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Molecular and cytogenetic analyses provide evidence of the introgression of chromosomal segments from the wild *Cucumis hystrix* into the cultivated cucumber through the bridge of a synthetic allotetraploid

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Abstract *Cucumis* × *hytivus* (2n = 4× = 38) is a synthetic allotetraploid obtained from interspecific hybridization between the cucumber (2n = 2× = 14) and its wild relative *C. hystrix* (2n = 2× = 24). The synthesis of this species built a bridge for cucumber improvement through gene introgression. Allotriploid and introgression lines (ILs) have previously been produced and characterized with respect to morphology, cytology, and molecular markers. However, no clear evidence of how the chromosomal segments of *C. hystrix* were introgressed and inherited was found owing to the small size of chromosomes. In the present study, cucumber-*C. hystrix* introgression lines were developed by backcrossing the allotriploid to North China cucumber breeding line "P01" followed by self-pollination. The introgressed segments of *C. hystrix* in the ILs were revealed by meiotic pachytene chromosome analysis. Fluorescence in situ hybridization (FISH) was performed on pachytene chromosomes using fosmid clones from cucumber, which confirmed that introgression occurred in the long arm of chromosome 7. Molecular analysis using a set of 53 simple sequence repeats (SSRs) indicated that the chromosomal segments of *C. hystrix* were introduced into 4 cucumber chromosomes, the short arms of chromosomes 2 and 6, and long arms of chromosomes 3 and 7. The inheritance of alien sequences in the long arm of chromosome 7 was investigated with 21 SSRs in self-pollinated progenies. *C. hystrix*-specific bands of several SSRs were still present in some individuals, indicating that the introgressed segment was partially preserved. The first unambiguous identification of alien chromosome segments in cucumber ILs using combined molecular cytogenetics could facilitate the determination of effects of wild alleles and promote cucumber improvement.

Keywords Introgression lines · Cucumber · *Cucumis hystrix* · Pachytene chromosome analysis · SSRs

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Introduction

Allopolyploidization, or the merging of two or more divergent genomes into a common nucleus, is a driving force in plant evolution (Jackson and Chen 2010; Zhang et al. 2013). Allopolyploids that arise from interspecific or intergenic hybridization and chromosome doubling could act as an efficient bridge for gene introgression. In wheat, a series of introgression lines (ILs) have been produced by crossing allohexaploid common wheat with various wild relatives, resulting in novel disease-resistant genes (including those against leaf rust, stem rust, stripe rust, and powdery mildew) and enhanced yield (Chen et al. 1995; Li et al. 2007; Jia et al. 2009; Zhao et al. 2010; Liu et al. 2011; Larson et al. 2012; Yaniv et al. 2015; Zhang et al. 2015). ILs have also been used in investigating leaf morphology and fruit metabolic profiling in the tomato (Schauer et al. 2006; Chitwood et al. 2013) and the improvement of fiber quality and the quantitative trait locus (QTL) mapping of disease resistance in upland cotton (Fang et al. 2014; Cao et al. 2014). Molecular markers and genomic in situ hybridization (GISH) are often used to identify introgression lines (Liu et al. 2006; Navabi et al. 2011; Zhang et al. 2015). However, most of the ILs are derived from interspecific hybridization between two parents with the same chromosome base, and the process of introgression has been comparatively overlooked.

The *Cucumis* allotetraploid *C. hytivus* (HHCC, 2n = 4× = 38) was successfully obtained through interspecific hybridization between the cultivated cucumber and its closest relative, *C. hystrix* (HH, 2n = 2× = 24), followed by chromosome doubling (Chen and Kirkbride 2000; Chen et al. 1997, 1998). Backcrossing between *C. hytivus* and the cucumber produced a partially fertile allotriploid (HCC, 2n = 3× = 26) (Chen et al. 2003a). The meiotic chromosome pairing of allotetraploids and allotriploids revealed a large scale of chromosome lagging, conglutination, and multivalency, which lays the foundation for homoeologous recombination and gene introgression (Chen et al. 2003b; Guo et al. 2004). Cucumber ILs with resistance to downy mildew have been produced through backcrossing cucumber cultivar "CC3" with the allotetraploid *C. hytivus*, identified by random amplified polymorphic DNA (RAPD) markers and the high frequency of multivalents in the metaphase and bivalent lagging in anaphase (Cao et al. 2005; Zhou et al. 2008). In a further cytological investigation conducted on the ILs (reported by Zhou et al. 2008), delayed sister chromatids were observed at the mitotic late metaphase, and 0.2 tetravalents, 0.05 hexavalents, and 0.05 octovalents per pollen mother cell were observed at the meiotic diakinesis (Zhou et al. 2009). The distribution and coverage of the introgressed segments, as well as variations in morphological traits, were evaluated in cucumber ILs (Shi et al. 2011, 2012). QTLs for resistance to gummy stem blight and downy mildew have been identified in three and one cucumber ILs, respectively (Lou et al. 2013b; Pang et al. 2013). Nonetheless, clear evidence of the occurrence and inheritance of introgression segments in cucumber-*C. hystrix* introgression lines is still lacking.

Complementary molecular and cytogenetic approaches are powerful tools for the identification and characterization of introgression lines, which can lay the foundation for crop improvement. In contrast to wheat, cucumber has very small chromosomes, and the repetitive sequences accumulate at the heterochromatin and centromeric region instead of being evenly distributed along the chromosomes. The resolution of genomic in situ hybridization (GISH) is not sufficient for identifying the cucumber introgression lines. The development of abundant molecular markers (simple sequence repeats, SSRs) and molecular cytogenetic techniques in cucumber enables the accurate identification and characterization of introgression segments. Fluorescence in situ hybridization (FISH) using pachytene chromosomes is a powerful cytogenetic technique that can detect short DNA sequences with lengths of 1.5~2.0 kb (Lou et al. 2013a, 2014; Han et al. 2015).

A clear cytogenetic identification of the introgression lines could facilitate the determination of effects of individual wild alleles. In the present study, the cucumber-*C. hystrix* introgression line was developed using a *Cucumis* allotriploid (HCC, 2n = 26) as the maternal parent and the cultivated cucumber P01 (CC) as the paternal parent. The introgressed chromosomal segments of *C. hystrix* in the IL and self-pollinated progenies were unambiguously identified and characterized with complementary molecular marker and pachytene chromosome analysis. The strategic development and identification of ILs reported herein is crucial for disease

resistance breeding in the cucumber and provides a new method for introgression in other crops.

