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J. Chen · J. Staub · Ch. Qian · J. Jiang · X. Luo
F. Zhuang

Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix* Chakr. × *Cucumis sativus* L.

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Abstract Interspecific hybrids between *Cucumis hystrix* Chakr. (2n = 2*x* = 24) and *Cucumis sativus* L. (2n = 2*x* = 14) were produced by means of F_1 (2n = 19) embryo rescue and subsequent chromosome doubling. The hybridity was confirmed by genomic in situ hybridization (GISH) and chromosome analysis. The amphidiploid (2n = 38) was self-pollinated and backcrossed to cucumber resulting in lines with improved crossability to *C. sativus*. Examination of shape, stainability, and germination rate of pollen grains and yield as a function of mature fruit set per ten pollinated flowers indicated a tendency for increased fertility in BC_1S_1 progeny when compared to F_1 and amphidiploid offspring. Cytogenetic characterization of F_1 and amphidiploid progeny was performed. Generally normal meioses produced viable pollen grains, and fertilization resulted in partial fertility restoration in amphidiploid progeny. Chromosome anomalies such as "frying-pan trivalent", chromosome lagging and spindle mis-orientation were also observed. In most of the PMCs of the F_1 diploid hybrid progeny, 19 univalents were observed at diakinesis and MI. In the amphidiploid, more than 90% of the configurations at MI consisted of the predicted 19 bivalents and less than 5% contained multivalents [trivalents (2.3%) + quadrivalents (0.3%)], suggesting the presence of preferential pairing, and a distinctive parental genome as well. The chiasmata observed between homoeologous chromosomes further demonstrated the introgression of the *C. hystrix* genome into that of *C. sativus*.

Keywords Interspecific hybridization · Amphidiploid · GISH · Chromosome pairing

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J. Chen (✉) · Ch. Qian · X. Luo · F. Zhuang
State Key Laboratory of Crop Genetics and
Germplasm Enhancement, College of Horticulture,
Nanjing Agricultural University, Nanjing, 210095 China
e-mail: jfchen@njau.edu.cn
Tel.: 025-4396279, Fax: 025-4396279

J. Staub
U.S. Department of Agriculture, Agricultural Research Service

J. Staub · J. Jiang
Department of Horticulture, University of Wisconsin-Madison,
1575 Linden Drive, Madison WI 53706 USA

Introduction

Intraspecific hybridization has been widely employed in crop species to produce improved germplasm for commercialization. Recently, however, the genetic diversity of horticulturally advanced germplasm has become increasingly narrow following continued use of germplasm with similar pedigrees in plant breeding programs. This phenomenon is especially true in crops such as cucumber (*Cucumis sativus* L., 2n = 2*x* = 14) where germplasm banks are typically comprised of relatively few accessions (e.g., only approximately 1,360 accessions in the U.S. National Plant Germplasm System) (Staub et al. 1999). The incorporation of wild *Cucumis* species to broaden the genetic base of cucumber has been a goal of plant geneticists and breeders for over 100 years. The identification and incorporation of resistance to economically important pests such as root knot nematode (Walters et al. 1993) and various foliar pathogens (Den Nijs and Custers 1987) is important to cucumber production. Genes for resistance to several crop-limiting pests and pathogens are, however, not found in cucumber. Attempts at interspecific hybridization of wild *Cucumis* species (e.g., *Cucumis metuliferus* E. Mey ex Schrad., *Cucumis melo* L.) with cucumber have been either unsuccessful or not repeatable. Thus, it has been concluded that mating of *C. sativus* with any other *Cucumis* species possessing a chromosome number of 2n = 24 or 48 would not be successful (Kristkova and Lebeda 1995).

In 1995, interspecific hybridization was accomplished between *Cucumis hystrix* Chakr. (2n = 2*x* = 24) and *C. sativus* (Chen et al. 1997a). The cross was attempted based on their phylogenetic relationship as revealed by isozyme analysis (Chen et al. 1995, 1997b). Since the

initial F_1 hybrids (2n = 19) were both male and female sterile [12 and 7 chromosomes contributed by *C. hystrix* (H) and *C. sativus* (C), respectively], chromosome-doubling experiments were initiated to restore fertility (Chen and Staub 1997). In 1998, an amphidiploid (HHCC, 2n = 4*x* = 38) was obtained through somaclonal variation during in vitro embryo culture resulting in the production of the first fertile amphidiploid in *Cucumis* (Chen et al. 1998a). An array of amphidiploid plants were obtained and subsequently self-pollinated to produce viable seeds (Chen and Kirkbride 2000).

