Octoploidy-induced Genome Downsizing and its Effects on Plant Morphology in *Arabidopsis thaliana*

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Abstract

It is known that the tardy asynchronous meiosis-2 (tam-2) mutant of Arabidopsis thaliana produces unreduced gametes by skipping meiosis II, resulting in genome duplication in the next generation. This process of polyploidization continues until it reaches octoploidy that becomes unstable. The progeny of octoploid tam-2 are of reduced ploidy as a result. The purpose of this study was to examine fertility and morphological changes over at least two generations from the octoploid tam-2 of A. thaliana. We hypothesize that the instability of the genome may result in new genome compositions that produce new phenotypes in the generations after the octoploid generation. We found a positive linear relationship between guard cell nuclear area and ploidy level; this relation will be used for determining the ploidy levels in the progeny of the octoploid tam-2. By observing morphological changes due to genomic decay in real time, we may find new varieties of Arabidopsis in the making over one or more generations, which has important implications to the mechanisms of speciation, and to technological development for engineering new crops in the agricultural industry.

Keywords: Polyploidy, *Arabidopsis*, Genomic Decay, TAM, Mutation

Introduction

Polyploidy refers to an organism with an extra set(s) of chromosomes compared to the genome of the corresponding diploid organism (Otto 2007). Polyploid organisms exist as either allopolyploid organisms or autopolyploid organisms. Allopolyploids are polyploid organisms whose chromosomes are from different species because of polyploidization in an F1 hybrid organism. An autopolyploid is an organism whose genome consists of more than two sets of chromosomes from a single species. Polyploidy may be detected by an increased size of the nucleus or an increased chromosome number (Otto 2007, Zielinski and Scheid 2012).

Polyploidy is common among plant species presumably due to the lack of a pachytene checkpoint to prevent meiosis in the case of meiotic error; in mammal and bird species, a meiotic error often results in cell death that precludes gamete formation (Otto 2007). Polyploidy can lead to genetic changes. In particular, the genomic composition of an allopolyploid organism originated from an inter-species hybridization differs from that of either of the parent plants. Moreover, both allopolyploids and autopolyploids often undergo chromosome loss (Otto 2007). These genetic changes are subject to natural selection, which may lead to formation of new species. In African clawed frogs, allopolyploidization has occurred six times and resulted in three new species from the original diploid (Evans et al., 2004). Polyploidy can also result in ploidy-specific lethality, which is when a gene deletion has little effect in a lower-ploidy species but causes death in higher-ploidy species (Storchová, et al. 2006). In yeast, the mutations responsible for this lethality impair meiotic processes such as recombination of homologues, mitotic spindle function, and the joining of sister

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chromatids (Storchová et al. 2006). Mayer and Aguilera's (1990) studies of yeast suggest that increased ploidy leads to increased chances of chromosome deletion, with the rate of deletion exponentially higher for each increase in ploidy level. In flowering plants, genome downsizing due to sequence deletion is a result of allopolyploid formation and is a frequent trend in the evolution of the angiosperms. An estimated 70% of angiosperms had instances of polyploidization in their evolutionary history (Matzke et al. 1999). Polyploidization to genome downsizing to new genome formation may have repeatedly occurred in plant evolution, but such a process has never been demonstrated in the lab.

Arabidopsis thaliana is a model organism for genetic studies because it has a fully sequenced genome, as well as a short lifecycle that lends itself to experiments that require multiple generations of specimens (Meinke et al. 1998). Increases in ploidy in Arabidopsis are commonly attributed to the formation of unreduced gametes as a result of errors in chromosome pairing or unbalanced chromosome segregation in meiosis I (Brownfield and Köhler, 2011). Genome duplication is also common in allopolyploids of Arabidopsis as shown by Madlung et al. (2004). The *Arabidopsis* genus has also been shown to exhibit unique epigenetic silencing as a consequence of change in ploidy level, which could significantly affect cell function (Matzke et al., 1999).

The *TARDY ASYNCHRONOUS MEIOSIS* (*TAM*) gene, is responsible for positively regulating cell cycle progression in *Arabidopsis* meiosis (Maganrd et al. 2001, Wang et al. 2004). The partial loss-of-function *tam-1* mutant displays delayed and asynchronous cell divisions in meiosis I and II (Maganrd et al. 2001, Wang et al. 2004, Wang et al. 2010). The *tam-2* null mutant produces unreduced gametes by skipping meiosis II entirely. The resulting polyploidization in *tam-2* continues until it reaches octoploidy that becomes unstable (Wang et al. 2010). The progeny of octoploid *tam-2* are of either octoploid or reduced ploidy as a result.

In this study, we will examine fertility and morphological changes over at least two generations from the octoploid *tam-2*. We expect that the instability of the genome will result in new genome compositions that produce new phenotypes. It will be the first time that such a process can be observed in real time in the lab. The findings of this study should have important implications to the mechanisms of angiosperm speciation, and to technological development for engineering new crops in the agricultural industry.

