Plantcytogenetics

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The Cytogenetics of cucumber, *Cucumis sativus* L.

Cucumber, *Cucumis sativus* L. is a succulent vegetable that can be eaten fresh or turned into pickles. Chromosome study in cucumber is a challenging endeavour because of its small genome size. For comparison: Cucumber, n = 7 chromosomes = 367 Mb; *Arabidopsis thaliana*, n = 5 chromosomes = 135 Mb; Corn, n = 10 chromosomes = 2500 Mb; Bread wheat, n = 7 chromosomes = 1700 Mb.

Useful Links

- Cucumber genome database
- Cucumber genome in nature genetics
- Synteny with melon
- HTML templates

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Introduction

Cucumber, *Cucumis sativus* L. comes from the Cucurbitaceae family, which also includes other well-known cultivated plants such as melon, watermelon, squash, and pumpkin (Nayar and More, 1998). Cucumber is mainly eaten in salads and could also be turned into pickles. There are four main varieties of cucumber; slicing, pickling, specialty, and container cucumbers (http://www.grow-it-organically.com/cucumber-varieties.html). The center of origin and domestication of cucumber was thought to be India (Ren et al., 2009). In 2013, the top producer of cucumber was China with 76% of global output, followed by Turkey, Iran, Russia, and Ukraine (Food and Agriculture Organization of the United Nations, 2013).

Cucumber is often used as a model system in sex determination studies because of its rich diversity of sex expression (Tanurdzic and Banks, 2004). In addition, its readily collected xylem and phloem sap made it a model plant for plant vascular biology studies (Lough and Lucas, 2006; Xoconostle-Cázares et al., 1999).

Genetics of cucumber

Cucumber is a diploid species with 2n = 2x = 14 chromosomes. The cucumber Genome Database (http://cucumber.genomics.org.cn/page/cucumber/index.jsp) published the first genomic sequences of cucumber based on the domestic cucumber variety, *C. sativus* var. *sativus* L. The haploid genomic size of the cucumber is 367 Mb and there are a total of 26,682 genes predicted. The genome size of cucumber is smaller than any other species in the Cucurbitaceae family: melon=480 Mb, watermelon=430 Mb, squash and pumpkin=539 Mb (Arumuganathan and Earle, 1991).

The genomic sequence of cucumber showed multiple tandem repeats in the telomeric and centromeric regions with tandem repeat type I/II, type III, type IV, and rDNA (Han et al., 2008, Huang et al., 2009). Type I/II, type III, and type IV accounted for 90% of the satellite DNA in the cucumber genome (Ganal and Hemleben, 1988). Type I/II have a repeat length of 182 bp with a 47% of GC content. Type II differs from type I for a single base pair, and because of that they are grouped together as type I/II. Type III has a repeat length of 177 bp with a 53% of GC content, while type IV has a repeat length of 360 bp with a 47% of GC content (Ganal et al., 1986; Ganal and Hemleben, 1988).

Huang et al. (2009) explained that the small number of genes in cucumber is due to the absence of recent whole genome duplication and the existence of few ancient tandem duplications. This was revealed by the collinear gene-order analysis, by comparing the paralogous gene pairs between the syntenic blocks in a common ancestor, *Arabidopsis thaliana*, and in cucumber. The study showed that cucumber chromosomes arose from the fusion of ancestral chromosomes, right after divergence from melon, *Cucumis melo* (n = 12) about 4-7 million years ago (Huang et al., 2009). Chromosome 1, 2, 3, 5, and 6 of cucumber's chromosomes arose from the fusion of chromosome 2 and 12, 3 and 5, 4 and 6, 9 and 10, and 8 and 11 of melon's chromosomes, respectively (Huang et al., 2009). This was demonstrated by the presence of collinear regions between the chromosomes in the two species. The study also suggested chromosome rearrangements that happened before the speciation event (Huang et al., 2009).

Cytogenetic studies in cucumber

The first chromosome staining in cucumber was conducted by Ramachandran and Seshadri (1986) using the C-banding technique. The study was done to compare the different banding patterns in cucumber and melon. Based on the cytological and morphological characteristics, center of origin, and the strong hybridization barrier of the species, the study suggested that cucumber and melon evolved separately in Asia and Africa. The karyotype formula was established by the authors using the Japanese cucumber cultivar, with one subterminal, two median, and four submedian chromosomes (Ramachandran and Seshadri, 1986).