This amphidiploid has been designated a new synthetic species, and can serve as genetic bridge in *Cucumis*, and may become a source for broadening the genetic base of *C. sativus* (Chen and Kirkbride 2000). As a bridge species, the synthetic species may prove useful for the transfer of economically important traits such as root knot resistance from *C. hystrix* to *C. sativus* (Chen et al. 2001), and tolerance to growth under low irradiance and temperature (unpublished data). The exploitation of this amphidiploid for cucumber germplasm improvement has, however, been hampered by a lack of understanding of meiotic and mitotic behavior. We report herein the characterization of the cytogenetics, fertility and reproduction of the F_1 interspecific hybrid between *C. hystrix* and *C. sativus*, and the synthetic amphidiploid derived therefrom.

Materials and methods

Materials

The *C. hystrix* accessions employed were those recollected from the original site in Xishuangbanna, Yunnan province, China (Chen et al. 1994). Cucumber seed of North (*C. sativus* cv Beijingjietou; designated North-China type) and South China (*C. sativus* cv Erzhaozi; designated South-China type) market classes were the same as those used by Chen et al. (1997b). To synchronize flowering time and avoid contamination by uncontrolled cross-pollination, accessions were planted in a greenhouse at Nanjing Agricultural University at different times. While the *C. hystrix* parent was planted on May 2, 2000, *C. sativus* accessions were planted on July 25, 2000.

Hybridization, embryo rescue and chromosome doubling

Reciprocal matings were made among *C. hystrix* and *C. sativus* accessions between October 5 and 20, 2000, according to Chen et al. (1997a). Floral buds were wrapped in parafilm the afternoon before anthesis to ensure controlled pollination.

Immature embryos varying from "early heart" to "rabbit-ear" in morphology were recovered from 16 day old fruits and cultured on a solid MS hormone-free medium, containing 30 g/l of sucrose, and 8–12 g/l of agar maintained at pH 6.0 (Murashige and Skoog 1962). The embryos were incubated in sterile conditions at 25 °C under fluorescent light (40 ± 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a photoperiod of 16 h/8 h (day/night) for 50 days until plantlets developed.

A doubling of the chromosome number of the F_1 interspecific dipoid hybrid progeny was accomplished by monitoring cultures for somaclonal variation according to Chen et al. (1998a). Putative chromosome-doubled somaclonal variants were initially identified by their unique morphological characters (e.g. curved leaf edge). Expanding leaves excised from putative diploid hybrid plantlets (2n = 19) were sliced into 0.5 cm and placed with abaxial side up, on a MS medium containing 1.0, 0.5, 0.4 and 10 $mg \cdot l^{-1}$ of 2.4 D, BA, ABA and $AgNO_3$, respectively. After 4 weeks incubation under fluorescent light [16 h/8 h (day/night), 40 ± 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$], plantlets were transferred to MS hormone-free medium. Three to four weeks later, shoots were removed from the adherent callus, and transferred to MS medium containing 1.0 $mg \cdot l^{-1}$ of IBA to induce root formation. When small roots (20 to 40 mm long) protruded from shoots, plantlets were transferred from artificial media to moist soil media (vermiculite/sand = 1/1 vol.:vol.) and held under a plastic canopy for greenhouse acclimatization under high relative humidity (>85% RH; 25 °C; natural irradiance) for 1 week. The canopy was then removed, and plants were matured in a greenhouse at 25 °C under fluorescent light irradiance (100 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and a photoperiod of 16 h/8 h (day/night).

Cytological analyses of the diploids and amphidiploids

Confirmation of hybridity

Tendrils were harvested from five amphidiploid plants, and 30 meristematic cells were observed in each sampled plant after fluorescence labeling. The chromosome number of putative amphidiploid progeny was counted by observing the meristematic cells of the tendrils. The genomic in situ hybridization (GISH) technique was used to assess the amphidiploid derived from chromosome-doubled F_1 interspecific hybrids.

