

Genomic *In Situ* Hybridization (GISH) as a Tool to Identify Chromosomes of Parental Species in Sunflower Interspecific Hybrids

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Abstract

Interspecific hybridization has been widely used to transfer genes from wild species into cultivated sunflower. Fluorescent genomic *in situ* hybridization (GISH) has been used to identify alien chromosomes or segments in other crops, but an equivalent technique for sunflower is lacking. The objective of this study was to develop a GISH procedure for identifying chromosomes or chromosome segments of wild species in the background of cultivated sunflower. Interspecific hybrids and backcross progenies involving four wild perennial species, *H. californicus*, *H. angustifolius*, *H. nuttallii* and *H. maximiliani*, were examined. With different blocking/probe ratios and washing stringencies, chromosomes or segments of the four wild species were clearly identified. Our results demonstrated that the GISH procedure is a practical tool for identification of alien chromosomes or chromosome segments during the process of interspecific gene transfer.

Introduction

The *Helianthus* genus is comprised of annual and perennial species. Interspecific hybrids have contributed to the improvement of agronomic traits and oil quality of sunflower. Genomic *in situ* hybridization (GISH) can detect alien chromosomes or segments in the interspecific or intergeneric hybrids, translocation breakpoints, chromosome pairing activity, and the genome composition of polyploidy plants. This technique has been applied in many crops since its establishment for plants by Schwarzacher et al. (1989), including rye (Heslop-Harrison et al., 1990), wheat (Liu et al., 2007; Qi et al., 2008), barley (Pickering et al., 1997), beet (Schmidt et al., 1997), rice (Jin et al., 2006), potato (Pendinen et al., 2008), tomato (Ji and Chetelat, 2003), brassica (Wang et al., 2005), and cotton (Guan et al., 2008). Due to the close relationship between cultivated sunflower and wild *Helianthus* species, an acceptable GISH technique specific to sunflower is lacking. The objective of the present study was to develop a GISH procedure for identifying the chromosomes or chromosome segments of wild species in the background of cultivated sunflower and to characterize the genome composition of interspecific hybrids.

Materials and Methods

Progenies from four interspecific crosses were used for this study; (1) BC₄F₁ progeny with 2n=35 of *H. californicus* (2n=102) × HA 410 (2n=34), (2) BC₄F₁, 2n=35 and BC₄F₂, 2n=34 progeny from cytoplasmic male-sterile (CMS) 514A (2n=34) × (*H. angustifolius* × P21, amphiploid) (2n=68), (3) F₁ progeny of nuclear male-sterile (NMS) HA 89 (2n=34) × *H. nuttallii* (2n=34), and (4) one amphiploid (2n=67) of NMS HA 89 ×

H. maximiliani ($2n=34$). Root tips collected from 3-week-old seedlings were placed in distilled water at 2 °C for 18 h. After fixation in 3:1 (v/v) ethanol:glacial acetic acid for 3-4 h, chromosome spreads were made following the method of Liu et al. (2007).

Genomic DNA of wild sunflower species was used as a probe after being sheared in boiling water for 10 min and labeled with digoxigenin-11-dUTP using the nick translation method (Roche Applied Science, Nutley, NJ, USA). Genomic DNA of HA 89 was used as blocking DNA after shearing, with ratios of blocking DNA to probe DNA ranging from 35:1 to 120:1. Different washing stringencies were used for different wild species. Labeled probes were detected with anti-dig-rhodamine (Roche Applied Science). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma) in Vectashield (Vector Laboratories, Burlingame, CT, USA). Slides were analyzed under a fluorescence Axioplan2 Imaging microscope (Carl Zeiss, Germany). Images were captured by a chargecoupled device (CCD) camera (Carl Zeiss AxioCam HRM, Germany), and processed using Axiovision 3.1 software and Adobe Photoshop 6.0.

Results and Discussion

Alien chromosome or chromosome segments were detected by the probe of *H. californicus* or *H. angustifolius* in their backcross progenies. *H. californicus* was crossed with HA 410 and backcrossed several times to reduce the chromosome number close to the $2n=34$ of the cultivated sunflower. When the chromosome number in the progenies was 34-37, the GISH procedure revealed a whole alien chromosome in the progenies with $2n=35$ (Fig. 1A), as well as part of an alien chromosome resulting from recombination between the two parents ($2n=35$, Fig. 1B).