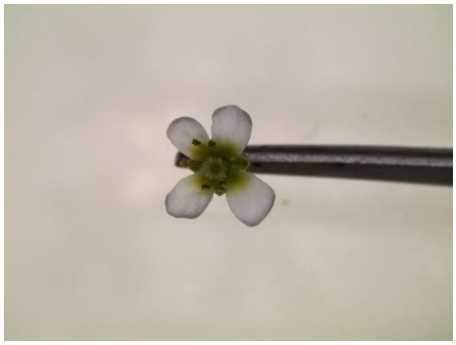

Figure 1 - Typical flower phenotype of octoploid tam-2 A. thaliana

Figure 2 - Octoploid tam-2 silique (top) compared to wild-type diploid silique (bottom). Note: The wild-type silique has dehisced and all the seeds and the fruit wall were missing.

Methods

Octoploid *Arabidopsis thaliana tam-2* seeds were grown with the same type of soil and under identical lab conditions. All plants were of the Colombia ecotype. We aimed to produce one hundred individuals to assure the production of enough fertile plants for supply of plant materials of subsequent generations, as the plants were expected to have poor fertility. Wild-type diploid, tetraploid, hexaploid, and octoploid of *A. thaliana* were also cultivated for comparison of morphology according to ploidy level.

During growth, we took cotyledon samples for examination using light microscopy. First, the samples were fixed and stored in 70% ethanol solution. We then stained the samples with 4'.6-diamidino-2-phenylindole (DAPI) to determine the nuclear size in guard cells using fluorescence microscopy. The series of guard cell nuclear sizes corresponding to the different ploidy levels served as the standard values for determining the ploidy levels in the progeny of the octoploid *tam-2* (Jha et al., 2014).

We will investigate the fertility of the plants in each generation by counting the number of seeds per individual. We will use a compound microscope and a dissecting scope equipped with a digital imaging system to detect morphological changes at the cellular level and organ level, respectively. If we observe significant phenotypic changes, we will attempt to identify the genomic changes responsible for the phenotypic changes using the polymerase chain reaction technique, or PCR. We will use electrophoreses with 0.8% agarose gel stained with Phenix's GelRed Nucleic Acid Stain to detect the lengths of the PCR products. If deemed necessary, we will also employ one-step Reverse Transcriptase PCR, or RT-PCR, technique to detect changes in the transcription of genes in the affected genomic regions.

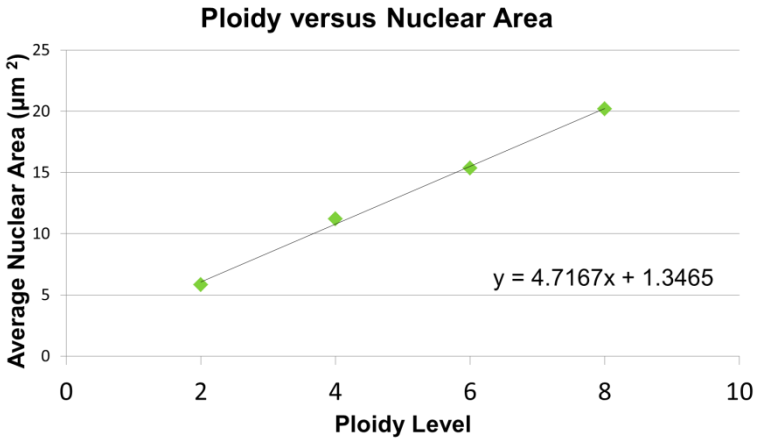

Figure 3 - Graph depicting the relationship between ploidy and nuclear area in wild-type A. thaliana

Progress to Date

At this point in time, eighty six octoploid *Arabidopsis thaliana tam-2* individuals have been grown to maturity. More seeds are likely needed for assuring the production of enough seeds for subsequent analysis, as most of the plants are expected to have poor fertility or may be sterile. Wild-type diploid, tetraploid, hexaploid, and octoploid of *A. thaliana* have also been grown for building a standard curve of guard cell nuclear size in relation to the ploidy level.

We have begun recording the average number of seeds obtained per individual plant over the plants' lifetime in order to gauge the overall fertility of the generation. Selected plants have been examined for morphological changes and photographed with a camera directly or a camera on a dissecting or compound microscope so that these images can be used to compare phenotype over generations (Figures 1 and 2). We have collected cotyledons from the *tam-2* individuals for

staining and viewing under the microscope but have yet to gather data on their nuclear sizes.