The *C. hystrix* genomic DNA was labeled with biotin-11-dUTP by nick translation. Shared *C. sativus* DNA (0.5 μg/μl) was added to the hybridization mixture. GISH experimentation proceeded according to Dong et al. (1999) such that 40 μl of hybridization mixture containing 100 ng of the *C. hystrix* probe (2 to 5 μl), and 4 μg of blocking DNA (about 8 μl), were applied to chromosome preparations. Post-hybridization washing and signal detection procedures were according to those described by Jiang et al. (1995).

Chromosome number assessment of amphidiploids

Interspecific F_1 plants produced few roots, and therefore young, actively growing tendrils (1 to 3 cm long) were used to determine the chromosome number of putatively doubled-hybrid plants. The procedure for chromosome preparation was according to Chen et al. (1998b) with slight modifications; tendrils were pretreated in 8-hydroxyquinoline for 50 min at 18 °C, and then hydrated in 1N HCl for 10 min at 60 ± 1 °C. Chromosomes of 30 cells from ten F_1 (among 70) and five amphidiploid plants (among 75 morphologically uniform plants) were observed. These chromosomes and mitotic figures were recorded at metaphase (M) on Kodak film with a single-lens reflex camera attached to an Olympus (BX-51) microscope system (Olympus, Japan) at 1,500 × magnification.

Meiotic observation of diploids and amphidiploids

Young floral buds from diploid *C. sativus* parents (1.0–2.5 mm long) and synthetic amphidiploid (2.0–3.0 mm long) progeny were selected for fixation in Carnoy's fluids (1 glacial acetic acid:3 chloroform:6 ethanol) for 24 h, and then transferred into 70% ethanol solution and stored at 4 °C for examination.

Chromosome preparation was that according to van Raamsdonk (1989). The anthers were tangentially cut into two parts with a razor blade, stained with acetocarmine, macerated and then gently squashed, and warmed under a cover slip for cytogenetic analyses. At least 100 pollen mother cells (PMCs) from each ploidy level were examined at different meiotic stages, and figures were recorded using the Olympus (BX-51) microscope system as described above.

Assessment of fertility

Fertility was assessed by observing shape, stainability and germination in pollen from 20 plants, and by recording the number of