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Basic staining with improved chromosome preparation methods were later used to characterize the cucumber chromosomes (Hoshi et al. 1998, Chen et al. 1998, Koo et al. 2002). Chen et al. (1998) utilized the C-banding method to perform cucumber karyotyping in the Chinese cultivar 'Beijin Jietou' and observed symmetric chromosomes, including one submetacentric and six metacentric chromosomes. The range of chromosome length was 1.48 - 2.31 µm. The bands detected on the chromosomes were centromeric, telomeric and intercalary. Hoshi et al. (1998) conducted orcein and C-banding techniques on diploid and tetraploid cucumbers of variety 'Borszczagowski'. The study reported five metacentric and two submetacentric chromosomes in these plants. Koo et al. (2002) used Feulgen staining to karyotype a Korean cucumber cultivar and reported one submetacentric and six metacentric haploid chromosomes (Figure 1). The range of chromosome length was 1.63 - 3.40 µm.

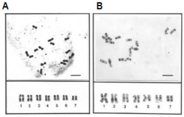

Figure 1: A - Feulgen staining in cultivar Winter Long, showing one submetacentric and six metacentric chromosomes. B - C-banded somatic metaphase chromosomes (Koo et al., 2002).

The researchers karyotyping the cucumber chromosomes using the conventional staining method faced many challenges due to the small size of the chromosomes, the similar chromosome lengths and/or arm ratio, and the lack of chromosome landmarks (Han et al., 2008). Because of these challenges, fluorescent staining and fluorescence *in situ* hybridization (FISH) are becoming increasingly popular method to conduct the cytogenetic studies in cucumber (Koo et al., 2002; Koo et al., 2005; Han et al., 2008; Hoshi et al., 2008; Hoshi et al., 2011; Lou et al., 2014). These techniques help in revealing individual chromosomes by the means of probes hybridization on the chromosome structure.

Hoshi et al. (2011) utilized fluorescent banding with GC-specific chromomycin A3 (CMA) and AT-specific 4',6-diamidino-2-phenylindole (DAPI), and FISH with the probes of 5S, 45S rDNA and telomere repeat sequences to distinguish two European pickling cultivars - Borszczagowski and Monastyrski - and one Japanese cultivar - Zibai. Han et al. (2008) used seven kinds of repeats as the probes for FISH procedure: type I/II, type III, type IV, CsRP1, CsRP2, 45S rDNA, and 5S rDNA. Type I/II and type IV probes were hybridized on the telomeric heterochromatin region, type III probe was hybridized on the specific centromeric region, and CsRP1 and CsRP2 were hybridized on the centromeric/pericentromeric regions (Han et al., 2008).

Lou et al. (2014) used single copy gene sequences as probes to 'paint' the cucumber chromosomes and performed comparative painting on three *Cucumis* species: cucumber, melon, and horned melon (*C. metuliferus*). The probes used in this technique were gene sequences free of repetitive sequences with sizes bigger than 2 kb (red signals in Figure 2). The study discovered three misaligned regions in the distal end of chromosome 4 and used these probes to study the chromosome rearrangements that happen during the speciation event within the *Cucumis* genus.

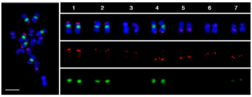

Figure 2: Chromosome painting using single copy gene probes. Red = gene probes on individual chromosomes; Green = 45S rDNA probes (Lou et al., 2014).

Comparative cytogenetics between cucumber and melon was also performed by Han et al. (2009) using a common set of fosmid clones (Figure 3). The comparative FISH mapping was specifically done on two chromosomes in each species: chromosomes 6 and 7 in cucumber and 1 and 11 in melon. The study suggested that the event of centromere repositioning through an inactivation of a centromere in the original region and activation of a new centromere (known as neocentromere) in the non-centromeric region happening because of a poorly understood epigenetic mechanism. Han et al. (2009) demonstrated this idea by using a centromeric probe specific for cucumber - type III repeat - and for melon - centM repeat. Sequence analysis between these two repeats did not show any similarity, and they did not generate any cross-hybridization signals when the two species tried to hybridize reciprocally. This analysis showed that the new sets of centromere were formed during the divergence of the species from a common ancestor.

The study also demonstrated the gain and loss of pericentromeric heterochromatin regions in the melon and cucumber, respectively, due to the centromere repositioning. The suggested chromosomal rearrangements that occurred were chromosomal fission in melon and chromosomal fusion in cucumber, and the alternative explanation was pericentric inversion involving the centromere and a large region of pericentric heterochromatin.

