considered for measurement of DNA content per nucleus. The average length of DNA per nucleus of *G. intraradices* was 14.37 SD ± 3.2 Mb. The mean fluorescence of the *G. intraradices* nuclei with 18 independent samples, were also measured and the length of DNA per nucleus was calculated using the *S. cerevisiae* standard curve. This gave a mean of 13.93 SD ± 3.75 Mb. The overall length of DNA per nucleus of *G. intraradices* using both methods was 14.07 SD ± 3.52 Mb. The low variation in fluorescence and the lack of a clear second peak suggest that most of the nuclei were in the same phase of the cell cycle and in that the nuclei were of the same ploidy level.

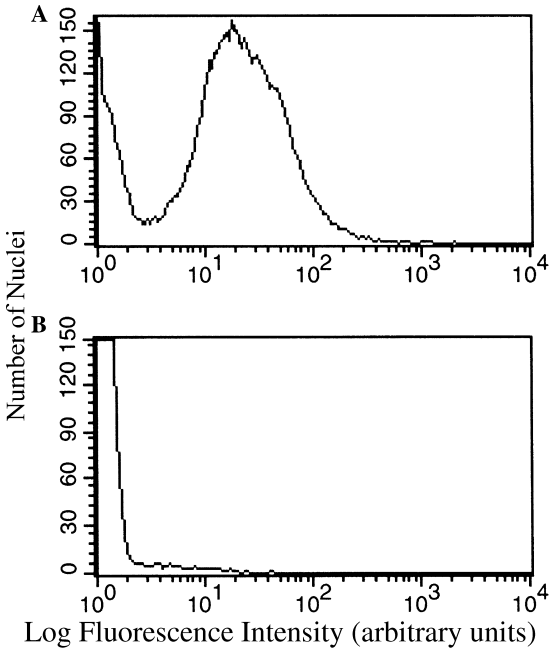

Fig. 2. Histograms showing (A) fluorescence intensities obtained by flow cytometry with propidium iodide-stained nuclei of *G. intraradices* and (B) unlabeled nuclei of *G. intraradices* (used as a negative control to check for autofluorescence).

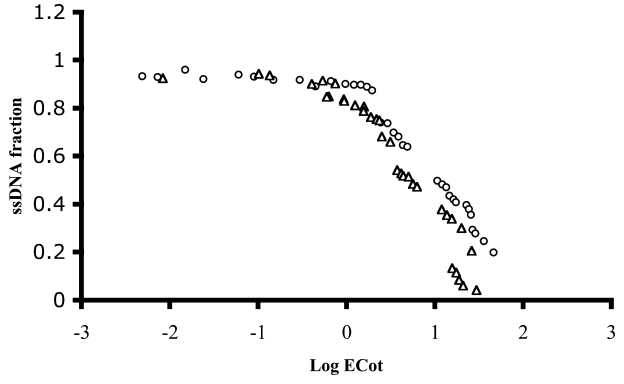

Fig. 3. Reassociation curve (EC_0t curve) of combined data from three independent experiments: (△) *E. coli* DNA; (○) *G. intraradices* DNA.

3.2. Reassociation kinetics

The reassociation curves obtained for *G. intraradices* and *E. coli* are shown in Fig. 3. Calculation based on the best fit curve indicated a total genome size of 16.54 Mb, where 88.36% of this (14.61 Mb) was single copy DNA. Another minor fraction of 1.59% (0.26 Mb) represented moderately repetitive DNA. A third component of 10.05% (1.66 Mb) was fold-back DNA (Table 1) and this most likely represents palindromic sequences and GC-rich short repeats, such as telomeres. The genome size estimate of 16.54 Mb represents the approximate length of DNA that makes up the haploid genome of *G. intraradices*.

