Received: 27 November 2024 | Accepted: 16 February 2025

DOI: 10.1002/tpg2.70015

The Plant Genome

ORIGINAL ARTICLE

Special Section: Tribute to Ron Phillips: Crop Genetics, Genomics and Biotechnology

Chronic water-deficit stress may increase meiotic recombination in maize

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Assigned to Associate Editor Feng Zhang.

Funding information
R.F. Baker Center for Plant Breeding at Iowa State University

Abstract

Meiosis and recombination lead to gametes with novel combinations of genes as key processes in evolution and plant breeding. Numerous extrinsic factors have been reported to affect meiotic recombination of plants. The goal of this research was to identify simple, low-cost, and effective treatments that affect recombination in maize (*Zea may*s L.). The treatments, water-deficit stress and defoliation, were separately applied to two F1-generation genotypes, B73/Mo17 and Mo17/H99. The F1 plants were backcrossed to an inbred line to produce the backcross populations that were genotyped at microsatellite loci on chromosomes 1 and 10. Overall, 1271 crossovers were observed in the progeny of the water-stressed plants while 1092 were observed in the progeny of the non-stressed plants. The water-deficit treatment may have increased the rates of recombination in both F1 genotypes while the defoliation treatment was ineffective.

Plain Language Summary

Plant gametes, cells that unite to make the next generation, carry chromosomes with new combinations of genes. Those chromosomes are important sources of genetic variation, a key driver of evolution and plant breeding. Herein, we describe a simple and low-cost method for increasing meiotic recombination in maize to produce a higher frequency of gametes that contain chromosomes with new combinations of genes.

1 | INTRODUCTION

The concept of the investigations described herein originated in the early 1980s when a coauthor (M.L.) was a teaching assistant for the cytogenetics course taught by Ron Phillips, his academic advisor. Prior to the start of a lecture to the class, Dr. Phillips casually described a series of experiments in which growing certain genotypes of cultivated flax with limited amounts of phosphorous fertilizer induced heritable and stable phenotypic changes in the next sexual generations (Durrant, 1962). Subsequently, that coauthor (M.L.) became a professor at Iowa State University and followed the advice of Dr. Phillips, given to him by his advisor, Dr. Charles Burnham, and assigned this project, the best idea available at that time to a new student and coauthor (L.A.V.) for his graduate studies. These experiments were inspired by the idea that changes in a plant's genome could be induced by common environmental variables. The goal of this research was to

identify simple, low-cost, and repeatable treatments that could influence meiotic recombination in maize.

The defining features of meiosis are the production of gametes with nuclei containing half the number of chromosomes as their somatic cells and some chromosomes with novel haplotypes, new combinations of genes, and possibly new alleles through the process of recombination. Some of the genes, their gene products, and functions that participate in meiosis and native recombination in plants have been gradually identified (Chen et al., 2024; Lambing et al., 2017). The investigation of the developmental deployment of the transcriptome of meiosis has been initiated at the cellular level for plants, as exemplified for maize (Nelms & Walbot, 2019, 2022). As the identification and understanding of the sequence of steps involved in meiosis and recombination advance, opportunities for directly manipulating those processes may be revealed.

Several comprehensive assessments of recombination have revealed some trends in plants. First, there are some clear differences among species with respect to the distribution of crossovers along the length of chromosomes (Chen et al., 2024; Lambing et al., 2017; Wang & Copenhaver, 2018). An obligatory one crossover per bivalent has been revealed (Lambing et al., 2017; Wang & Copenhaver, 2018). Also, the total number of crossovers per bivalent seems to be constrained at one or two, and rarely three (Lambing et al., 2017). The number of crossovers per bivalent has been inferred by genomic sequencing of single microspores (Li et al., 2015), direct cytological observations of chiasmata at pachynema (Sidhu et al., 2015), and analyses of transcripts of single pollen precursor cells (Nelms & Walbot, 2022). In those studies, a range of one–two crossovers per pair of homologous chromosomes has been reported.

Numerous extrinsic factors have been reported to affect meiosis and, possibly, recombination in plants (Fuchs et al., 2018). Herein, the effects of two possible stress treatments, chronic water deficit and defoliation, on meiotic recombination in pollen mother cells of maize (*Zea mays* L.) have been described.