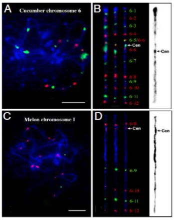

Figure 3: Comparative FISH mapping between cucumber chromosome 6 and melon chromosome 1, suggesting centromere repositioning (Han et al., 2009).

FISH was also used several times to develop the cucumber cytogenetic map by integrating the information from the genetic map (using SSR markers) and the physical genome map (Figure 4) (Sun et al., 2013, Han et al., 2011, Han et al., 2009, and Ren et al., 2009). Sun et al. (2013) used 8 - 14 fosmid clones per chromosome (a total of 76 FISH loci) to construct the molecular cytogenetic map of cucumber. The fosmid clones were distributed at regular intervals along the cucumber chromosome's draft genome assembly maps; some of these clones represented the major genetic markers in cucumber (Sun et al., 2013). The physical positions of these formid clones on the chromosomes correlate with their respective position on the draft genome assembly map Sun et al. (2013), except for seven misassembled fosmid clones. Nonetheless, Sun et al. (2013) concluded that the draft genome assembly sequences covered the cucumber chromosomes almost entirely, with gaps being only from the tandem repeat sequences. The cytogenetic map in this respect has helped to verify the quality of the draft genome assembly map.

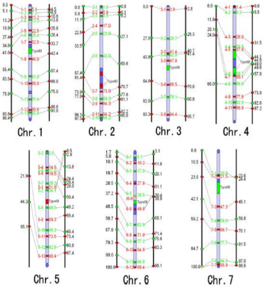

Figure 4: Integrated genetic (left)/cytogenetic (middle)/genome assembly (right) maps of cucumber inbred line 9930 (Sun et al., 2013).

Study objectives

1. To document the different stages of cell mitosis in the cucumber root tips.
2. To have an unambiguous count of cucumber chromosome number.
3. To find the optimum condition for chromosome staining by testing the following conditions:

Plant type:

a. Seeds germinated in a petri dish on moist filter paper for 2-3 days.
b. Seeds grown in a vermiculite near the lab window for 5 days.

Pretreatment:

a. Distilled water at 4°C temperature for 18 hours.
b. 8-hydroxyquinoline solution at 4°C for 4-6 hours.

Cell wall hydrolysis:

a. 1M hydrochloric acid at 60°C for 5 minutes.
b. 1M hydrochloric acid at 60°C for 10 minutes.

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Materials and Methods

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1. Cucumber seeds from these cultivars, Straight eight heirloom and Cross country were purchased from the local market. Straight eight heirloom is an all-America selections winner with good flavor and has wide adaptation (http://www.burpee.com/vegetables/cucumber/cucumber-straight-eight-prod000699.html). Cross country is a hybrid cultivar that originated from India; it has a high fruit yield and its crisp exterior and tender flesh make it suitable to be eaten as salad or turned into pickles (https://myfolia.com/plants/26-cucumber-cucumis-sativus/varieties/131348-cross-country-hybrid).
2. Seeds from these two cultivars were divided into two: the first half was grown on wet filter paper in a petri dish for 2.5 days in dark (Figure 5), and the second half was grown in a vermiculite placed next to the lab window for 4 days (Figure 6). The seeds were watered once a day to induce germination.

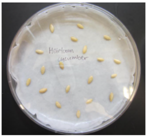

Figure 5: Seeds were grown on wet filter paper in a petri dish.

Figure 6: Seeds were grown in vermiculite and placed next to the lab window.

3. Roots from the petri dish seedlings (Figure 7) were cut to about 1 cm in length and placed in a tube containing:
   a. Cool distilled water: these tubes were pretreated at 4°C for 18 h (Koo et al., 2002).
   b. 8-hydroxyquinoline solution: these tubes were pretreated at 4°C for 4 - 6 hours (Singh, 2002).

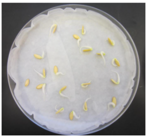

Figure 7: The 2.5-day-old seedlings grown on a wet filter paper in dark. 1 cm roots were excised and placed in the tubes containing pretreatment solution.

4. Roots from the seedlings grown in vermiculite (Figure 8) were excised, washed with tap water (Figure 9) and placed in the similar pretreatment solutions used for seedlings grown in the petri dish.

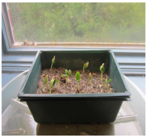

Figure 8: Four day-old-seedlings grown in vermiculite placed near the lab window.

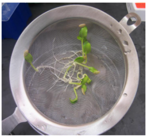

Figure 9: Roots were washed with tap water before being pretreated with cool distilled water (4°C) for 18 h or 8-hydroxyquinoline at 4°C for 4-6 h.