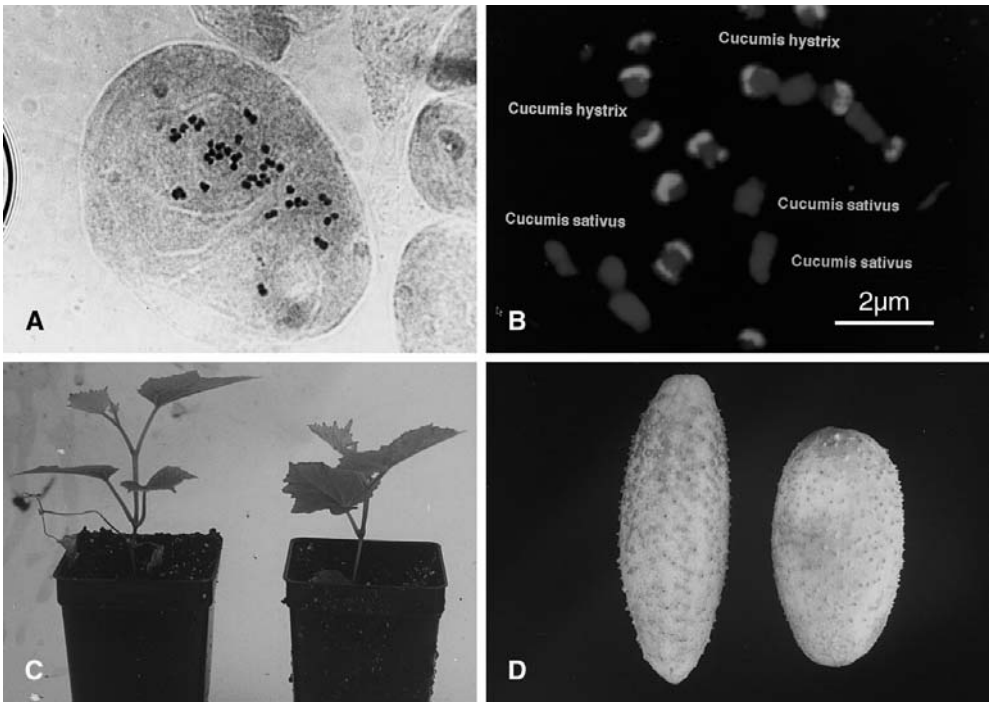

Fig. 1A–D Production and verification of an interspecific hybrid and amphidiploid from a mating between *C. sativus* (2n = 14) and *C. hystrix* (2n = 24). **A** Amphidiploid chromosomes at metaphase in a somatic cell. **B** GISH verification of parentage of chromosomes in amphidiploid with 12 chromosomes from *C. hystrix* having a fluorescence signal and seven from *C. sativus* without a signal. **C** Plants at different ploidy levels: diploids (left) with flat leaf edges (*C. sativus* parental line) and amphidiploids (2n = 4*x* = 38; right) with curved leaf edges. **D** Fruits of diploids (left) with a short pole shape and amphidiploids (right) with an ellipsoid shape

mature fruits and seeds per fruit obtained from ten hand-pollinated flowers drawn from 20 randomly pollinated plants of each diploid, and amphidiploid and backcross (from amphidiploids × *C. sativus* inbred lines) progeny identified. Pollen grains were stained with acetocarmine, and germinated in a medium containing 0.8% agar, 10% sucrose and 0.05% boric acid.

Results

Reproduction

Hybridization, embryo rescue, and chromosome doubling

Over 90% of the crosses between *C. hystrix* and cucumbers resulted in mature fruits with putative hybrid embryos. A total of 185 embryos from interspecific hybridization (*C. hystrix* × *C. sativus*) were removed from fruits produced by mating to both *C. sativus* genotypes (North- and South-China). Seventy (37.8%) of the embryos that were subsequently cultured in vitro matured into flowering plants.

Organogenesis of embryos and the subsequent culturing of the leaf tissue from hybrid plantlets resulted in 993 plantlets. Among those regenerants, there was a group of 75 (7.6%) uniform plants with a unique leaf morphology. Leaf margins of these chromosome-doubled plants (2n = 38) were curved, and regenerants possessed relatively short internodes (4.8 ± 1.2 cm) (Fig. 1A).

The induction rate leading to the production of chromosome-doubled plants was genotype-dependent. Of the 840 regenerants derived from the *C. hystrix* × 'Beijing Jietou' mating, 47 (5.6%) were chromosome-doubled plants (data not presented). In contrast, the culture of hybrid embryos derived from crosses with 'Er Zhaozi' resulted in 36 (23.5%) chromosome-doubled plants from 153 initial regenerants. The frequency and genotype-dependent recovery of chromosome-doubled plants in our experiments agrees closely with the frequency obtained by Adelberg and Chen (1998) during regeneration of tetraploid melon (*C. melo*) plants.

Assessment of hybridity

The general leaf and fruit morphology of the putative interspecific F_1 hybrid differed from both parents (Fig. 1C and D). In general, however, these hybrids were more characteristic of the *C. sativus* parents than of the *C. hystrix* parent. Nevertheless, hybrid plants were intermediate in internode length (5.7 ± 1.4 cm) when compared to the *C. sativus* (10.6 ± 2.4 cm) and *C. hystrix* (4.8 ± 2.7 cm) parents (data not presented). Likewise, the leaf size (191.2 ± 27.8 cm^2) and fruit length (9.2 ± 1.8 cm) of hybrid plants were intermediate to the *C. sativus* (leaf size = 321.5 ± 34.5 cm^2; fruit length = 25 ± 2.5 cm) and *C. hystrix* (leaf size = 102 ± 13.9 cm^2; fruit length = 5.5 ± 1.0 cm) parents.

GISH was used to assess the hybridity of interspecific F_1 progeny by examination of the 19 chromosomes present (Fig. 1B). Fluorescence labeling revealed that 12 chromosomes were associated with a green signal corresponding to that demonstrated by the hybridization of probes to the *C. hystrix* parent. The remaining seven chromosomes demonstrated the red color-labeling characteristic of the *C. sativus* parents, thus confirming progeny hybridity. The 12 chromosomes associated with the green labeling could be divided into two types based on the position of the labels: ten chromosomes had a label