Materials and methods

Plant materials

Previously, an allotetraploid *Cucumis* species (*C. hytivus* (HHCC, 2n = 38)) was obtained through interspecific hybridization and chromosome doubling, followed by the synthesis of the allotriploid via backcrossing with *C. sativus* (Chen and Kirkbride 2000; Chen et al. 2003a). The *Cucumis* allotriploid (HCC, 2n = 26) was used as the maternal parent and crossed with the paternal parent, the cultivated cucumber P01 (CC, 2n = 14). The allotriploid normally bears elliptical fruits with black spines and a length of 12–14 cm, whereas P01 is a typical North Chinese fresh market cucumber with slim white-spine fruits of 20–30 cm in length (Fig. S1). Seeds of the allotriploid and P01 were germinated at 28 °C and grown in a greenhouse at Jiangpu Cucumber Research Station of Nanjing Agricultural University (JCRSNAU), Nanjing, China. The soil media was 25% peat + 25% cinder + 50% perlite. The seed of introgression line was obtained in the fruit of the allotriploid in autumn 2014. To evaluate the stability of alien chromosomal segments, self-pollinated progenies of one IL were obtained in spring 2015. The scheme for developing cucumber-*C. hystrix* introgression line is shown in Fig. S1.

Cytological analysis

Pollen grains from the male flowers of allotriploid, cucumber, and one IL (three flowers for each material) were stained with 1% acetocarmine (Momotaz et al. 1998); more than 250 pollen grains were observed under an Olympus BX51 microscope (http://www.olympus-global.com). The percentage of stained pollen grains was calculated to represent the pollen fertility of the introgression lines.

Chromosome preparation

Root tips of the cucumber-*C. hystrix* IL were harvested and fixed in Carnoy's solution (methanol/acetic acid = 3:1) at 4 °C. The procedure for mitotic and meiotic pachytene chromosome preparation was the same as that described in Lou et al. (2013a). For pachytene chromosome preparation, young flower buds with a size of 1.0–2.5 mm were harvested and fixed in Carnoy's solution (ethanol/acetic acid = 3:1) for at least 1 day. The anthers at the meiotic pachytene stage were digested with enzyme mixtures containing 4% cellulase and 2% pectinase for 1.5 h at 37 °C and fixed in Carnoy's fixative solution (ethanol/acetic acid = 3:1). Slides with well-spread pachytene chromosomes were obtained by macerating the digested anthers on glass slides in 60% acetic acid solution with a fine-pointed forceps and then "flame-dried."

Fluorescence in situ hybridization

The fosmid clones from cucumber chromosome 7 used in the FISH experiment were from the cucumber fosmid library constructed from the cucumber inbred line 9930 (Han et al. 2009), kindly provided by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. Different types of satellite DNA sequences, including Type III, and 45S rDNA (Han et al. 2008) were used for the identification of the mitotic chromosomes.

The fosmid DNA was isolated using the QIAGEN (Valencia, CA) plasmid Midi kit. Fosmid clones and repetitive sequences were labeled with biotin-16-UTP or digoxigenin-11-dUTP using nick-translation and were subsequently detected with a fluorescein isothiocyanate-conjugated anti-biotin antibody and a rhodamine-conjugated anti-digoxigenin antibody (Roche Diagnostics, Indianapolis, IN), respectively. The experimental procedure for FISH was as previously described (Lou et al. 2013a). Chromosomes were counterstained with DAPI in the antifade solution VectorShield (Vector Laboratories, Burlingame, CA), and images were captured with a SenSys CCD camera attached to an Olympus BX51 microscope (http://www.olympus-global.com). The CCD camera was controlled using Applied Spectral Imaging FISH view 5.5 software (Applied Spectral Imaging, Inc., USA), and the FISH images were processed with Adobe Photoshop 6.0 (Adobe Systems, http://www.adobe.com).

SSR analysis

Unexpanded young leaves were collected from the allotetraploid *C. hytivus*, allotriploid, cucumber variety P01, and ILs. Genomic DNA was extracted using the

CTAB method (Murray and Thompson 1980) with some modifications. For the identification of the introgression segments in the IL, 95 SSRs selected from Ren et al. (2009) and Yang et al. (2012, 2013) were screened for polymorphism, and 53 polymorphic SSRs were subsequently used. For the analysis of the inheritance of the *C. hystrix* introgression segment in self-pollinated individuals, a set of 21 polymorphic SSRs from the long arm of chromosome 7 (Ren et al. 2009; Yang et al. 2012, 2013) was used. Each polymerase chain reaction (PCR) contained 25 ng template DNA, 0.5 μM each of two primers, and 1× PCR master mix (Fermentas, Glen Burnie, MD) in a total volume of 10.0 μL. A "touchdown" PCR program was used for all primer sets (Weng et al. 2005). Each primer pair amplified a major band in each sample of template DNA, in which the variation in PCR product size allowed for banding morphotypes to be resolved in 8% polyacrylamide gels and silver-stained.

Results

Generation of cucumber-*C. hystrix* introgression line

The allotetraploid *C. hytivus* was crossed with the cucumber breeding line P01 to generate allotriploid, which was subsequently planted and backcrossed with P01 (scheme shown in Fig. S1). For the development of the IL, 18 crosses between allotriploid and cucumber P01 were performed, generating three fruits. A total of four seeds were harvested and grown, whereas only one fully fertile seed geminated. The IL exhibited extensive variations in morphology compared with two parents, with strong growing vigor and more branches, similar to the allotriploid (Fig. 1). The shape of the leaves was a heart-shaped pentagram in the cucumber and pentagonal in the *Cucumis* allotriploid, whereas the leaf shape of the IL was a palmate pentagram with a significantly elongated angle, as shown in Figs. 1b and S2. The leaf color was light green in IL, in contrast to the green leaf of the cultivated cucumber and the yellow-green leaf of the allotriploid.

The sizes of male and female flowers were both intermediate between the allotriploid and P01 (Fig. 1c, d). The tomentum on the receptacle of male flower observed in IL was white, which the same as in cucumber P01 but differing from the dark brown color found in allotriploid and allotetraploid (shown in Fig. S3). However, the tomentum on the receptacle of female flower observed in IL was brown, which is most likely inherited from the allotriploid parent (Fig. S4). Moreover, from allotetraploid, allotriploid, IL to P01, the tomentum color on female flowers decreased gradually from dark brown to white, which may reflect the decrease in the genome dosage of *C. hystrix*. Another possible influence of the *C. hystrix* genome dosage can be seen in the fruit length, which significantly increased moving across the allotetraploid, allotriploid, and introgression line to P01 (Fig. 1c). The fruits on the IL had black spines when observed under a stereomicroscope, consistent with those of *C. hystrix* and the allotetraploid and allotriploid but in contrast to the white spines observed in P01 (Fig. 1e). Thereby, we speculated that the black spines were inherited from *C. hystrix*. Mature pollen grains of the cucumber, allotriploid, and IL were stained with 1% acetocarmine to examine pollen fertility. The fertile pollen grains were stained red, whereas those that were aborted were of irregular shape and only lightly stained (Fig. 1f). As shown in Table 1, the pollen fertility of the IL was 87.7%, which is higher than the 14.9% of the allotriploid but lower than that of the cucumber (98.1%).