Core Ideas

- When plants make gametes, the cells that unite to create the next generation, the gametes contain some chromosomes with new combinations of genes made through the process of recombination.
- The chromosomes with the new combinations of genes are important sources of genetic variation that drives evolution and plant breeding.
- A simple and low-cost method for increasing the rate of recombination of maize is described.

2 | MATERIALS AND METHODS

These experiments were conducted to separately assess the effects of two stress treatments, water-deficit stress and defoliation, on meiotic recombination in maize. The stress treatments and controls (non-stress) were applied to F1-generation plants grown in the Agronomy Greenhouse at Iowa State University. After the treatments were applied, the F1-plants were mated as males to plants of one of the parental inbred lines to produce kernels of the backcross populations (BC1F1) used to assess recombination as described.

2.1 | Water-deficit stress treatment

This treatment involved growing F1-generation plants, side by side, at either chronic water-deficit stress of 25% field capacity (FC) or at constant FC. FC was determined for the soil mixture, which would enable adjustment of water content for the treatment. FC is the total amount of water retained in a particular soil after it has been drained from saturation by gravity (Miller & Donahue, 1990). The 12-L pots contained an equal bulk density (weight of soil solids per unit volume) of a dry, sterile mixture of soil, peat, and perlite (1:2:2) with one plant per pot. Six randomly chosen pots were saturated with water, placed on a bench, and allowed to drain until dripping stopped. Prior to planting, each pot was weighed and recorded. Weight measurements for each pot were taken every 15 min until the weight was stable. The average weight difference between the pots, before and after saturation, was designated as the weight of a pot at FC. Based on the results, the total weight at FC and at 25% FC was calculated for each pot. Since plant growth affects the total weight, biomass curves for each treatment were used to adjust for water content. Water content was adjusted by weighing the pots daily, subtracting the plant weight estimated according to the biomass curves, and adding water until the correct weight for each treatment was reached. Thus, the treatment of 25% FC involved maintaining the pots with 75% less water than the FC treatment.

Pots were fertilized once with 20 g of Scotts Sierra fertilizer (17-6-12) and watered to promote adequate germination. The temperature in the greenhouse was 25/20 ± 3°C day/night. Additional light was provided with high discharge lights (400-watt bulb; Philips, r/i00s51) to achieve a 14-h photoperiod. Light intensity during the day averaged 400 µmol/m-2s-1. The F1 kernels were planted at a soil depth of 2.5 cm in individual 12-L pots. All F1 plants were watered to FC until pre-meiosis stage (V7-V8) (Hanway & Ritchie, 1984), when water was withheld to maintain 25% FC for the water-deficit treatment.

The F1 plants were randomly assigned to each treatment, which in turn was randomly placed on the greenhouse

benches. Growth stages were assessed by dissecting F1 plants and examining tassel size, development, and position (Chang & Neuffer, 1989). At pre-meiosis, water content of each pot was adjusted according to the treatments. The treatments were maintained until the end of male meiosis when tassels emerged from the whorl. To assess the effect of the treatments on plant growth and development, phenotypic (plant height and the number of days to anthesis) measurements were recorded.

The genotypes exposed to the water-deficit treatments were the F1 generation B73/Mo17 and Mo17/H99 created by mating inbred lines B73 with Mo17 and Mo17 with H99 (female inbred line is listed first). Water-deficit treatments were initially applied to B73/Mo17, and later repeated with Mo17/H99. When the F1 plants reached anthesis, they were mated as males to a parental inbred genotype—B73/Mo17 to B73, and Mo17/H99 to H99—to create the F1BC1 populations with one population for each F1 plant. For each F1 genotype, there were three F1BC1 populations created for each treatment, three for the 25% FC and three for the FC. Each F1 genotype, B73/Mo17 and Mo17/H99, was grown at a separate time in the greenhouse.

2.2 | Defoliation stress

A third experiment was conducted to ascertain if there are any indistinguishable differences in patterns of meiotic recombination resulting from defoliated and non-defoliated plants of genotype Mo17/H99. All F1 plants of this experiment were grown under FC and fertilized as described. This experiment was conducted at the same time, side by side, with the water-deficit experiment with F1 genotype Mo17/H99. This enabled measurement of the effect of defoliation on genetic recombination, and at the same time, comparing recombination rates in the two types of stress. At pre-meiosis (V7–V8), three F1 plants were randomly selected for the defoliation stress treatment. Defoliation was performed by clipping all extended leaves up to the sheath at V7 stage of development, approximately 40% of the leaves of each F1 plant. At the same time, another three F1 plants were randomly selected as controls (non-stress treatment). Each F1 plant was mated as the male to a plant of inbred line, H99. Control plants were grown under the same conditions with the exception that they were not defoliated. All F1 plants in this experiment were grown at FC with the previously described fertilizer regime. Procedures for DNA extraction, PCR, and genetic analysis were the same as for the water-deficit experiments.

