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Asynchronous meiosis in *Cucumis hystrix*–cucumber synthetic tetraploids resulting in low male fertility

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ABSTRACT

Interspecific hybridization and allopolyploidization contribute to the improvement of many important crops. Recently, we successfully developed an amphidiploid from an interspecific cross between cucumber (*Cucumis sativus*, $2n = 2x = 14$) and its relative *C. hystrix* ($2n = 2x = 24$) followed by chemical induction of chromosome doubling. The resulting allotetraploid plant was self-pollinated for three generations. The fertility and seed set of the amphidiploid plants were very low. In this study, we investigated the meiotic chromosome behavior in pollen mother cells with the aid of fluorescence *in situ* hybridization, aiming to identify the reasons for the low fertility and seed set in the amphidiploid plants. Homologous chromosome pairing appeared normal, but chromosome laggards were common, owing primarily to asynchronous meiosis of chromosomes from the two donor genomes. We suggest that asynchronous meiotic rhythm between the two parental genomes is the main reason for the low fertility and low seed set of the *C. hystrix*–cucumber amphidiploid plants.

1. Introduction

Cucumber (*Cucumis sativus* L., $2n = 2x = 14$, genome CC) is an economically important crop. However, in the U.S., cucumber yield has reached a plateau in the last two decades. The lack of genetic diversity and emerging or persistent pests are some of the reasons for this yield stagnancy.

Wild relatives of crops often contain valuable traits, such as disease resistance, for crop improvement [1,2]. Such traits can potentially be introgressed into crops by crossing with the wild species and development of introgression lines [3].

A wild relative of cucumber, *C. hystrix* Chakr. ($2n = 2x = 24$, genome HH), possessing multiple disease resistances, is the only species that is sexually compatible with cucumber in genus *Cucumis* [4]. Cucumber and *C. hystrix* diverged from a $2n = 24$ common ancestor approximately 5 million years ago [5,6], making it difficult to make direct crosses between the two species for transferring useful genes into cucumber. The

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synthetic tetraploid of the two species may be a useful bridge for overcoming the long reproductive isolation. Chen et al. [4] was the first to make a successful interspecific cross between cucumber and *C. hystrix*, obtaining a synthetic allotetraploid named *Cucumis* × *hytivus* (2*n* = 4*x* = 38, genomes HHCC) resulting from spontaneous chromosome doubling of the F_1 plant [7]. We recently developed an interspecific F_1 hybrid between the *C. hystrix* accession TH1 [6] and a North American pickling cucumber inbred line, Gy14, from which amphidiploid plants were successfully obtained (Fig. 1) by chemical induction of chromosome doubling in TH1 (female) × Gy14 (pollen donor) mating (Pan et al., unpublished data). The amphidiploid plants were subsequently self-pollinated and were able to set fruits with viable seeds. However, the fruits contained only a few viable seeds, suggesting low pollen fertility, as reflected in pollen stainability. Low seed set restricts the exploitation of the amphidiploid for cucumber germplasm improvement. It is accordingly desirable to identify the reasons for lower fertility and seed set in amphidiploids.

Chromosome behavior during meiosis plays an important role in plant fertility. Meiotic irregularities are thought to be related to low fertility in allopolyploids [8–11]. A fertile allopolyploid requires diploid-like meiotic behavior to establish disomic inheritance and full fertility. The coexistence of genetically closely related genomes in an allopolyploid can lead to homoeologous chromosomes pairing during meiosis, preventing the formation of functional gametes and reducing fertility [12]. In the present study, the meiotic chromosome behavior in *C. hystrix*–cucumber amphidiploids was investigated using squashes and fluorescence *in situ* hybridization (FISH) to identify the cytological mechanism of the low fertility and low seed set in amphidiploids.

2. Material and methods

2.1. Plant materials

Plant materials included self-pollinated S1 to S3 progeny of amphidiploid plants (2*n* = 4*x* = 38, HHCC) generated by chemical induction of chromosome doubling in the TH1 (female) × Gy14 (pollen donor) cross. The amphidiploid plant from the F_1 hybrid was assigned as S0 and was self-pollinated for three consecutive generations resulting in S1, S2, and S3 plants.