Molecular and pachytene chromosome analysis of introgression segments

FISH using satellite repeat Type III (green) and 45S rDNA (red) as probes was performed on the mitotic metaphase chromosomes of the IL, showing 2n = 14 chromosomes, 5 pairs of 45S signals, and 7 pairs of Type III signals (Fig. 2a–c). However, the resolution is not sufficient to determine the introgressed *C. hystrix* segments, so the meiotic pachytene chromosomes were observed. As shown in Fig. 2d, e, there were at least three unpaired regions at the end of three individual chromosomes (indicated by black arrows), which is likely caused by homoeologous chromosome recombination between *C. hystrix* and the cucumber. Several double exchange regions were also observed, visually shown as short unpaired strings in the middle of the chromosomes (Fig. 2d, indicated by blue arrows). According to the chromosome length and arm ratio, we assumed that the unpaired chromosome end (indicated by red arrow) might occur at the end of chromosome 7. FISH was performed on meiotic pachytene chromosomes of the IL to confirm the proposed introgression event using four fosmid clones from cucumber

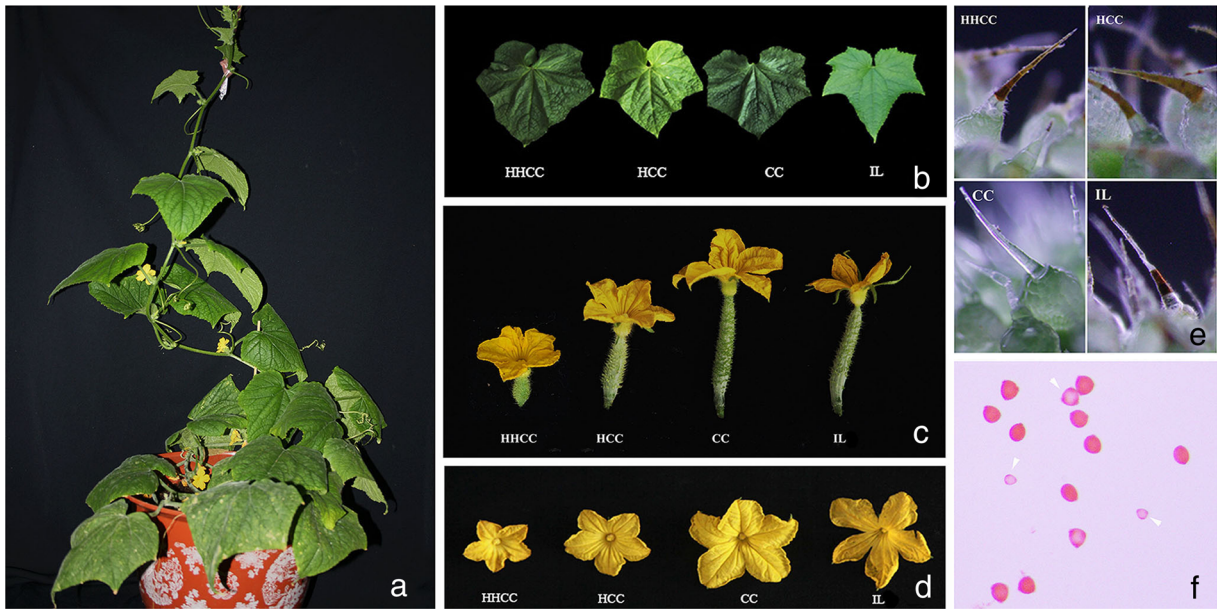

Fig. 1 Morphology and fertility of cucumber-*C. hystrix* introgression lines. **a** Plant architecture of the IL. Differences in leaf shape and color (**b**), female flowers (**c**) and male flowers (**d**) of the IL (on the right) compared with those of the allotetraploid, allotriploid, and cultivated cucumber (from left to right, marked below). **e** Fruit spine colors of allotetraploid (*black*), allotriploid (*black*), cucumber (*white*), and IL (*black*). **f** Pollen fertility of the IL. *Arrows* indicate aborted pollen grains, which are lightly stained and have a smaller shape compared to fertile pollen grains

chromosome 7 (7–3, 7–4, 7–6, and 7–7). Details on the fosmid clones are provided in Table S1. Fosmid 7–7 (red) had just one FISH signal on the chromosome string, whereas the other string had no FISH signal (Fig. 2f), which demonstrates that recombination had occurred between cucumber chromosome 7 and one of the *C. hystrix* chromosomes, leading to the introduction of *C. hystrix* chromosome segments.

To detect all the introgression events that occurred in the IL, we selected 95 SSR primers from a previously constructed cucumber linkage map (Ren et al. 2009) to screen for polymorphism among *C. hystrix*, the allotriploid, *C. sativus*, and the IL. Four primers were selected for each chromosome arm, except for chromosome 7, where 39 primers were selected to detect the *C. hystrix* sequences in the long arm of chromosome 7 of the IL. Fifty-three polymorphic SSRs were subsequently used (21 SSRs were polymorphic from the long arm of chromosome 7). Detailed information on the SSR primers used in the present study is provided in Table S2. Only the SSRs that were polymorphic among *C. hystrix*, the allotriploid, and the cucumber were subsequently used to detect the introgression line. As shown in Fig. 3a, seven SSRs from the long arm of chromosome 7 (chr7–28, chr7–29, chr7–35, chr7–36, chr7–38, and chr7–40) showed heterozygous bands, all with *C. hystrix*-specific bands in the allotriploid and allotetraploid and a comparatively weaker band in the IL. Likewise, chr2–3, chr3–8, chr6–2, and chr7–5 exhibited *C. hystrix*-specific bands in allotriploid and allotetraploid and comparatively weaker bands in the IL (Fig. 3b). The results indicate that the introgression of the chromosomal segments occurred at the short arms of chromosomes 2 and 6 and the long arms of chromosomes 3 and 7 (Table S2). Additionally, a double exchange was observed in the short arm of chromosome 7 (Fig. 3). The chromosomal location of the

Table 1 Pollen fertility (represented by stain capability) of allotriploid (HCC), cucumber (CC), and the IL

Materials	Chromosome number (2n)	Stained capability of pollen		
		Stained number	Total number	Rate of stained (%)
HCC	19	38	254	14.96
ILs	14	307	350	87.71
CC	14	265	270	98.14