3.3. Genomic reconstruction

A linear relationship was found between the hybridization of each probe to genomic DNA and hybridization of the probe to known amounts of the cloned gene. The R^2 values of each linear fit were: 0.93 for the malate synthase probe, 0.88 for the Rad32 probe, 0.93 for the RecA probe, and 0.79 for the Hsp88 probe (Fig. 4). From these standard curves the genome size was estimated separately using each probe. The mean genome size estimated using the malate synthase probe, was 14.52 Mb SD ± 0.97 ($n = 3$); Rad32 probe, 17.47 Mb SD ± 2.34 ($n = 3$); RecA

Table 1
Reassociation kinetics for genomic DNA from *G. intraradices*

Component	Fraction[a]	k[b]	$C_0t_{1/2}$[c]	k_{pure}[d]	Complexity (Mb)[e]	Size (Mb)[f]
Fold-back	0.1005					1.66
Single copy	0.8836	0.094	10.55	0.107	14.61	14.61

[a] Fraction of each component.
[b] Reassociation rate expressed as $M^{-1}\,s^{-1}$.
[c] Product of the molar dsDNA concentration (in nucleotides) and the time (s) for half of reassociation completion ($C_0t_{1/2} = 1/k$).
[d] Modified second-order rate constant for each component, if pure.
[e] Size of component.
[f] Total size of each component.