Cotyledon samples from the wild-type individuals were collected and fixed in 70% ethanol. The fixed cotyledons were stained with 4'.6-diamidino-2-phenylindole (DAPI), and examined and photographed using fluorescence microscopy (Figures 4-7). The series of guard cell nuclear sizes corresponding to the different ploidy levels were recorded and plotted on a graph to construct a standard curve (Table 1; Figure 3). Our results indicate that the ploidy level is positively and linearly correlated with the guard cell nuclear area, which lays the foundation for determining the ploidy levels in octoploid *tam-2* and its progeny.

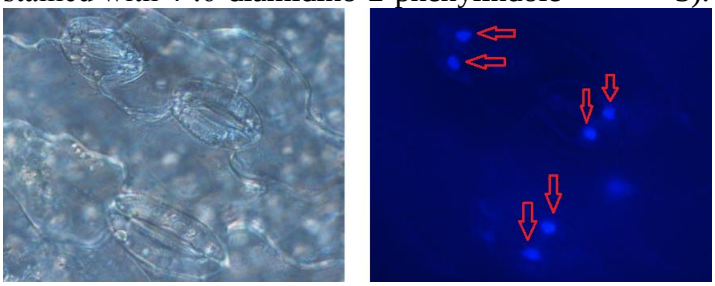

Figure 4 - Diploid (2C) bright-field and DAPI microscopy images taken at 100x objective, with arrows highlighting the guard cell nuclei

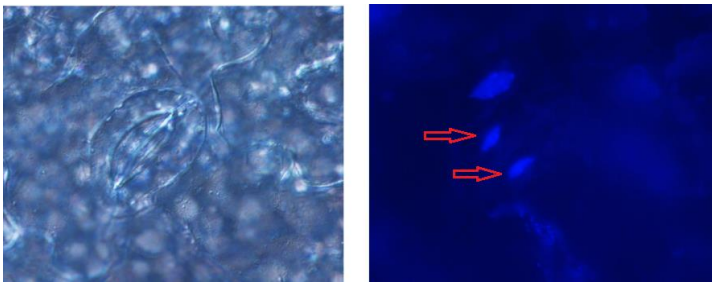

Figure 5 - Tetraploid (4C) bright-field and DAPI microscopy images taken at 100x objective, with arrows highlighting the guard cell nuclei

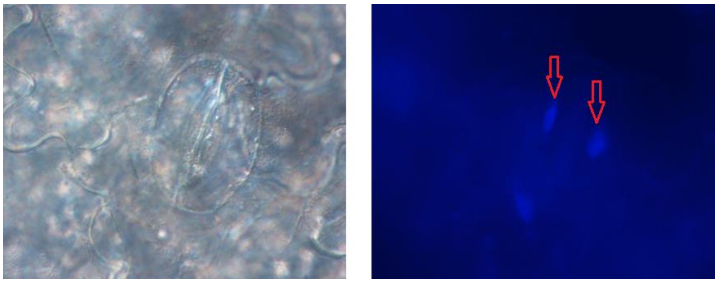

Figure 6 - Hexaploid (6C) bright-field and DAPI microscopy images taken at 100x objective, with arrows highlighting the guard cell nuclei

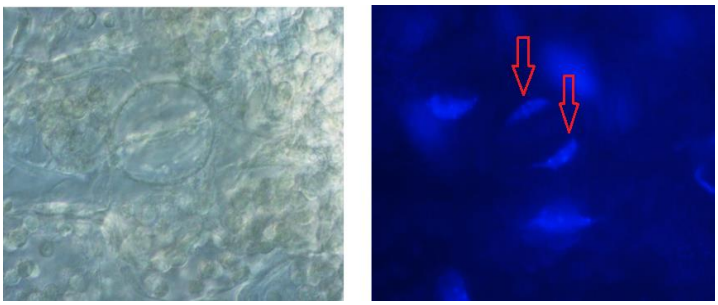

Figure 7 - Octoploid (8C) bright-field and DAPI microscopy images taken at 100x objective, with arrows highlighting the guard cell nuclei

Future work

The strong and positive linear relationship between the ploidy and the guard cell nuclear area suggests that higher ploidy levels result in larger nuclear areas. This was expected, since it was assumed that a larger genome with more genetic material would require more space, and therefore a larger nucleus. With the seeds that have been collected from octoploid *tam-2*, we are ready to investigate how the progeny of octoploid *tam-2* change in respect to morphology, genome size, and structure.

Presently, nuclear areas are being recorded for individuals of the *tam-2* variety that have produced seeds thus far. Their seeds will be planted and the process of recording guard cell areas repeated. We plan to compare the nuclear areas of the *tam-2* individuals to the standard curve to determine their ploidy levels. The same experiments will be conducted with the *tam-2* progeny as well. If deemed necessary, PCR technique will be used to detect genomic changes in the progeny.

Acknowledgements

This research was supported by a scholarship granted to freshman researchers at Oklahoma State

University of Stillwater, Oklahoma by the Howard Hughes Medical Institute.

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