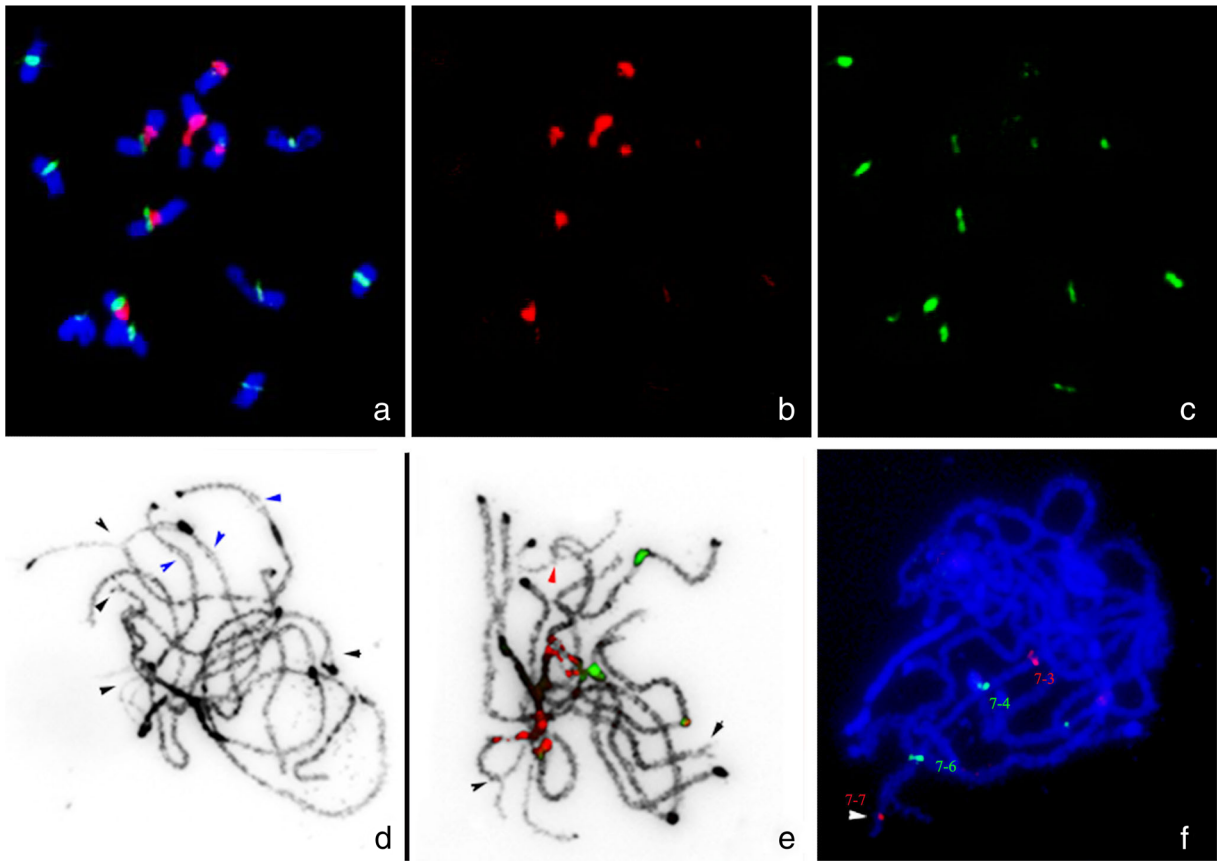

Fig. 2 **a**–**c** FISH signals of Type III (*green*) and 45S rDNA (*red*) on mitotic metaphase chromosomes of the IL, indicating 7 pairs of Type III signals and 5 pairs of 45S rDNA signals. **d**–**f** Meiotic pachytene chromosomes of cucumber ILs. **d** DAPI-stained chromosomes, where *black arrows* indicate introgression regions of *C. hystrix* at chromosome ends and *blue arrows* indicate potential double crossovers that occurred in the middle of individual chromosomes. **e** FISH signals of Type III (*green*) and 45S rDNA (*red*) on pachytene chromosomes, where *red arrow* indicates the assumed introgression in chromosome 7. **f** FISH signals of 4 fosmid clones from chromosome 7; 7–7 has a FISH signal on just one chromosome string (indicated by the *white arrow*)

fosmid 7–7 is approximately 19.1 Mb determined by its anchor SSR (Table S1). The chromosomal locations of the SSRs (chr7–28, chr7–29, chr7–35, chr7–36, chr7–38, and chr7–40) from the long arm of chromosome 7 that exhibited heterozygous bands are inferred by sequence alignment (Table S2). The chromosomal locations for chr7–28 and chr7–29 are 18.28 and 18.90 Mb, and that for chr7–38 and chr7–40 are 20.16 and 19. 61 Mb. Thus, we speculate that the chr7–38 and chr7–40 are located within the unpaired region of the long arm of chromosome 7. These results elucidated that multiple chromosome recombination events have occurred during meiotic chromosome pairing between *C. hystrix* and *C. sativus* and that a genomic component of the wild *C. hystrix* has been successfully introduced into the cucumber genetic background.

Inheritance of alien chromosomal segments in self-pollinated progeny

To investigate the inheritance stability of the introgression segments, the introgression line was then self-pollinated, generating approximately 200 seeds, from which 38 were randomly selected for the SSR analysis of the presence of introgression segments at the long arm of chromosome 7. The leaf morphology and fruit tuberculate distribution were highly variable among individuals (Fig. S5). In addition, the spine colors of the fruits of the IL descendants were apparently segregated, i.e., there were individuals with white and black spines (Fig. S6), in contrast to the black spines of the IL fruits. Twenty-one polymorphic SSRs from the long arm of chromosome 7 were used to screen 38 individuals to