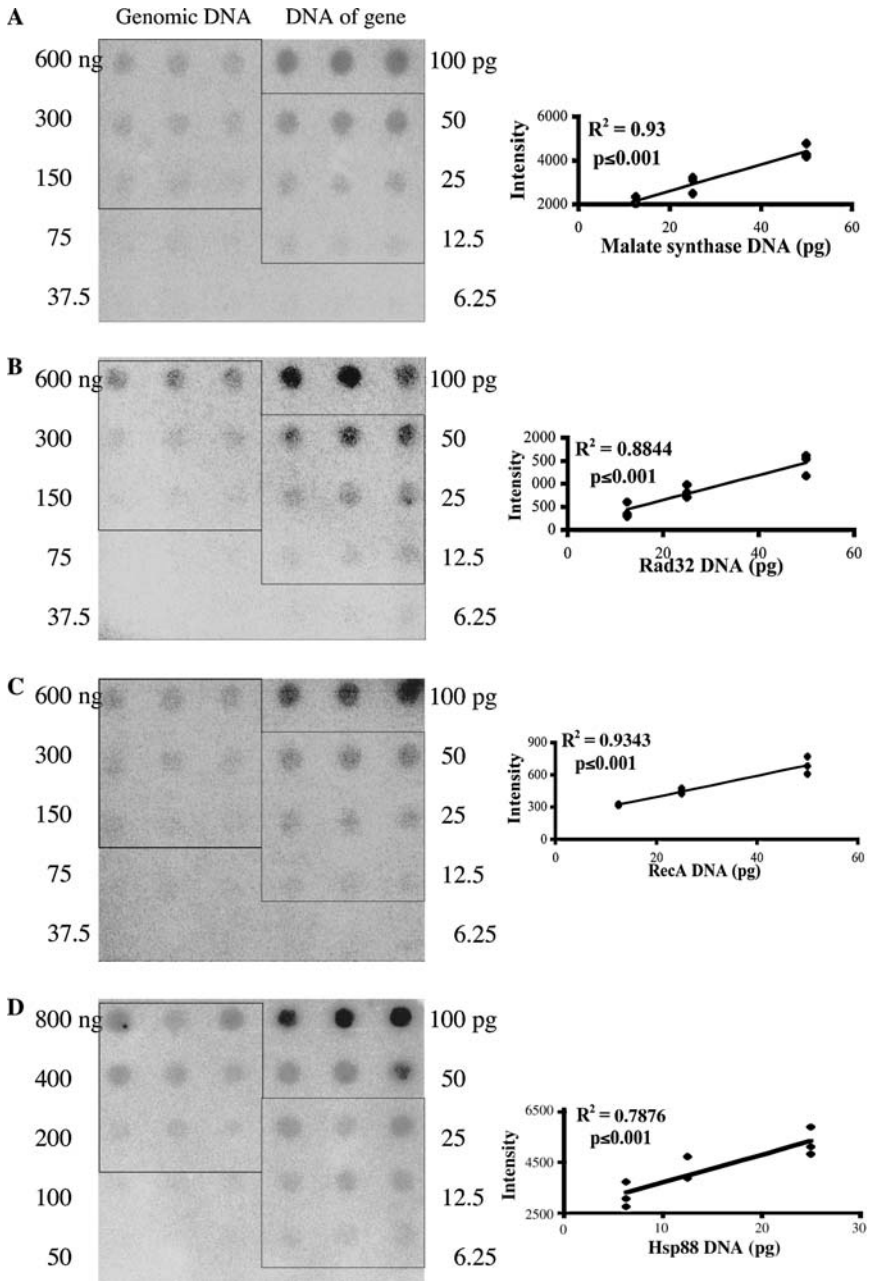

Fig. 4. Genomic reconstruction of *G. intraradices* DNA for estimation of genome size or copy number. Left panels show an autoradiography of *G. intraradices* DNA using malate synthase probe (A); Rad32 probe (B); RecA probe (C); and Hsp88 probe (D). The three lanes on the left show twofold serial dilutions of *G. intraradices* DNA with three replicates. The three lanes on the right show twofold serial dilutions of each probe with three replicates. The graphs in the right panel represent the spot intensity plotted against the amount of DNA of each spot. The spots enclosed by the two squares were used for estimating the genome size of *G. intraradices* because the spot intensities were in a similar range. The lines show the best-fit linear curves for each of the four genes.

probe, 15.06 Mb SD ± 2.53 ($n = 3$); and Hsp88 probe, 15.93 Mb SD ± 0.9 ($n = 3$) under the assumption that each gene is single copy. Using the estimates derived from the four genes the average genome size for *G. intraradices* was 15.74 Mb SD ± 1.69 if each of the four genes is single copy. The formula for estimation genome size using genomic reconstruction assumes that the gene is single copy. If the genome size (haploid genome per nucleus) is already known using flow cytometry and reassociation kinetics then an equivalent genome size estimate using genomic reconstruction confirms that the gene used as a probe is single copy. This was the case for the four genes malate synthase, Rad32, RecA, and Hsp88, where values were within the ranges predicted from flow cytometry and reassociation kinetics.

3.4. Ploidy

Ploidy can be determined by calculating the DNA index (DI), which is defined as the ratio between the length of DNA per nucleus (as measured by flow cytometry) and that of the genome size estimate (by either reassociation kinetics or genomic reconstruction). A DI of approximately 1, represents a haploid genome; approximately 2 represents a diploid genome, etc. Analysis by flow cytometry estimated the length of DNA per nucleus to be 14.07 ± 3.52 Mb. The estimated genome size by reassociation kinetic analysis was 16.54 Mb. This gives a ratio of 0.85 and this suggests that *G. intraradices* is haploid. Calculation of DI using values from flow cytometry genomic reconstruction (using the average value for the four genes, 15.74 ± 1.69) gives a DI of 0.89. It is usual that the DI is less than 1 when flow cytometry data are used as the numerator because values of genome size derived from reassociation kinetics or genomic reconstruction include DNA outside the nucleus, e.g., mitochondrial DNA which can be considerable in fungi.

4. Discussion

We used three independent methods to determine the basic features of the genome of the arbuscular mycorrhizal fungus *G. intraradices*, regarding DNA content per nucleus, genome size, complexity, and ploidy.

The fungal kingdom shows a huge genome size variation of approximately an 1000-fold range from 8 Mb, reported for *Bolbitius vitellinus* (Latzelsberger and Krisai-Greilhuber, 2000), to 8036 Mb found in the insect-infecting genus, *Entomophaga* (Murrin et al., 1986). However, the size of most basidiomycete, ascomycete, and some zygomecete genomes, are in the range 8–46 (Ayad-Durieux et al., 2000; Dusenberry, 1975; Keijer et al., 1996; Randhir and Hanau, 1997; Wöstemeyer and Burmester, 1986). Therefore, the genome size of *G. intraradices* is on the lower size range for fungal genomes and for eukaryotes. The *G. intraradices* Γ value appears very small in comparison to other the AMF reported by Hosny et al., 1998, and Bianciotto and Bonfante, 1992, which were in the range of 127.4–1058.4 Mb. Our results on genome size are in agreement with random *G. intraradices* genome sequencing survey (GSS) (Peter Lammers, New Mexico State Unversity, http://darwin.nmsu.edu/~plammers/). Analysis of the GSS data did not reveal any highly repetitive sequence. Given that *G. intraradices* can easily be produced in large amounts in a clean culture system and that it has a very small genome size this makes it an excellent candidate for a genome sequencing project.