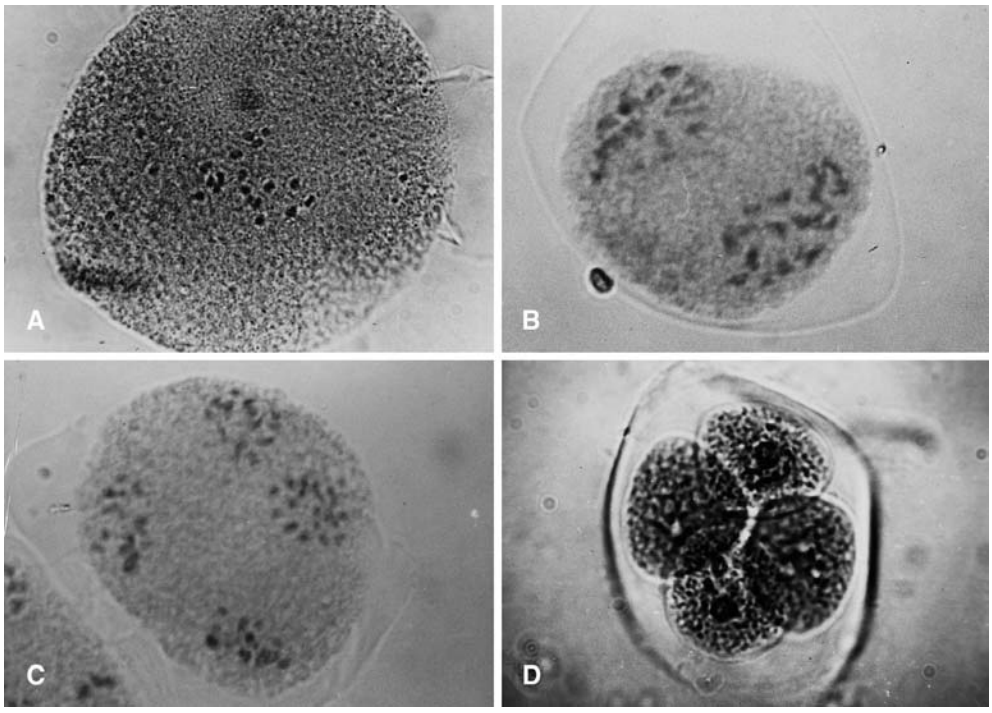

Fig. 2A–D Normal meiotic stages in PMCs of amphidiploid plants derived from *C. hystrix* × *C. sativus*. **A** Metaphase-I showing 19 bivalents. **B** Telophase-I showing 19 dyads at each pole. **C** Telophase-II showing 19 chromosomes at each pole. **D** Tetrads

Table 1 Fertility of F_1 hybrids (2n = 19), amphidiploids (2n = 38) and progenies (BC_1, 2n = 26) from backcrossing

Genotype	Pollen grain stainability	Pollen grain shape	Pollen grain germination (%)	Number of fruits with seeds per ten self-pollinated flowers	Number of seeds per fruit	Number of plants examined
F_1 hybrid	Colorless or light color	Deformed and Irregular	0–2	0	0	20
Amphidiploid	~25% dark color	~25% full and plump	10–40	2–4	0–20	20
BC_1	~13% dark color	~13% full and plump	5–20	1–2	0–10	20

at one terminal region and two chromosomes had a label at both terminal regions.

Of the 30 meristematic cells observed in each of the five amphidiploid progenies examined, 28–30 (93.3–100%) of the cells possessed a chromosome number of 38 (Fig. 1A). These data recapitulated those recorded in a previous flow cytometry study where chromosome number was established to be 2n = 38 (Chen et al. 1998a). The appearance of aneuploid cells was rare (0 to 6.7%), but in such cases chromosome number varied between 30 and 37.

Fertility investigation

The interspecific F_1 hybrids recovered were vigorous and morphologically uniform. Nevertheless, when these hybrids were reciprocally backcrossed to either parent (as maternal or paternal donors) or self-pollinated, flowers aborted prior to anthesis and thus hybrids were designated as male and female sterile. As revealed by pollen stainability, shape, germination percentage and fruit number data, an increased fertility from the F_1 hybrid to amphidiploids during polyploidization was recorded (Table 1). Viable pollen grains of amphidiploids absorbed stain, were full and plump, and germinated well on artificial media. In contrast to the F_1 hybrid, seeds (0–20 per fruit) were recovered from self-pollinated amphidiploid plants, indicating fertility restoration after chromosome doubling. Fertility restoration was further confirmed by the production of vigorous, fertile BC_1 progeny when amphidiploid plants (*C. hystrix* × *C. sati-vus*) were used as the maternal parent in backcrossing to *C. sativus* (Table 1). Amphidiploid progeny derived from the reciprocal crossing (*C. sativus* × *C. hystrix*) failed to set fruit with seeds after selfing or backcrossing (data not presented).