To allow comparisons with the water-deficit stress experiments, chromosomes 1 and 10 were chosen for this investigation. The same microsatellite loci used in the water-deficit stress experiment were selected for this analysis.

2.3 | DNA procedures and genetic analyses

The kernels of each F1BC1 population from each treatment were planted in sand benches, and leaf tissue was collected from individual seedlings. Techniques used for tissue collection and DNA extraction have been described (Dietrich et al., 2002). Each population, which had originated from an F1 plant mated as a male to a plant of an inbred lines (either B73 or H99), was represented by a sample of 93 F1BC1 seedlings. DNA was extracted by using the PURGENE isolation Kit (Gentra). Sample collection and isolation protocols were those suggested by PURGENE for 50–100 mg of fresh solid tissue. Chromosome 1 was selected for analysis because it had the highest number of loci detected by microsatellites at the time of the study, and it has been analyzed in other studies (Tulsieram et al., 1992; Williams et al., 1995). Loci on chromosome 10 were included to provide an assessment of another region of the genome. Microsatellite loci were used with primers that amplified one PCR product in each inbred parent and the two expected PCR products in the F1 being selected for the analysis. Selection of primers was based on separation of alleles (PCR products) on metaphor gels, reproducibility, and clarity of the PCR products (Senior et al., 1998). Screening of primers was conducted to identify at least one microsatellite locus in each chromosome bin, so that intervals between adjacent loci were <25 cM based on published genetic maps available at the time of the investigation (Sharopova et al., 2002). MAPMAKER3.0 was used to estimate recombination frequency in stress and non-stress plants and to construct genetic maps (Lander et al., 1987). Closely linked loci (±3 cM) were not assayed in the rest of the populations because they were less informative. Loci with codominant alleles were screened and mapped on a non-stress population (control treatment) of each F1 genotype until all bins of the genetic maps of chromosomes 1 and 10 were represented, and intervals between adjacent loci were intended to be smaller than 20 cM. The numbers of microsatellite loci used to assess recombination were 16 on chromosomes 1 and nine on chromosome 10 for B73/Mo17, and seventeen on chromosomes 1 and 7 on chromosome 10 for Mo17/H99. The segregation ratio at each locus was tested against the expected 1:1 ratio with Pearson's chi-square analysis. All segregation data and number and distribution of crossovers have been presented in the Supporting Information (Tables S1–S7). Crossovers were detected when alleles at adjacent loci were from different parents.

For each population, 96-well microtiter plates containing DNA from the 93 BC1 F1 individual seedlings, the F1 genotype, and the appropriate parental inbred lines were created. Techniques used for tissue collection and DNA extraction have been described (Dietrich et al., 2002). PCR reactions were performed using a PTC-100 (MJ Research) thermal

TABLE 1 Observed crossovers in chromosomes 1 and 10 of six maize backcross populations (B73[B73/Mo17]) from water-deficit stress and non-stress treatments.

	Chromosome 1							
	Water-stress populations				Non-stress populations			
	1	2	3	Mean	4	5	6	Mean
Crossovers per chromosome								
0	22	15	16	18	20	15	20	18
1	28	38	34	33	40	41	33	38
2	29	22	25	25	23	27	30	27
3	8	18	16	14	10	10	10	10
4	5	0	2	2	0	0	0	0
5	1	0	0	0	0	0	0	0
Total number of crossovers	135	136	140	137	116	125	123	121
Average crossovers by individual	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3
	Chromosome 10							
	Water-stress populations				Non-stress populations			
	1	2	3	Mean	4	5	6	Mean
Crossovers per chromosome								
0	39	40	37	39	46	45	47	46
1	44	39	43	42	39	36	35	37
2	9	14	13	12	8	12	11	10
3	1	0	0	0	0	0	0	0
Total number of crossovers	65	67	69	67	55	60	57	57
Average crossovers by individual	0.7	0.7	0.7	0.7	0.6	0.7	0.6	0.6