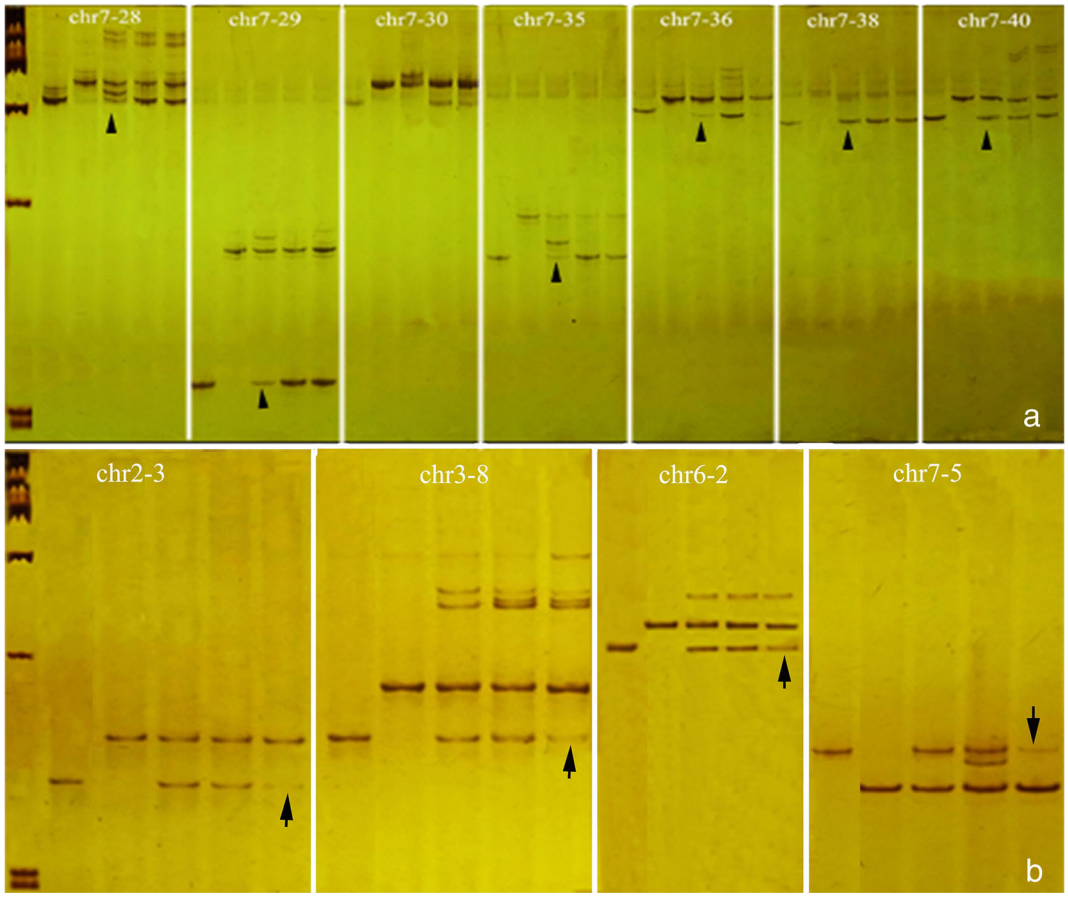

Fig. 3 A set of 53 polymorphic SSR markers were used to identify the alien chromosome segments of *C. hystrix* (bands indicated by *arrows*) in the cucumber IL. **a** Introgression regions in long arm of chromosome 7 identified by SSRs. From left to right, each pair of primers represents *C. hystrix*, "P01," IL, allotetraploid, and allotriploid. **b** Introgressions occurred in the short arms of chromosomes 2 and 6 and long arms of chromosomes 3 and 7. From left to right, each pair of primers represents *C. hystrix*, P01, allotetraploid, allotriploid, and the IL

examine the inheritance of the alien segment. Heterozygous bands of several SSRs were still observed in the self-pollinated populations, indicating that the introgression segment was partially preserved. For example, SSR chr7–29 showed heterozygous bands of *C. hystrix* in 8 individuals, whereas SSR chr7–36 showed them in 3 individuals (Fig. 4a, b). However, two SSRs at the unpaired region of chromosome 7 (chr7–38 and chr7–40) did not show *C. hystrix*-specific bands (Fig. 4c, d), which could be attributed to a crossover between homoeologous sequences. Nonetheless, the inheritance of the *C. hystrix* genomic component in other chromosomes, as well as the mechanisms underlying the morphological variations and introgression segment loss, remains in need of further investigations.

Discussion

Strategies for introducing wild alleles into the cultivated cucumber

Cucumbers have undergone extensive domestication and selection since their divergence from the melon approximately 10 million years ago (Kupper and Staub 1988), resulting in very limited genetic diversity and a lack of resistance genes. Advanced backcross populations derived from interspecific hybridization, such as introgression lines (ILs), chromosome segment substitution lines (CSSLs), and monosomic alien additional lines (MAALs), are valuable resources to increase the genetic diversity and improve the commercial quality of cultivated species (Caruso et al. 2016; Ali et al. 2010; Xu et al.

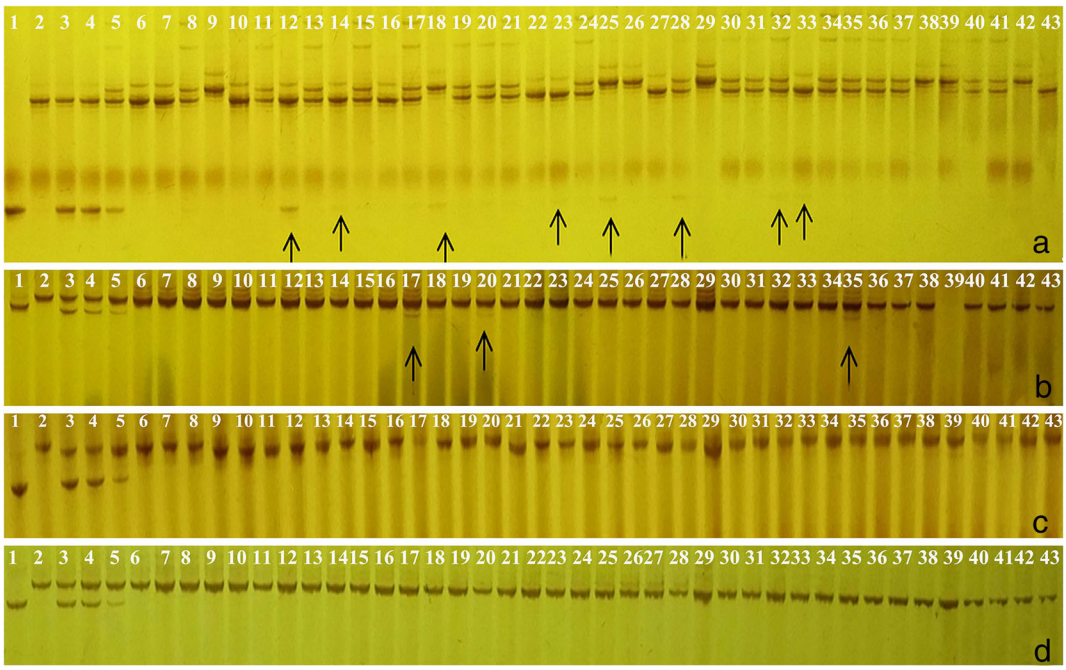

Fig. 4 Identification of introgression segments in self-pollinated progeny using SSRs. *Columns 1–5* represent "*C. hystrix*," P01, "allotetraploid," "allotriploid," and "IL," respectively. *Columns 6–43* represent individuals derived from the self-pollination of IL. Two SSRs, chr7–29 (**a**) and chr7–36 (**b**), had heterozygous bands in 8 and 3 individuals examined, respectively (marked by *arrows*), whereas chr7–38 (**c**) and chr7–40 (**d**) did not show *C. hystrix* bands, indicating that the alien chromosome segments were lost owing to chromosome recombination