The combination of CMS and its respective fertility restoration gene (*Rf*) is essential for commercial sunflower hybrid production. In our search for the *Rf* gene for a CMS line with *H. tuberosus* cytoplasm (CMS 514A), progenies of CMS 514A pollinated by one amphiploid involving *H. angustifolius* were all male-fertile with $2n=51$. After several backcrosses with HA 89 and selection for male-fertile progenies, male-fertile plants with $2n=35$ were isolated. Testcross progenies of CMS ($2n=34$) pollinated with two male-fertile plants with $2n=35$ resulted in 55 male-sterile $2n=34$ plants and four male-fertile $2n=35$ plants, suggesting that the *Rf* gene is located on the extra chromosome. With our GISH procedure, the extra alien chromosome can be easily identified in the background of the 34 cultivated line chromosomes (Fig. 1C). Self-pollination of eight $2n=35$ male-fertile plants resulted in one male-fertile progeny with $2n=36$, 10 with $2n=35$, and two with $2n=34$, and the remaining 49 were male-sterile. In F_2 progeny of a cross between one male-fertile plant ($2n=34$) and the CMS, GISH detected alien chromosome segments in a combination of patterns, with one plant having two translocations on different chromosomes (Fig. 1D, other data not shown). This technique, in combination with BAC-FISH, may expedite linking the *Rf* gene to a specific chromosome.

When the hybrids of NMS HA 89 \times *H. nuttallii* were probed with the genomic DNA of *H. nuttallii*, 17 out of 34 chromosomes displayed hybridization signals (Fig. 1E), proving that the chromosome of *H. nuttallii* can be distinguished from those of *H. annuus*, and that the GISH technique can track the *H. nuttallii* chromosomes or chromosome segments in interspecific progenies.

In a $2n=67$ amphiploid of NMS HA $89 \times H. maximiliani$, 33 out of 67 chromosomes were detected with the genomic DNA of *H. maximiliani* (Fig. 1F). The results suggested two genomes belong to *H. annuus*, and two from *H. maximiliani* with one chromosome missing. This also indicated that tracking the *H. maximiliani* chromosomes or chromosome segments while selecting for specific traits.

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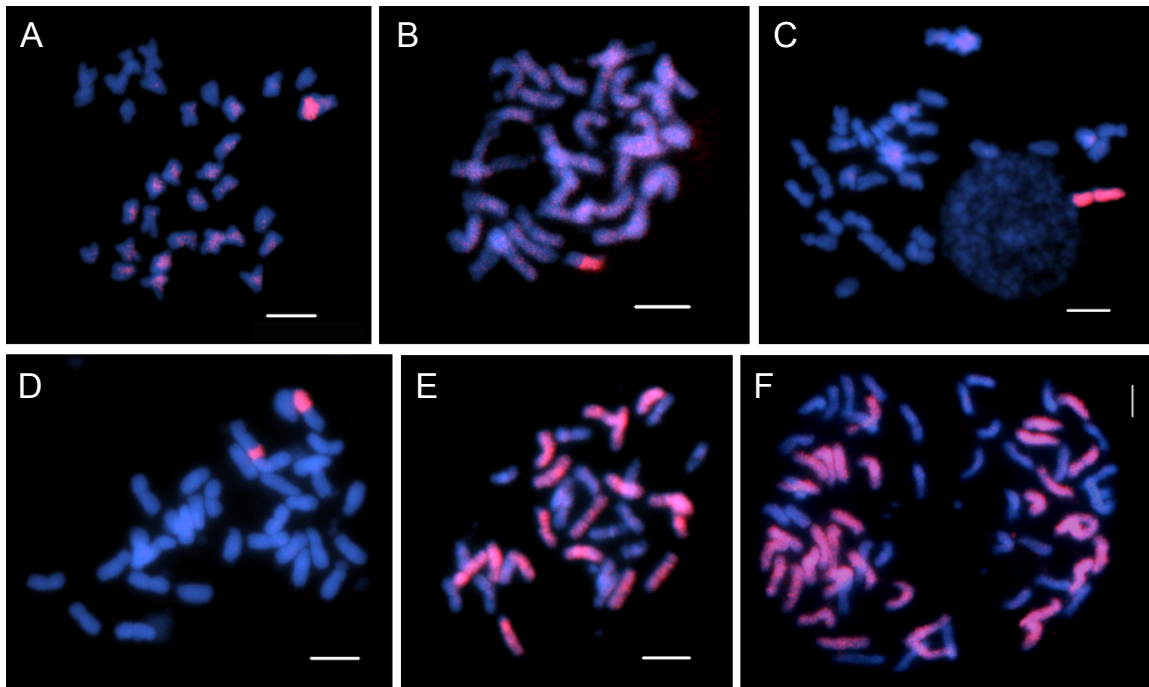


Figure 1. GISH analysis of backcross progenies and interspecific hybrids. The genomic DNA of wild sunflower species was labeled with digoxigenin-11-dUTP and detected with anti-dig-rhodamine (red). Chromosomes were counterstained with DAPI (blue). The alien chromosome (A) or segment (B) identified in the backcross progenies of *H. californicus* × HA 410. The additional alien chromosome (C) or translocations (D) detected in the progenies of CMS 514A × (*H. angustifolius* × P21, amphiploid). (E) The genome of *H. nuttallii* detected in the F₁ hybrid of nuclear male-sterile HA 89 × *H. nuttallii*. (F) The *H. maximiliani* chromosomes detected in the amphiploid of nuclear male-sterile HA 89 × *H. maximiliani*. Bars=5 μm.