Note: Each population, stress and non-stress, was respresented by a sample of 93 seedlings.

cycler. After amplification, products were separated using a modified version (Senior et al., 1998). In this version, a 4% METAPHOR gel (FMC Byproducts) containing 13 μg mL^{-1} of ethidium bromide was used for separation and detection of PCR products. Products were separated by electrophoresis in an A3-1 model gel system (23 by 40 cm; Owl Scientific) using 1X TBE buffer. Eight 50-tooth combs, spaced 4 cm apart, were placed in each gel so that samples from four 96-well plates ran on the same gel. DNA fragments were separated by electrophoresis at 300 V in a cold room (6°C) for 2–4 h depending on the expected molecular weight of PCR products (i.e., alleles). The 1X TBE buffer was cooled by circulation through an ice bucket with a CP-600 peristaltic pump (Life Technologies). Gels were observed in a transilluminator (FOTO/UV 300, FOTODYNE) and photographed with a digital imaging system (Alpha Imager 2000). The allelic constitution of each individual was determined on the basis of visual comparisons among PCR products observed for the appropriate parental inbred lines and the F1 genotype for a given pair of primers. The determination of alleles and data entry was repeated twice, and discrepancies were resolved by repeating the PCR for the samples in question. Individuals with loci that were homozygous for the allele of the recurrent parent (inbred lines B73 or H99) were coded as "B" and those individuals with loci presenting one allele from each respective parental inbred line were coded "H" (i.e., heterozygous). The PCR was repeated for all individuals involved in putative double crossovers (crossovers in adjacent intervals) and for unclear products or results.

MAPMAKER3.0 (Lander et al., 1987) was used to estimate recombination frequency in stress and non-stress plants and to construct genetic maps. The option "F2 backcross" and the default program setting were used. Genetic maps for each population and treatment were constructed for chromosomes 1 and 10 (Tables S4–S6). The Student *t*-test was performed to assess treatment differences in map length, assuming that estimates of map lengths have a normal distribution (Tables S1–S3). The linkage maps have been presented (Tables S4–S6). The least significant difference (LSD) was calculated to have an estimate of the map distance (cM) necessary to declare the population means different. The likelihood-ratio test (*G*-test, Beavis & Grant, 1991) was used to compare the three maps within each treatment (Table S7). This test is a generalization of the two-map case (Beavis & Grant, 1991; Liu, 1998), used to test homogeneity of recombination.

3 | RESULTS AND DISCUSSION

The assessments of morphological and physiological traits indicated that the water-deficit stress treatments affected the growth and development of the F1-generation plants. The average plant height of the water-deficit stress treatment for B73/Mo17 was 196 cm, while the value for the non-stress plants was 262. Furthermore, the average number of days to anthesis was 73 for the water-deficit stress and 63 for the non-stress treatment of B73/Mo17. Similarly, the average plant height of the water-deficit stress treatment for Mo17/H99 was 153 cm, while the value for the non-stress plants was 215. The average number of days to anthesis was 75 for the water-deficit stress and 63 for the nonstress treatment of Mo17/H99. The defoliation treatment has less prominent effects on the growth and development of the F1 generation, Mo17/H99. The defoliated plants had an average height of 231 cm and reached anthesis in 67 days, while the values for the non-stress plants were 215 cm and 63 days.

The crossovers observed for chromosomes 1 and 10 of the six backcross populations involving B73/Mo17 have been summarized in Table 1. For chromosome, the stress and non-stress treatments had nearly equivalent numbers of individuals with zero, one, or two crossovers. However, there were more individuals with crossovers for the stress treatment in the other categories. For three crossovers, the stressed populations had a total of 42 (8, 18, and 16) such individuals, while the non-stress populations had 30 (10, 10, and 10). For the four and five crossovers categories, the stressed populations had a total of 7 and 1, respectively, while the nonstress populations had zero for both categories. Overall, 411 and 364 crossovers were observed in the stress and nonstress populations, respectively, on chromosome 1 for this genotype.

For chromosome 10, there was also evidence of more frequent recombination in the water-deficit stress treatment involving B73/Mo17. The stress populations had fewer individuals with zero observed crossovers, that is, 116 (39, 40, and 37), than the nonstress populations, that is, 138 (46, 45, and 47). For the category of 1 crossover, the stress populations had 126 (44, 39, and 43) individuals, and the nonstress populations had 110. The category of two crossovers was nearly equivalent, with 36 and 33 individuals observed in the stress and nonstress populations, respectively. There was one individual observed with three crossovers, a member of the stress populations. Overall, for chromosome 10, there were 237 crossovers observed in the stress populations, while 172 were observed in the three nonstress populations involving B73/Mo17.

