FISHy Analysis of Tetraploid Cotton Species

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Fluorescent *in situ* hybridization (FISH) is an important technique in plant genome research, because it provides integrated information about DNA, chromosomes and genomes. Genomic *in situ* hybridization (GISH) is a modification of FISH that can be used to rapidly compare genome content, relatedness, organization and/or behavior. GISH results often provide insight into genome evolution and species relationships. The cotton genus *Gossypium* consists of about 50 species, including 5 AADD tetraploids (2n=4x=52) thought to have originated from two diploid genomes (2n=2x=26), one A-like genome (as female) and D-like genome (as male). Here we report on GISH-based comparative studies on the four tetraploids: *G. hirsutum* [$(AD)_1$], *G. barbadense* [$(AD)_2$], *G. mustelinum* [$(AD)_4$] and *G. darwinii* [$(AD)_5$]. Probes were made from genomic DNAs (gDNAs) of two A genome species [*G. herbaceum* (A_1), *G. arboretum* (A_2)] and five D genome species [*G. thurberi* (D_1), *G. davidsonii* ($D_{3\text{-}d}$), *G. klotzschianum* ($D_{3\text{-}k}$), *G. raimondii* (D_5) and *G. gossypioides* (D_6)], as well as from 45S rDNA to detect nucleolar organizer regions (NORs). For all four tetraploids, GISH of A- and D-genome gDNAs resulted in clear signals on A and D sub-genome chromosomes, respectively, but the signal intensities were noticeably stronger on A- than D-genome chromosomes. Exceptionally, D_6 gDNA generated signal in both A- and D-subgenome chromosomes and the signal intensities were similar Three pairs of NORs were detected by FISH of 45S rDNA probe. GISH of gDNA from all of the D-genome species including D_6, resulted in strong signals that co-localized with the NORs. We have thus dubbed these as "GISH-NORs". The GISH-NORs were not detected by GISH of A-genome gDNAs. In $(AD)_1$, $(AD)_2$ and $(AD)_5$ tetraploids, the three pairs of major NOR and GISH-NOR loci were situated similarly, one in the A sub-genome and two in D sub-genome; the relative sizes and signal intensities were also similar across species. But in $(AD)_4$, all three pairs of NORs and GISH-NORs were located in the A sub-genome, and one pair was exceptionally large ("super-major") and two pairs were minor. The super-major rDNA locus accounted for about one half of its chromosome length at metaphase, and the signal its middle region was absent or greatly diminished, and similar in appearance, at least superficially, to a centromere. Little difference was observed among AD genomes after GISH of A_1- or A_2-gDNA, but considerable variation was noted after GISH of various D genomes. Especially strong signals occurred in $(AD)_1$ and $(AD)_5$ after GISH of $D_{3\text{-}d}$, in $(AD)_2$ after GISH of D_4, and in $(AD)_4$ after GISH of D_1. The differential responses of AD genomes to GISH of various D-genomes presumably reflects contemporary differences in repeated sequences among the tetraploid species, especially $(AD)_4$, and thus differences in their origins and/or evolution.