Reassociation kinetics experiments with *G. intraradices* DNA indicated a haploid genome size of 16.54 Mb with approximately 1.59% (263 kb) repetitive DNA. This result is not particularly surprising when considering values of repetitive DNA in some other fungi. For example, *Histoplasma capsulatum* (G186AS) was found to have a genome of approximately 23 Mb with less than 0.5% repetitive sequences (Carr and Shearer, 1998). A similar result was found with *Aspergillus nidulans* DNA, which has a haploid genome size of 26 Mb and only slightly over 2% repetitive sequences (Timberlake, 1978). The repetitive fraction in *H. capsulatum* and *A. nidulans* most likely represents ribosomal DNA. It has previously been reported that rDNA copy number is very low (75 copies per genome) in the AMF *S. castanea*; a species that has much bigger genome than *G. intraradices* (Hosny et al., 1999). However, *G. intraradices* showed a fold-back DNA of 10.05% (1.66 Mb) which is high in comparison to ascomycetes and basidiomycetes. This probably represents palindromic sequences which have been observed by us many times in *G. intraradices* sequences (M. Hijri, unpublished) and GC-rich short repeats, such as telomeres and subtelomeric regions.

The number of protein-coding genes in organisms is thought to vary over 50-fold (Cavalier-Smith, 1985) while genome size varies over 80,000-fold. Gene number is positively correlated with structural complexity, whereas genome size is not (Graur and Li, 2000). Based on a total sequence of 13,026,500 bp per haploid genome of *S. cerevisiae*, 12,057,500 bp (92.56%) are unique sequences and another 969,000 bp (7.44%) is repetitive sequences. The number of putative protein-coding genes identified is approximately 6200. We could speculate that *G. intraradices* with a complexity of 14.61 Mb may have a similar number of protein-coding genes.

Because of the complementary information given by each of the three methods, we can also predict that the haploid genome size is near enough to the DNA content in each nucleus and, that therefore, *G. intraradices* is haploid. Furthermore, because the haploid genome size matches the DNA size per nucleus, that was predicted by genomic reconstruction, it is almost certain that the four genes malate synthase, Rad32, RecA, and Hsp88 are single copy.

The ploidy and the low amount of repetitive sequences in the *G. intraradices* genome is, however, particularly interesting when considering the arrangement of genetic variation within AMF individuals. Although direct evidence for genetic differences among nuclei has been shown in *S. castanea* (Kuhn et al., 2001) there has also been the suggestion that high within-spore genetic variation in AMF is due to high ploidy or high copy number of a given variable gene. Our results showing that *G. intraradices* is haploid, leads us to reject the hypothesis that genetic variation within this fungus is due to very high ploidy. Despite this, Kuhn et al. (2001) have seen considerable variation in the Bip gene in *G. intraradices*, with a large

proportion of the substitutions being non-synonymous. Furthermore, given that the *G. intraradices* genome is on the lower limit for eukaryotes and that a high proportion of the genome is single copy DNA it also seems unlikely that such high genetic variation is due to high gene copy number either.

This information will be particularly useful for understanding the arrangement of genetic variation in AMF among and within nuclei. By knowing that *G. intraradices* is haploid and by knowing that given genes occur as one copy per nucleus, any investigations of variation in such genes within *G. intraradices* will show how much genetic variation is partitioned among nuclei within AMF. This will undoubtedly be useful in future for understanding the evolutionary genetics of AMF.

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