2010; Niu et al. 2011; Chen et al. 2014). However, most species in *Cucumis* have 2n = 2× = 24 chromosomes or multiples of *n* = 12 (Kirkbride 1993), whereas cucumber (*C. sativus* L., CC, 2n = 2× = 14) is the only taxon with an *n* = 7 chromosome base. As a consequence, hybridization between the cucumber and most wild *Cucumis* species has encountered great difficulties because of the different chromosome bases, incompatibility barriers, limited homoeologous recombination, and low fertility of interspecific hybrids (Deakin et al. 1971; Franken et al. 1988). Embryos were recovered from interspecific hybridization between *C. sativus* and *C. melo* but consistently aborted at an early stage (Bates and Robinson 1995). The African *Cucumis* species possess variable degrees of cross-compatibility; for example, *C. ficifolius* is cross-fertile with *C. dipsaceus*, *C. figarei*, and *C. zeyheri* (F_1 seed sterile) but cross-incompatible with *C. leptodermis* and *C. myriocarpus* (Staub et al. 1992; Staub et al. 2005).

Serious barriers exist during the process of wild hybridization in *Cucumis*, such as the sterility of the F_1 hybrids, embryo imbalance, and lack of homologous chromosome pairing. Therefore, it is not possible to hybridize the cucumber with most wild *Cucumis* species, except *C. hystrix*, which produces male and female sterile F_1 hybrids (HC, 2*n* = 19, Chen et al. 1997). However, there are still substantial crossing barriers during the hybridization between *C. hystrix* and *C. sativus*, especially when *C. sativus* is the maternal parent. The fertility can be restored when using the species with the higher chromosome number as the maternal parent. This phenomenon has also been observed in the *Brassica campestris* L. and *Raphanus sativus* L. *complex* (Huang et al. 2001). Chromosome doubling of F_1 hybrid produced an amphidiploid species *C. hytivus* (HHCC, 2n = 4× = 38) that generated viable seeds (Chen and Kirkbride 2000; Chen et al. 2003b). Subsequently, an allotriploid (HCC, 2n = 3× = 26) with partial fertility was obtained by a backcross between *C. hytivus* and *C. sativus* (Chen et al. 2003a). These materials could act as a "bridge" to introduce wild alleles into cucumber gene pool by introgression breeding. Colchicine treatment was performed on the allotriploid to increase the ploidy level, and two MAALs (14 CC + 1H,2n = 15) were recovered from 252 viable plants instead of the expected allohexaploids (Chen et al. 2004). In addition to MAALs, cucumber introgression lines have been previously reported with

resistance to downy mildew generating from a cross between North China cucumber "CC3" and the allotetraploid *C. hytivus* (HHCC) in which *C. hytivus* was used as male parent (Zhou et al. 2008; Pang et al. 2013). One introgression locus was assigned to the introgression lines by the analysis of 23 SSRs. Nevertheless, efficient transition of *C. hystrix* sequences and clear cytogenetic evidence of introgression is still lacking. In the present study, we developed cucumber IL by following an effective strategy that could be summarized as (1) synthesis of interspecific F1 hybrids (Chen et al. 1998), (2) production of allotetraploids through chromosome doubling (Chen and Kirkbride 2000), (3) production of allotriploids by backcrossing allotetraploids to diploid species (Chen et al. 2003a), and (4) generation of introgression lines through backcrossing allotriploids with diploid species and self-pollination. This strategy provides the key steps in realizing gene transfer from wild relatives and cultivated species and can be further used to produce ILs that cover the whole genome of *C. hystrix*.

Characterization and potential applications of cucumber introgression lines

Introducing wild beneficial alleles into crops has been widely reported in many species such as wheat (Jia et al. 2009; Liu et al. 2011; Larson et al. 2012), rice (Huang et al. 2014; Arbelaez et al. 2015), cotton (Fang et al. 2014; Cao et al. 2014), sorghum (Kuhlman et al. 2008), and coffee (Herrera et al. 2007). The introgression lines possess small chromosomal segments from wild donors that are systematically introduced into cultivated crops using MAS (marker-assisted selection). Applications of cucumber ILs have been reported in the genetic mapping of genes/QTLs resistant to downy mildew and gummy stem blight (Zhou et al. 2009; Lou et al. 2013a; Pang et al. 2013). Molecular markers and cytological techniques, such as GISH and meiosis observations, are often used to identify introgression segments. For example, the wheat-*Agropyron cristatum* 6P chromosomal translocation was identified with P-genomic DNA as probes and 461 pairs of SSR primers and 13 STS markers from wheat and was found to exhibit enhanced thousand-grain weight and spike length (Zhang et al. 2015). However, the repetitive sequences of the cucumber genome accumulate at the heterochromatin and centromeric regions instead of evenly distributing along the chromosomes, which means that GISH cannot be used to identify small alien segments in cucumber ILs. FISH is a powerful cytogenetic technique for identifying introgression spots, and its resolution has been dramatically increased to detect short DNA sequences of 1.5~2.0 kb in length (Lou et al. 2014; Han et al. 2015). In a previous study, cucumber introgression lines were characterized by molecular markers and cytological observations at the mitosis late metaphase (delayed sister chromatids) and the diakinesis of meiosis (0.2 tetravalents, 0.05 hexavalents and 0.05 octovalents per pollen mother cell) (Cao et al. 2005; Zhou et al. 2008; Zhou et al. 2009).

Meiotic pachytene analysis combined with FISH provides an excellent opportunity to characterize introgression segments in ILs with small chromosomes and unevenly distributed repetitive sequences. Pachytene chromosome analysis (Fig. 2d, e) and a set of 53 SSRs were performed in the present study, revealing 5 introgression events in the short arms of chromosomes 2, 6, and 7 and the long arms of chromosomes 3 and 7 (Fig. 3). The unpaired introgression region in the long arm of chromosome 7 was verified using four fosmid clones, with fosmid clone 7–7 showing just one signal at chromosome end (Fig. 2f). The phylogenetic relationships between *C. hystrix* and cucumber chromosomes (Yang et al. 2014) suggest that the alien chromosome segments in the short arms of chromosomes 2 and 6 might come from *C. hystrix* chromosomes 3 and 11, respectively. Likewise, the introgression segments in the long arms of chromosome 3 and chromosome 7 might come from *C. hystrix* chromosomes 6 and 1, respectively. However, additional work is still needed to refine the understanding of the origins and effects of the alien sequences. Faris et al. (2008) proposed that the introgression segments are more likely to be stably inherited in progeny with a lesser chance of loss. We thus used 21 polymorphic SSRs from the long arm of chromosome 7 to examine the inheritance of the wild alleles in 38 self-pollinated individuals, and the results indicated that introgression sequences were partially preserved (Fig. 4). Moreover, the segregations of fruit spine color and tubercle distribution provide additional evidence for the loss of wild alleles in self-pollinated offspring. The unambiguous identification of cucumber-*C. hystrix* introgression line reported herein will facilitate the understanding of the effects of *C. hystrix* alleles, broadening the genetic base of cultivated cucumber and enabling resistance breeding.