The crossovers observed for chromosomes 1 and 10 of the six backcross populations involving Mo17/H99 have been summarized in Table 2. For chromosome 1, the stress and non-stress treatments had nearly equivalent numbers of individuals in the categories of zero, one, two, and three crossovers: 48 versus 46, 100 versus 113, 83 versus 79, and 34 versus 37, respectively. In the category of four crossovers, the stress populations had 11 individuals, and the nonstress populations had 4. The stress populations had 3 individuals with five crossovers, but such individuals were not observed in the nonstress populations. Overall, for chromosome 1, there were 427 crossovers observed in the stress populations while 398 were observed in the nonstress populations involving Mo17/H99.

For chromosome 10 of populations involving Mo17/H99, there was evidence that the water-deficit stress treatment resulted in more frequent recombination. The stress populations had fewer individuals with zero observed crossovers, 121, than the nonstress populations, 146. For the category of 1 crossover, the stress populations had 122 while the nonstress populations had 108 observed crossovers. For the category of two crossovers, 32 crossovers were observed in the stress populations and 25 in the nonstress populations. For the categories of three and four crossovers, there were three crossovers in the stress populations and zero in the non-stress populations. Overall, for chromosome 10, there were 196 crossovers observed in the stress populations and 158 in the nonstress populations involving Mo17/H99.

In the defoliation treatment, the number of crossovers, overall and for any category, was not discernably different when comparing the stress and nonstress populations (Table 3). Overall, 400 crossovers were observed in the stress populations and 398 were observed for chromosome 1. For chromosome 10, there were 170 crossovers observed in the stress populations, and 158 were observed in the nonstress populations because there were more individuals with two crossovers in the stress populations (30) than in the nonstress populations (25).

The linkage maps for the populations and related analyses have been summarized in Tables S2–S7. Graphical presentations of all linkage maps have been reported (Verde, 2003). There may be some evidence of recombination "hot spots" in some stress populations. However, such assessments may be limited by the low number of loci, the lack of integration with a physical map, and the limited sample sizes of the progeny. So, it is difficult to discern if the extra recombination events have a stochastic pattern or not. Such considerations, while of interest, are not ancillary to this research. Overall, the frequency of recombination events per chromosome observed herein has a similar range as those inferred in previous reports in maize (Nelms & Walbot, 2022; Sidhu et al., 2015).

When this investigation was conducted, microsatellites were among the best methods for linkage analysis of DNA polymorphism. Like all methods of that time, a major limitation was a complete lack of integration with detailed physical maps of chromosomes. Such integration only became possible

TABLE 2 Observed crossovers in chromosomes 1 and 10 of six maize backcross populations (H99[Mo17/H99]) from water-deficit stress and non-stress treatments.

	Chromosome 1							
	Water-stress populations				Non-stress populations			
	1	2	3	Mean	4	5	6	Mean
Crossovers per chromosome								
0	14	20	14	16	15	16	15	15
1	32	32	36	33	36	35	42	38
2	32	23	28	28	26	24	29	26
3	11	11	12	11	15	15	7	12
4	3	5	3	4	1	3	0	1
5	1	2	0	1	0	0	0	0
Total number of crossovers	146	141	140	142	137	140	121	133
Average crossovers by individual	1.6	1.5	1.5	1.5	1.5	1.5	1.3	1.4
	Chromosome 10							
	Water-stress populations				Non-stress populations			
	1	2	3	Mean	4	5	6	Mean
Crossovers per chromosome	42	41	39	41	47	46	53	49
0	43	40	39	41	41	37	30	36
1	6	12	14	11	5	10	10	8
2	2	0	0	1	0	0	0	0
3	0	0	1	0	0	0	0	0
Total number of crossovers	61	64	71	65	51	57	50	53
Average crossovers by individual	0.7	0.7	0.8	0.7	0.5	0.6	0.5	0.5

Note: Each population, stress and non-stress, was represented by a sample of 93 seedlings.

several years after the completion of this study with the advent of a near complete sequence of the maize genome. So, it is quite likely that some regions of chromosomes such as telomeres were not surveyed herein. As with all investigations, only what has been observed can be reported.