5. After pretreatment roots were transfered to new tubes containing Farmer's solution (3:1 ethanol and glacial acetic acid) for 24 h for chromosome fixation (Koo et al., 2002).
6. Roots were rinsed with 70% ethanol and stored in 70% ethanol at 4°C until further use.
7. Roots were cleared with 1M hydrochloric acid at 60°C in a water bath for:
   a. 5 minutes (Koo et al., 2002).
   b. 10 minutes.
8. Roots were washed 3 times with 70% ethanol. The root tip (about 2 mm long) was excised and placed on a glass slide. One drop of modified carbol fuchsin was placed on the root tip (Kao, 1975).
9. The cover slip was slowly placed on the stained root tip, and the root was squashed gently using the blunt end of a pen. The slide was then turned upside down and further squashed onto folded paper towel.

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10. Slides were viewed using a Zeiss Axio Imager light microscope, and pictures were taken using the AxioCam HRC built in camera. Images were processed using the AxioVision 4.8 software with automatic setting.

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Results and Discussion

a) The different stages of mitosis in cucumber

All stages of mitosis were successfully observed in the root tips of cucumber using the modified carbol fuchsin stain. The following images are of the mitosis stages:

Interphase

This stage is where the cell prepares itself for cell division. The stage is divided into three checkpoints: G1 (first gap), S (synthesis), and G2 (second gap). In this stage, the cell grows by producing proteins and cytoplasmic organelles, and chromosomes are replicated in the S phase. The nucleus in the interphase appears round, and the nuclear membrane is visible (Figure 10 - 12).

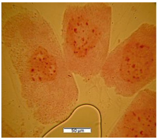

Figure 10: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes.

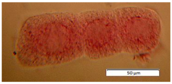

Figure 11: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes.

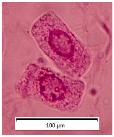

Figure 12: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes.

Prophase

The prophase occurs after the G2 checkpoint of the interphase. During the prophase the chromosomes are further condensed (Figure 13 - 15) and the mitotic spindle is formed. This stage allows the chromosomes to be viewed under the high magnification of the light microscope; the chromosomes in this stage consist of two chromatids joined at centromere. In the late prophase, the nuclear membrane is dissolved, chromosomes start to move to the metaphase plate (Figure 16), and kinetochores (protein around the centromere) begin to attach to the spindle fibers.

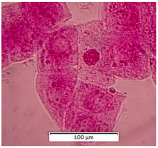

Figure 13: Heirloom cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell in the middle underwent early prophase. Note that the chromosomes began to condense.

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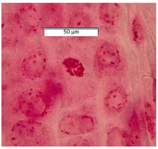

Figure 14: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. The chromosomes in the middle cell are condensed and clearly visible.

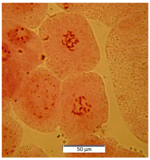

Figure 15: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. Chromosomes in the two middle cells were clearly visible.

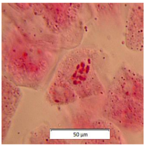

Figure 16: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. The cell in the middle underwent late prophase. Note that the chromosomes have started to move to the equator in this polar view.

Metaphase

In the metaphase, chromosomes move and align at the equator/metaphase plate (Figure 17 - 20). In this stage the kinetochores are attached to the spindle fibers, and the chromosome arms float on either side of the nucleus (Singh, 2002).

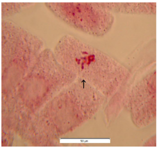

Figure 17: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell shown by the arrow is in early metaphase where its chromosomes have started to move to the equator.

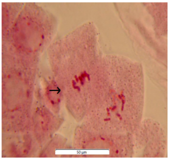

Figure 18: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The chromosomes in the cell shown by the arrow were aligned at the equator.

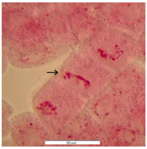

Figure 19: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell that has undergone metaphase is shown with arrow.

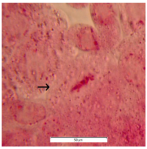

Figure 20: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell that has undergone metaphase is shown with arrow (polar view).

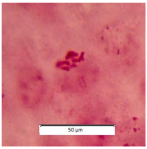

Figure 21: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. This cell is in late metaphase. Note that the sister chromatids have started to move to the opposite pole.

Anaphase

In the anaphase sister chromatids separate from each other and are pulled toward opposite poles (Figure 22 - 25). In this stage kinetochores double and are pulled apart to opposite poles by the spindle fibers (Singh, 2002). The spindle fibers disappear at the end of the anaphase.