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Authors' contributions JFC and QFL conceived the study and designed the experiments. YZW and ZTZ conceived the study, participated in the experimental design, performed data analysis, and drafted the manuscript. LJ, ZAL, and JL helped with the synthesis of materials, statistics collection, and analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards The experiments comply with the ethical standards in the country where they were performed.

References

Ali ML, Sanchez PL, Yu S, Lorieux M, Eizenga GC (2010) Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from Oryza wild species into cultivated rice (*O. sativa*). Rice 3:218–234

Arbelaez JD, Moreno LT, Singh N et al (2015) Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice, *O. meridionalis* and *O. rufipogon*, in a common recurrent parent, *O. sativa cv. Curinga*. Mol Breed 35:1–18

Bates DM, Robinson RW (1995) Cucumbers, melons and watermelons. In: Smartt J, Simmonds NW (eds) Evolution of crop plants, 2nd edn. Longman, Harlow, pp 89–96

Cao QH, Chen JF, Guo JY, Qian CT (2005) Observation on micronucleolus during meiosis of pollen mother cell in cucumber. J Wuhan Bot Res 23:11–14 **(In Chinese)**

Cao ZB, Wang P, Zhu XF, Chen H, Zhang T (2014) SSR marker-assisted improvement of fiber qualities in *Gossypium hirsutum* using *G. barbadense* introgression lines. Theor Appl Genet 127:587–594

Caruso G, Gomez LD, Ferriello F et al (2016) Exploring tomato *Solanum pennellii* introgression lines for residual biomass and enzymatic digestibility traits. BMC Genet 17:56

Chen JF, Kirkbride JH (2000) A new synthetic species of *Cucumis* (*Cucurbitaceae*) from interspecific hybridization and chromosome doubling. Brittonia 52:315–319

Chen P, Qi L, Zhou B, Zhang S, Liu D (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91:1125–1128

Chen JF, Staub JE, Tashiro Y, Isshiki S, Miyazaki S (1997) Successful interspecific hybridization between *Cucumis sativus* L. and *C. hystrix Chakr*. Euphytica 96:413–419

Chen JF, Adelberg JW, Staub JE, Skorupska HT, Rhodes BB (1998) A new synthetic amphidiploid in Cucumis from a *C. sativus*× *C. hystrix* F1 interspecific hybrid. Cucurbitaceae 336–339

Chen JF, Luo XD, Staub JE, Jahn MM, Qian CT, Zhuang FY, Ren G (2003a) An allotriploid derived from a amphidiploid× diploid. Euphytica 131:235–241

Chen JF, Staub JE, Qian CT, Jiang J, Luo XD, Zhuang FY (2003b) Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix Chakr.* × *Cucumis sativus* L. Theor Appl Genet 106:688–695

Chen JF, Luo XD, Qian CT et al (2004) *Cucumis* monosomic alien addition lines: morphological, cytological, and genotypic analyses. Theor Appl Genet 108:1343–1348

Chen Y, Wang YY, Wang K, Zhu XF, Guo WZ, Zhang TZ, Zhou BL (2014) Construction of a complete set of alien chromosome addition lines from *Gossypium australe* in *Gossypium hirsutum*: morphological, cytological, and genotypic characterization. Theor Appl Genet 127:1105–1121

Chitwood DH, Kumar R, Headland LR et al (2013) A quantitative genetic basis for leaf morphology in a set of precisely defined tomato introgression lines. Plant Cell 25:2465–2481

Deakin JR, Bohn G, Whitaker TW (1971) Interspecific hybridization in *Cucumis*. Econ Bot 25:195–211

Fang H, Zhou HP, Sanogo S et al (2014) Quantitative trait locus analysis of Verticillium wilt resistance in an introgressed recombinant inbred population of upland cotton. Mol Breed 33:709–720

Faris JD, Xu SS, Cai X, Friesen TL, Jin Y (2008) Molecular and cytogenetic characterization of a durum wheat–*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. Chromosom Res 16:1097–1105

Franken J, Custers J, Bino R (1988) Effects of temperature on pollen tube growth and fruit set in reciprocal crosses between *Cucumis sativus* and *C. metuliferus*. Plant Breed 100:150–153

Guo JY, Chen JF, Cao QH, Luo XD, Chen LZ (2004) Cytological studies on microsporogenesis and male gametophyte development of a *Cucumis* allotriploid derived from *C. hytivus* × *C. sativus*. Cytologia 69(3):335–340

Han YH, Zhang ZH, Liu JH, Lu JY, Huang SW, Jin WW (2008) Distribution of the tandem repeat sequences and karyotyping in cucumber (*Cucumis sativus* L.) by fluorescence in *situ* hybridization. Cytogenet Genome Res 122:80–88

Han YH, Zhang ZH, Liu CX, Liu JH, Huang SW, Jiang JM, Jing WW (2009) Centromere repositioning in cucurbit species: implication of the genomic impact from centromere activation and inactivation. Proc Natl Acad Sci 106:14937–14941

Han YH, Zhang T, Thammapichai P, Weng YQ, Jiang JM (2015) Chromosome-specific painting in *Cucumis* species using bulked oligonucleotides. Genetics 200:771–779

Herrera JC, D'Hont A, Lashermes P (2007) Use of fluorescence in situ hybridization as a tool for introgression analysis and chromosome identification in coffee (*Coffea arabica* L.) Genome 50:619–626

Huang BQ, Chang L, Ju CM, Chen JG (2001) Production and cytogenctics of intergeneric hybrids between Ogura CMS *Brassica campestris* var. *purpuraria* and *Raphanus sativus*. Acta Genet Sin 6:009

Huang SW, Li RQ, Zhang ZH et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Huang LY, Zhang F, Wang WS, Zhou YL, Fu BY, Li ZK (2014) Comparative transcriptome sequencing of tolerant rice

introgression line and its parents in response to drought stress. BMC Genomics 15:1026

Jackson S, Chen ZJ (2010) Genomic and expression plasticity of polyploidy. Curr Opin Plant Biol 13:153–159

Jia J, Yang Z, Li G et al (2009) Isolation and chromosomal distribution of a novel Ty1-copia-like sequence from Secale, which enables identification of wheat—Secale africanum introgression lines. J Appl Genet 50:25–28

Kirkbride JH (1993) Biosystematic monograph of the genus Cucumis (Cucurbitaceae): botanical identification of cucumbers and melons. Parkway Publishers, Inc.