Meiotic analysis

Normal meiotic process

In F_1 hybrid progeny, 19 univalents were observed at MI in most (>95%) PMCs, indicating a low level affinity

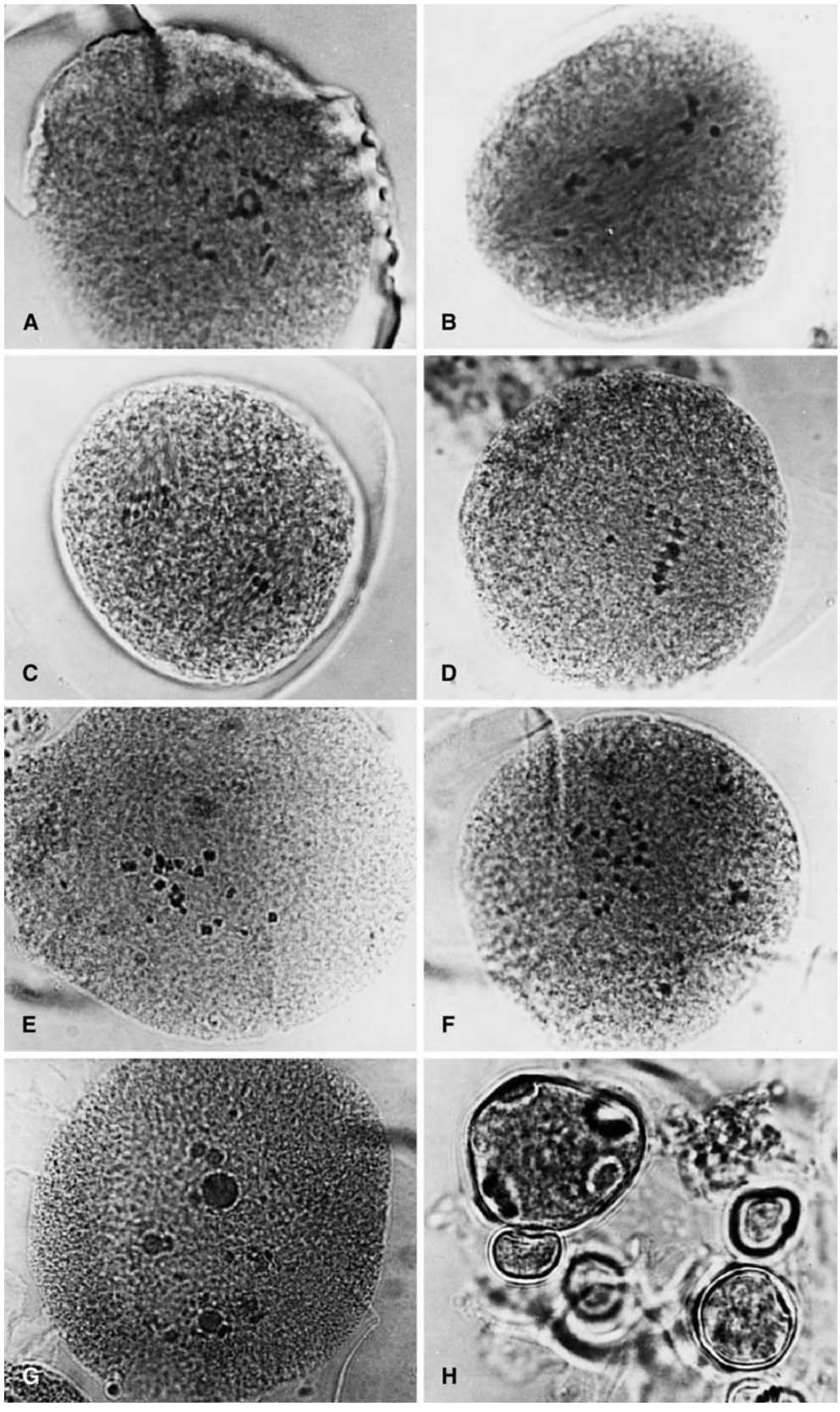

Fig. 3A–H Abnormal meiotic figures in pollen mother cells (PMC) of interspecific hybrids between *C. hystrix* and *C. sativus*. **A** A frying-pan trivalent at metaphase-I in PMC of the diploid (2n = 19). **B** Disorder disjunction of chromosomes at anaphase-I in the PMC of the diploid. **C** Spindle misorientation in the PMC of the diploid. **D** Laggards at metaphase-I in the PMC of the amphidiploid (2n = 2*x* = 38). **E** Multivalent formation at metaphase-I in the PMC of the amphidiploid. **F** Unequal number of chromosomes at four poles at telophase-II in the PMC of the amphidiploid. **G** Redundant micronucleus at the tetrad stage in the PMC of the amphidiploid. **H** Malformed pollen grains (most inviable) of the amphidiploid