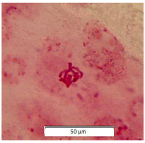

Figure 22: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. The cell in the middle underwent early anaphase. Note that the sister chromatids have just begun to move to the opposite pole.

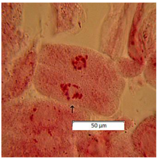

Figure 23: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell shown by the arrow has undergone anaphase.

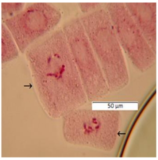

Figure 24: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cells denoted by arrows have undergone anaphase.

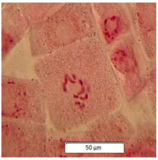

Figure 25: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell in the middle has undergone anaphase.

Telophase and Cytokinesis

In the telophase all sister chromatids have migrated to their respective poles (Figure 26 - 29) and form dense chromatid balls (Singh, 2002) (Figure 29 - 31). Nucleoli and nuclear membranes begin to reappear. The telophase is followed by cytokinesis, a process where cytoplasm and organelles divide between the two daughter cells. The cell plate is formed by the end of cytokinesis (Figure 27, 29 - 31).

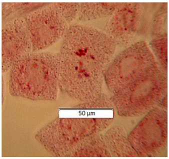

Figure 26: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell in the middle is in the late anaphase and early telophase. Note that the cell was undergoing cytokinesis: the cytoplasm has started to divide, and the cell has expanded into two cells.

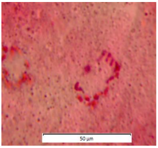

Figure 27: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell was in late anaphase and early telophase. Note that the cell plate has just begun to form in the middle.

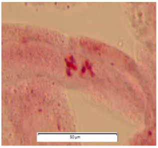

Figure 28: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. Chromosomes were at their respective poles.

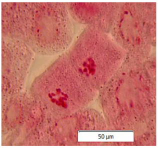

Figure 29: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. The cell plate has formed between the cells.

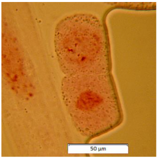

Figure 30: Heirloom cucumber with 8-hydroxyquinoline pretreatment and cleared in 1M HCL for 10 minutes. The cell plate has formed between the cells.

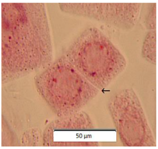

Figure 31: Cross country hybrid cucumber with cool water pretreatment and cleared in 1M HCL for 10 minutes. Cells were separated by the cell plate (shown by an arrow).

b) Chromosomes count

Total chromosome count was successfully done on the cell undergoing mid-prophase (Figure 32). The chromosomes in this stage are more spread out because they are no longer constrained by the nuclear membrane and are not concentrated in the middle of the cell, which would happen during the metaphase. The total number of chromosomes in the picture is 14.

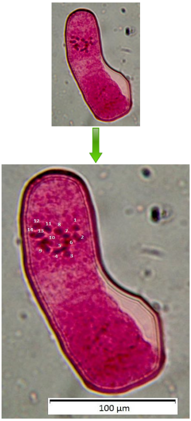

Figure 32: Heirloom cucumber planted in vermiculite, pretreated with cool water, and cleared with 1M HCL for 10 minutes. The individual chromosomes are numbered in the picture for the purpose of total chromosome count. Note that the cucumber chromosomes in the picture are 2n = 2x = 14 chromosomes.

c) Optimal conditions for chromosome staining

Overall, the mitotic stages of cucumber cells were successfully observed and the following conclusions were drawn:

1. Roots from seeds germinated in a petri dish in the dark at 2.5 days old gave more mitotic cells than roots from seedlings grown in vermiculite at 4 days old.
2. Roots pretreated in cool distilled water for 18 hours yielded more visible chromosomes than roots pretreated with 8-hydroxyquinoline at 4°C for 4-6 hours.
3. Only roots hydrolyzed with 1M HCL at 60°C for 10 minutes have stain permeability compared to digestion for only 5 minutes.

Acknowledgements

I would like to thank Edward Mcassey for being a great instructor and demonstrator throughout our lab sessions and during my chromosome viewing work. I also want to thank Dr Wayne Parrott for sparking my interest in cytogenetics, my coursemates Luellan and Chandler for giving me suggestions and help throughout this project, and last but not least, April Legg and Christine Brady for proofreading my paper.

Plantcytogenetics

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Useful Links

Cucumber genome database

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