Kuhlman LC, Burson BL, Klein PE, Klein RR, Stelly DM, Price HJ, Rooney WL (2008) Genetic recombination in Sorghum bicolor× S. macrospermum interspecific hybrids. Genome 51:749–756

Kupper R, Staub JE (1988) Combining ability between lines of *Cucumis sativus* L. and *Cucumis sativus* var. *hardwickii* (R.) Alef. Euphytica 38:197–210

Larson SR, Kishii M, Tsujimoto H et al (2012) Leymus EST linkage maps identify 4NsL–5NsL reciprocal translocation, wheat-Leymus chromosome introgressions, and functionally important gene loci. Theor Appl Genet 124:189–206

Li HJ, Conner RL, Liu ZY et al (2007) Characterization of wheat-triticale lines resistant to powdery mildew, stem rust, stripe rust, wheat curl mite, and limitation on spread of WSMV. Plant Dis 91:368–374

Liu SB, Zhou RH, Dong YC, Li P, Jia JZ (2006) Development, utilization of introgression lines using a synthetic wheat as donor. Theor Appl Genet 112:1360–1373

Liu WX, Jin Y, Rouse M, Friebe B, Gill B, Pumphrey MO (2011) Development and characterization of wheat-*Ae. searsii* Robertsonian translocations and a recombinant chromosome conferring resistance to stem rust. Theor Appl Genet 122: 1537–1545

Lou QF, He YH, Cheng CY, Zhang ZH, Li J, Huang SW, Chen JF (2013a) Integration of high-resolution physical and genetic map reveals differential recombination frequency between chromosomes and the genome assembling quality in cucumber. PLoS One 8:e62676

Lou LN, Wang HJ, Qian CT, Liu J, Bai YL, Chen JF (2013b) Genetic mapping of gummy stem blight (Didymella bryoniae) resistance genes in Cucumis sativus-hystrix introgression lines. Euphytica 192:359–369

Lou QF, Zhang YX, He YH et al (2014) Single-copy gene-based chromosome painting in cucumber and its application for chromosome rearrangement analysis in *Cucumis*. Plant J 78:169–179

Momotaz A, Kato M, Kakihara F (1998) Production of intergeneric hybrids between *Brassica* and *Sinapis* species by means of embryo rescue techniques. Euphytica 103:123–130

Murray M, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Navabi ZK, Stead KE, Pires JC et al (2011) Analysis of B-genome chromosome introgression in interspecific hybrids of *Brassica napus* × *B. carinata*. Genetics 187:659–673

Niu Z, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, Xu SS (2011) Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021

Pang X, Zhou XH, Wan HJ, Chen JF (2013) QTL mapping of downy mildew resistance in an introgression line derived from interspecific hybridization between cucumber and *Cucumis hystrix*. J Phytopathol 161:536–543

Ren Y, Zhang ZH, Liu JH et al (2009) An integrated genetic and cytogenetic map of the cucumber genome. PLoS One 4:e5795

Schauer N, Yaniv S, Ute R et al (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nat Biotechnol 24:447–454

Shi JL, Lou QF, Qian CT, Wan HJ, Zhou XH, Chen JF (2011) Construction and genetic evaluation of chromosome segment introgression lines in cucumber. J Nanjing Agric Univ 34:20–24 (In Chinese)

Shi JL, Chen JF, Lou QF, Qian CT, Meng JL (2012) Analysis of genetic variation and QTL detection in cucumber introgression progenies. Acta Hortic Sin 39:687–694 (In Chinese)

Staub JE, Knerr L, Holder D, May B (1992) Phylogenetic relationships among several African *Cucumis* species. Can J Bot 70:509–517

Staub J, Chung SM, Fazio G (2005) Conformity and genetic relatedness estimation in crop species having a narrow genetic base: the case of cucumber (*Cucumis sativus* L.) Plant Breed 124:44–53

Weng Y, Li W, Devkota RN, Rudd JC (2005) Microsatellite markers associated with two *Aegilops tauschii*-derived greenbug resistance loci in wheat. Theor Appl Genet 110:462–469

Xu JJ, Zhao Q, Du PN et al. (2010) Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (*Oryza sativa* L.). BMC Genomics 11: 656

Yang LM, Koo DH, Li Y, Zhang X, Luan F, Havey MJ, Jiang J, Weng Y (2012) Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. Plant J 71:895–906

Yang LM, Li DW, Li YH, Gu XF, Huang SW, Garci-Mas J, Weng Y (2013) A 1,681-locus consensus genetic map of cultivated cucumber including 67 NB-LRR resistance gene homolog and ten gene loci. BMC Plant Biol 13:53

Yang LM, Koo DH, Li DW et al (2014) Next-generation sequencing, FISH mapping and synteny-based modeling reveal mechanisms of decreasing dysploidy in *Cucumis*. Plant J 77:16–30

Yaniv E, Raats D, Yefim R et al (2015) Evaluation of marker-assisted selection for the stripe rust resistance gene *Yr15*, introgressed from wild emmer wheat. Theor Appl Genet 35:1–12

Zhang HK, Yao B, Gou XW et al (2013) Persistent whole-chromosome aneuploidy is generally associated with nascent allohexaploid wheat. Proc Natl Acad Sci 110:3447–3452

Zhang J, Zhang JP, Liu WH et al (2015) Introgression of *Agropyron cristatum* 6P chromosome segment into common wheat for enhanced thousand-grain weight and spike length. Theor Appl Genet 128:1827–1837

Zhao W, Qi L, Gao X, Zhang G, Dong J, Chen Q, Friebe B, Gill BS (2010) Development and characterization of two new *Triticum aestivum–Dasypyrum villosum* Robertsonian translocation lines T1DS· 1V# 3L and T1DL· 1V# 3S and their effect on grain quality. Euphytica 175:343–350

Zhou XH, Wan HJ, Qian CT, Chen JF (2008) Development and characterization of *Cucumis sativus-hystrix* introgression lines exhibiting resistance to downy mildew. Cucurbitaceae 353–358

Zhou XH, Qian CT, Lou QF, Chen JF (2009) Molecular analysis of introgression lines from *Cucumis hystrix Chakr.* to *C. sativus* L. Sci Hortic 119:232–235