2.2. FISH

The FISH procedure was essentially the same as regular FISH protocols developed for meiotic pachytene chromosomes [13]. The type III repeat, a satellite repeat located in all cucumber

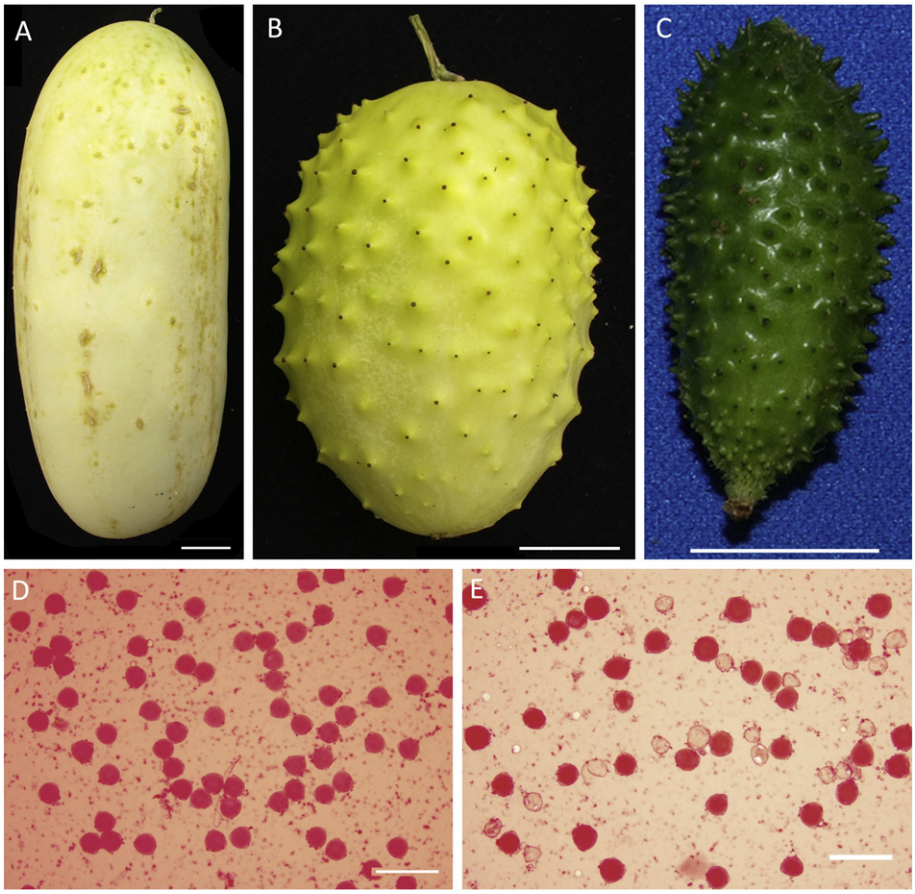

Fig. 1 – Fruit images of the pickling cucumber Gy14 (A), the amphidiploid (B), and the *C. hystrix* parental line TH1 (C). The amphidiploid (S2, selfed F_2, genome HHCC, E) has lower fertility than Gy14 (D, genome CC) as evaluated by stainability with acetocarmine dye. Bars are 2 cm in Figs. A–C and 200 μm in Figs. D–E.

centromeres [14], was used to identify cucumber chromosomes in the *C. hystrix*–cucumber amphidiploids. A type III probe was labeled with biotin-dUTP *via* nick translation and detected with avidin conjugated with FITC (Vector Laboratories). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in VectaShield antifade solution (Vector Laboratories, Burlingame, CA). FISH images were processed with Meta Imaging Series 7.5 software. The final contrast of the images was processed using Adobe Photoshop CS3 software.

3. Results

The different meiotic stages of the *C. hystrix*–cucumber amphidiploids are shown in Fig. 2. In pollen mother cells (PMCs), the chromosomes paired mainly as bivalents in cells from pachytene (Fig. 2-C) to metaphase I (Fig. 2-E). Occasionally univalents and multivalents appeared in some cells, but the frequency was generally very low (Table 1). For example, univalents and multivalents were detected in only 2 or 4 metaphase I (MI) PMCs from S1 to S3, respectively.

The most obvious feature of meiosis in the amphidiploid plants was asynchronous meiotic rhythm, as shown by the following observations. First, PMCs from the same flower showed different meiotic stages. As shown in Table 1, in plants of all three generations (S1, S2, and S3), PMCs from the same male flower but in different meiotic stages were visible. All meiotic stages could be observed in PMCs from two S1 male flowers examined (Fig. 2). Second, two daughter cells from the same PMC were asynchronous. For example, at metaphase II, chromosomes from one daughter cell had organized into the metaphase plate while chromosomes from the other daughter cell remained dispersed (Fig. 2-H). Third, both parental genomes in the same cell did not display the same meiotic rhythm. For example, when chromosomes from the cucumber genome (with green signals, indicated by arrows) were in diakinesis, those from the *C. hystrix* genome were in diplotene (Fig. 2-D). In many PMCs at metaphase I, the cucumber chromosomes (with green signals, indicated by arrows) reached the metaphase plate well ahead of those of *C. hystrix* (Fig. 2-E). In the subsequent phases, only some chromosomes from the *C. hystrix* parent reached the poles in time to be included in telophase nuclei, resulting in chromosome laggards at anaphase I (Fig. 2-F) and formation of micronuclei at telophase I (Fig. 2-G). Chromosome laggards prevailed at later stages (Fig. 2-H–J). The male gametes from these PMCs will contain unbalanced chromosome complements. The asynchronous meiotic rhythm between two parental genomes seemed to start in the zygotene. For example, most homologous chromosomes began to pair at early zygotene (Fig. 2-A). At late zygotene (Fig. 2-B), a few chromosomes remained unpaired (indicated by arrow) when most chromosomes were fully paired.