Table 2 Meiotic configurations at diakinesis and metaphase-I in amphidiploids (2n = 4*x* = 38) derived from crossing *C. hystrix* (2n = 24) with *C. sativus* (2n = 14)

Stage	PMCs observed	PMCs with chiasma*	Mean no. of chromosome configurations**			
			Bivalents	Univalents	Trivalents	Tetravalents
Diakinesis	100	70 (0–3)	17.5 (91.1%)	1.1 (5.7%)	0.5 (2.6%)	0.1 (0.5%)
Metaphase-I	100	50 (0–3)	17.6 (90.9%)	1.25 (6.5%)	0.45 (2.3%)	0.05 (0.3%)

* and **, for values in parenthesis, indicate the range of chiasma and configuration percentage in one PMC, respectively

between the H (*C. hystrix*) and C (*C. sativus*) genomes (data not presented). In amphidiploids, leptotene was characterized by chromatin threads contracted in a bundle such that one end of the bundle was connected to the nucleolus. Chromosomes observed at late diplotene were well-spread and the decrescent nucleolus was present. At MI, more than 90% of the configurations were bivalents (Table 2), most of which formed symmetric rings (Fig. 2A). Heteromorphic bivalents were observed in amphidiploids but not in the normal meiotic process, indicating a high degree of preferential genome pairing in the amphidiploids examined. At anaphase-I and -II, chromosomes were symmetrically separated, resulting in 19 chromosomes at each pole of the PMCs (Fig. 2B, C). Simultaneous cytokinesis was detected in the amphidiploids. Given these observations, viable pollen was most likely a result of normal tetrad development which consequently led to fertility restoration in amphidiploid progeny (Fig. 2D).

Special meiotic behavior

F_1 hybrid (diploid). About 5% of the meiotic configurations observed at MI were trivalents having a "frying-pan" (a ring bivalent connected to a rod univalent) appearance (Fig. 3A). Chromosome lagging (25%), asymmetric division (20%) (Fig. 3B) and spindle disorientation (30%) were also observed in MI and telophase-I (TI) (Fig. 3C). Although cytokinesis was generally synchronous, cells containing simultaneous and successive cytokinesis stages were detected in the same plant. For example, MI and TI as well as microspores were observed in maturing PMCs.

Amphidiploid. Abnormal meiotic behavior was observed in amphidiploid progeny. At prophase-I, leptotene and zygotene figures were observed simultaneously in the same pollen mother cell (about 5%), and micronuclei (about 25%) were recorded in PMCs (data not presented). Likewise, laggards (Fig. 3D; 20%) at MI, asymmetric separation of chromosomes (15%) at TII, and redundant micronuclei (Fig. 3G) were observed. These anomalies may be responsible for the resultant inviable pollen grains observed (Fig. 3H).

The frequency of chiasmata during homoeologous chromosome pairing (as indicated by the presence of multivalents) was 0.7 (70 per 100 cells) as observed at diakinesis and 0.5 (50 per 100 cells) as observed at MI (Table 2). But the number of chiasmata actually formed per PMC was comparatively small, only varying from one to three per cell. Bivalent figures in chromosome-doubled F_1 hybrid progeny (>90% configurations) ranged from 15 to 19 in PMCs, with a mean of 17.5 and 17.6 at diakinesis and MI, respectively (Table 2). While the percentage of bivalent and univalent figures in diakinesis was 92.1 and 5.8% respectively, the percentages of bivalent and univalent figures in MI were 92.6% and 6.6%, respectively. In contrast, the percentage of trivalents (2.6%) and tetravalents (0.5%) in diakinesis decreased to 2.3% and 0.3% in MI, respectively.