The word "may" has been chosen, in the title of this report, to indicate uncertainty because the effects of stress on recombination and the related biological mechanisms are mostly unknown and only defined in an elementary manner at this time. The authors have neither stated nor implied the results were statistically significant with respect to the numbers of crossovers observed in the stress and non-stress populations. The significance of the results reported herein, while possibly statistically insignificant or not, should be assessed on the basis of the observations from several simple experiments of potential biological significance.

Given the vital and central nature of the roles of water in plant growth and development, it may be reasonable to expect a variety of responses when such a resource becomes limited and stress has been imposed on an organism (Parsons, 1988). Such responses may also hinder the ability to immediately identify the pathways engaged by the plant's response (McClintock, 1984). Herein, chronic water scarcity may have elicited a response native to maize that resulted in higher rates of meiotic recombination. Water deficits result in other physiological adaptations by the plant that may be closer to the true cause of the elevated recombination. For example, when plants are exposed to water deficits, the stomata close and that could lead to a cascade of other reactions. That response may elevate temperatures in some tissues and cells. Elevated temperatures have been reported to increase meiotic recombination in plants (De Storme & Geelen, 2020; Fuchs et al., 2018). Other pathways and responses, such as abscisic acid signaling due to temperature stress, may be somehow related to an increase in recombination. As with many systems found in nature, there are many interacting components, moving parts, that have assisted the survival of the species and confounded our understanding of them. Perhaps, simple experiments as described herein may have a role in elucidating some of nature's enigmas.

AUTHOR CONTRIBUTIONS

Luis A. Verde: Formal analysis; investigation; methodology; validation; writing—original draft. **Tatenda R. Musimwa**: Data curation; formal analysis; validation; writing—original draft; writing—review and editing. **Michael Lee**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision;

TABLE 3 Observed crossovers in chromosomes 1 and 10 of six maize backcross populations (H99[Mo17/H99]) from defoliation stress and non-stress treatments.

	Chromosome 1							
	Defoliation populations				Non-stress populations			
	7	8	9	Mean	4	5	6	Mean
Crossovers per chromosome								
0	16	12	13	14	15	16	15	15
1	39	35	43	39	36	35	42	38
2	27	33	26	29	26	24	29	26
3	10	11	9	10	15	15	7	12
4	0	2	2	1	1	3	0	1
5	1	0	0	0	0	0	0	0
Total number of crossovers	128	142	130	133	137	140	121	133
Average crossovers by individual	1.4	1.5	1.4	1.4	1.5	1.5	1.3	1.4
	Chromosome 10							
	Defoliation populations				Non-stress populations			
	7	8	9	Mean	4	5	6	Mean
Crossovers per chromosome								
0	49	47	45	47	47	46	53	49
1	36	33	38	36	41	37	30	36
2	8	13	9	10	5	10	10	8
3	0	0	1	0	0	0	0	0
Total number of crossovers	52	59	59	57	51	57	50	53
Average crossovers by individual	0.6	0.6	0.6	0.6	0.5	0.6	0.5	0.5

Note: Each population, stress and non-stress, was represented by a sample of 93 seedlings.

validation; visualization; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

We would like to thanks Wendy Woodman–Clikeman for instructions related to DNA manipulations and the PCR, and for assistance in collecting some of the segregation data. The corresponding author of the paper thanks Ronald Lewis Phillips for inviting him to be a research assistant in his laboratory at the University of Minnesota, a source of many great moments and people in my life, so far. We would also like to thank R.F. Baker Center for Plant Breeding at Iowa State University for partial financial support. This research was conducted in partial fulfillment of the MS and PhD degrees of L.A.V at ISU. In 1836, Charles R. Darwin returned from a global cruise on the HMS *Beagle*. In 1859, his well-known book, *On the Origin of Species*, was published. Some things take time: Darwin took his, we had ours, and both needed >20 years to complete the journey. We are grateful to have had the opportunity to make this report in honor and memory of Dr. Phillips.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its supplementary information files: https://doi.org/10.5061/dryad.4j0zpc8pg).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Verde, L. A., Musimwa, T. R., & Lee, M. (2025). Chronic water-deficit stress may increase meiotic recombination in maize. *The Plant Genome*, *18*, e70015. https://doi.org/10.1002/tpg2.70015