The proportion of PMCs with lagging chromosomes seemed to decrease with the increase in self-pollination. For example, chromosome laggards were found in 96% (22/23) and 91% (21/23) MI cells from two S1 flowers. In two S2 flowers, chromosomal laggards were found in 69% (11/16) and 35% (16/46) of cells. In one S3 male flower examined, chromosome laggards were found in 47% (7/15) of MI PMCs (Table 1). However, the tendency was not clear, with no significant differences in MI PMCs with lagging chromosomes between S2 and S3 flowers, and the proportions were also quite different in the two S2 flowers.

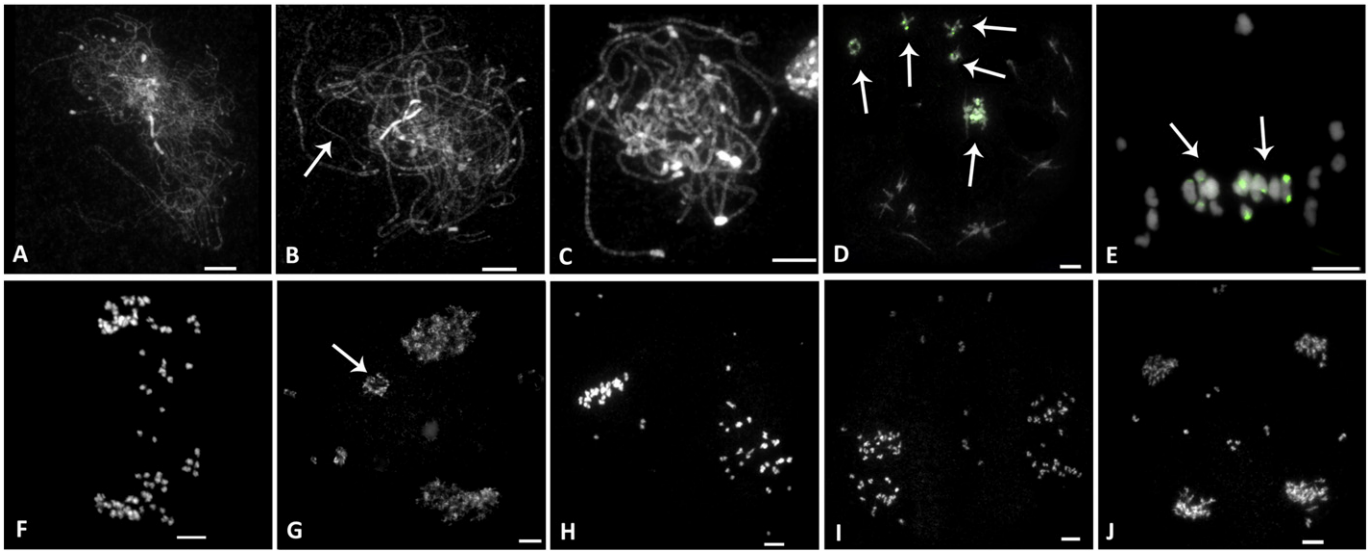

Fig. 2 – Meiosis in an amphidiploid S3 plant. Green FISH signals are from the type III repeat associated with cucumber centromeres. (A) An early zygotene cell. (B) A late zygotene cell. (C) Pachytene. (D) PMC showing that the cucumber chromosomes (green signals) were in diakinesis while the *C. hystrix* TH1 chromosomes were in diplotene. (E) PMC showing that the cucumber chromosomes (bivalents with green signals) reached the metaphase plate well ahead of those of *C. hystrix* chromosomes. (F) An anaphase I cell with lagging chromosomes. (G) A telophase I cell with laggards and micronucleus (arrow). (H) A metaphase II cell showing that two daughter cells were asynchronous. Anaphase II (I) and early telophase II (J) cells with lagging chromosomes. Bar, 10 μm.