Discussion

Wild *Cucumis* species are cross-incompatible with *C. sativus* and *C. melo* (Staub et al. 1992). However, these species are potentially important for plant improvement since they house genes for resistance to economically important pathogens that incite diseases such as powdery mildew, downy mildew, anthracnose and fusarium wilt (Leppick 1966; Lower and Edwards 1986; Kirkbride 1993). The first repeatable interspecific hybridization with *C. sativus* was accomplished by Chen et al. (1997a). Recently, amphidiploid progeny resulting from this *C. sativus* × *C. hystrix* mating have been shown to possess genes for root knot nematode resistance (Chen and Lewis 2000), high nutritional qualities, and tolerance to growth under low irradiance (unpublished data). The fact that these progeny are cross-compatible with *C. sativus* (Table 1) suggests their potential utility for cultivar improvement.

Chromosomes of *C. sativus* are relatively small, varying in length from 1.2 μm to 2.5 μm at mitotic metaphase (Chen et al. 1998b), and stain poorly with acetocarmine (Dane 1991). Our observations indicate that the majority of *C. hystrix* chromosomes are about one-half the length of those of *C. sativus*, and also stain poorly. GISH has proven useful for determining the parentage of chromosomes in somatic hybrids (Dong et al. 1999), and was effective in our studies for the assessment of chromosome constitution after interspecific hybridization.

The high frequency of bivalents and the low frequency of multivalents in amphidiploid hybrids, as revealed in the present study, indicates a considerable divergence between the genome *C. hystrix* (H) and the genome *C. sativus* (C). This hypothesis is supported by results of an isozyme analysis of *C. hystrix*, *C. sativus* and their in-

terspecific hybrid (Chen et al. 1997b). Since the genomes H and C are distinct, and each genome is represented twice in amphidiploids, the preferential pairing of species chromosomes in the interspecific hybrid might have been predicted. Usually high fertility will result from preferential pairing in amphidiploids (Jackson 1982). However, the fertility of the amphidiploid species examined in our study was relatively low (Table 1). This may be partially explained by the presence of multivalents and their activity during chromosome separation (Table 2), and by the yet unexplained maternal effects observed in the hybridization experiments described herein.

Pairing of chromosomes as multivalents provides a mechanism for chiasma formation between chromosomes of distinct genomes (Doroszewska and Berbec 2000). Multivalent formation in our interspecific hybrid progeny (possessing complements of the H and C genomes) described herein may provide a mechanism for genomic crossing-over. Chiasmata observed between homoeologous chromosomes in the *Cucumis* amphidiploids examined were abundant (70%) at diakineses, but frequency within any one PMC was relatively low (1 to 3 per PMC observed) (Table 2). The low frequency of chiasmata, however, does not necessarily negate the possibility of gene exchange between different genomes in fertile amphidiploids.

The chromosome-doubled F_1 hybrids derived from *C. sativus* × *C. hystrix* matings were sterile, while the chromosome-doubled F_1 hybrids derived from *C. hystrix* × *C. sativus* matings produced fruits containing only a few viable seeds. These observations indicated that a substantial crossing barrier exists when *C. sativus*, the parent with fewer chromosomes, is used as the maternal parent. The observation that fertility restoration can occur after exotic crossing when accessions with the higher chromosome number are used as the maternal parent has been made in the *Triticum araraticum* JaRubz and *Triticum aestivum* L. complex (Qi et al. 1998), and in crossing experiments between *Brassica campestris* L. and *Raphanus sativus* L. (Huang et al. 2001).

Variation in leaf and fruit shape and size, lateral branching, and resistance to several diseases (e.g. powdery mildew) within and between BC_1 and BC_1S_1 progeny derived from interspecific amphidiploids was observed (data not presented). The observed phenotypic variation in these BC populations mirrors variation detected at the protein (i.e. isozyme) and DNA (random amplified polymorphic DNA) level (unpublished data). These data suggest that amphidiploid derivatives examined herein may provide the breeder with a broad source of variation that is of potential value for germplasm enhancement in *Cucumis*.

Even though the initial introgression of *C. hystrix* genes into *C. sativus* has been accomplished, a highly effective gene-transfer protocol (bridge) for the enhancement of cucumber has not been established. Cytogenetic assessment using C-banding (chromosome structure), GISH (pairing analysis) and DNA analyses (i.e. synteny comparison) is critical for further description of the parental genome structure variation, and for tracing alien chromosome segments in backcross progeny. Such assessments will allow for the mapping and cloning of economically important genes.

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