Table 1 – Numbers of cells at specific meiotic phases from the same male flower at three generations (S1, S2 and S3) of *C. hystrix*–cucumber amphidiploid plants.

Phases	S1-male flower 1	S1-male flower 2	S2-male flower 1	S2-male flower 2	S3-male flower 1
Leptotene	0	1	0	0	0
Zygotene	11	0	0	0	2
Pachytene	2	0	0	0	1
Diplotene	12	1	0	6	0
Diplotene-Diakinesis	37	5	4	36	6
Diakinesis	10	5	32	19	12
Metaphase I	23[a]/22[b]/2[c]	23/21/2	16/11/4	46/16/4	15/7/2
Anaphase I	1/1	29/29	8/5	6/2	3/2
Telophase I	1/1	18/18	3/0	5/1	1/1
Metaphase II	0	4/4	2/0	0	2/2
Anaphase II	2/2	5/5	0	0	0
Telophase II	0	3/3	1/0	0	4/1

[a] Number of cells at specific meiotic phases in the same flower.
[b] Number of cells with lagging chromosomes.
[c] Number of cells with univalents or multivalents.

4. Discussion

Distant hybridization has been widely used as an important tool for crop improvement [15]. However, distant hybrids are often highly sterile, owing to the absence of homologous chromosomes and the failure of synapsis [16]. To overcome the sterility, distant hybrids are usually induced to form amphidiploids [15,17,18]. However, synthetic or neo-allopolyploids commonly display genetic instability and low fertility, a major constraint on polyploid establishment and persistence [19]. Explanations for the instability and low fertility are complex. Broadly, three causes have been identified: meiotic aberrations, genic factors, and incidental phenotypic effects [20,21]. Meiotic aberrations probably represent the most general factor affecting polyploidy fertility. An allopolyploid has a high risk of homoeologous chromosome pairing during meiosis, owing to the relatively close relationship of the parental genomes [12,22,23]. Homoeologous pairing and recombination can result in the formation of multivalents and univalents at MI that lead to the production of chromosomally and genetically unbalanced gametes, prohibiting the formation of functional gametes and reducing its fertility [24]. Studies in resynthesized *Brassica napus* confirmed that homoeologous recombination could lead to aberrant meiotic behavior and reduced fertility [9,10,25,26]. A recent study in *Tragopogon* provided evidence that deletions and rDNA changes detected in recent allopolyploids also result from homoeologous rearrangement [27].

In our previous study, we found that, in the interspecific F_1 hybrid of *C. sativus* CV Gy14 × *C. hystrix* TH1, cucumber chromosome C7 and *C. hystrix* chromosome H1, which were highly conserved during evolution [6], showed homoeologous pairing in 71% of prophase I cells and 25% of metaphase I cells [28]. In the present study, pairing between homeologous chromosomes was rare in a *C. hystrix*–cucumber interspecific amphidiploid: multivalents were detected in only 2 or 4 MI cells from S1 to S3, respectively. Instead, the most obvious feature of meiosis was asynchronous meiotic rhythm. The *C. hystrix*–cucumber amphidiploid displayed three asynchronous forms including multiple meiotic stages in PMCs from the same flower, asynchronous daughter cells in the same PMC, and different meiotic rhythms of parental genomes in the same cell. The first asynchronous form has previously been shown to occur in two mutants of *Arabidopsis* [29,30], intersubspecific autotetraploid rice (*Oryza sativa*) hybrids [21], and a *C. hystrix*–cucumber amphidiploid [31]. In general, the asynchrony did not affect pollen development, with all of meiocytes completing meiosis sooner or later and eventually forming normal pollen grains [29,30]. However, pollen fertility was highly affected in the *C. hystrix*–cucumber interspecific amphidiploids. We propose that different meiotic rhythms of two parental genomes in the same cell are the main reason for lower fertility, given that asynchrony led directly to the production of lagging chromosomes from meiotic anaphase I to telophase II in these amphidiploids. The male gametes from these PMCs will contain incomplete chromosome complements. Consequently, lower pollen fertility and lower seed production appeared (Fig. 1). This asynchronous form has previously been found in a tetraploid accession of *Paspalum subciliatum* [32], a triploid interspecific *Brachiaria* hybrid [33], two pentaploid accessions of *B. decumbens* [20], two hexaploid accessions of *P. jesuiticum* [34], and a nonaploid accession of *B. humidicola* [35]. In previous studies, the second asynchronous form has not been reported. However, given that the proportions of PMCs with lagging chromosomes tended to decrease with increased self-pollination, the fertility of the amphidiploid we developed could be improved in more advanced inbred lines.

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