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Germination of Twins in Intermediate Wheatgrass

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ABSTRACT

This research studied the frequency of twins in various genotypes of intermediate wheatgrass. Intermediate wheatgrass was germinated for a week and then sorted for twins. The twins were grown for several months and then analyzed to determine if either of the twins was haploid. Twins are rare in plants and haploid and dihaploid seedlings are even rarer. There have been very few studies of the frequency of twins in intermediate wheatgrass; however, this could be very beneficial and practical information for plant geneticists and plant breeders. This research project germinated 118,000 total seedlings, and found 58 (.0049%) twins. The results seem to indicate that twinning is not influenced by genotype. One haploid twin was identified in a parallel study at The Land Institute.

Keywords: *perennial*, *wheatgrass, twins, haploid, diploid, dihaploid*

INTRODUCTION

The world's population is now more than 7 billion people, and as the population continues to increase, it becomes increasingly more important to grow crops that are environmentally sustainable. The development of perennial wheatgrass offers many advantages over the traditional varieties of annual wheat. Annual wheat must be planted each year. This requires fossil fuels to run the tractors, fertilizers, and pesticides, and hours of human labor. Then when the wheat is harvested, the combine is driven over the fields. Each time these heavy machines drive over the fields, they compact the soil. This compaction prevents water from being absorbed into the soil easily. Each winter, bare soil is vulnerable to wind and rain erosion. It is estimated that an inch of soil is lost to erosion each year. Perennial wheatgrass would continue to cover the fields with vegetation, thus reducing soil erosion. The perennial wheatgrass *Thinopyrum intermedium* also has the advantage of greater root length and root depth than annual wheat. (Glover et al. 2009).

Scientists at the Land Institute in Salina, Kansas are working on developing "perennial versions of major grain crops,"including sunflowers, sorghum, legumes, maize, and over 70 genotypes of perennial wheatgrass (Glover et al 2010). One advantage to perennial crops is the longer growing season since they are in the ground year round (Glover et al 2010). The year-long growing season allows the soil ecosystems to be stable (Culman et al 2009). A disadvantage of perennial plants is the seed yield. Annual wheat typically produces greater seed yield than perennial wheatgrass. Farmers will be more likely to switch to perennial wheatgrass if the new plants are proven to provide seed yield comparable to the seed yield of annual wheat, and if their operating costs are lower. It is important to find the best genotype of intermediate wheatgrass so that farmers are more likely to profit from it, and so that the genetic variety is hardy in a variety of conditions as climate change will likely affect annual wheat crop yield.

Three years ago, The Land Institute collaborated in a S.A.R.E Grant with McPherson College in Kansas and Dordt College in Iowa. Seventy genotype varieties of intermediate wheatgrass were planted in three plots at each location. Students at each college were able to conduct research on the intermediate wheatgrass .They studied genetic traits of maternal plants and how they affect the seed yield. This study has focused on the frequency of haploid and dihaploid twins among genotypes of intermediate wheatgrass.

A normal plant has two sets of chromosomes (2n); one set from the male gametophyte (pollen), and one set from the female gametophyte (ovule). Haploid plants only have a single set of chromosomes (n). A haploid seedling is rare, and often much smaller than a normal seedling. If the seedling survives, it would not produce pollen when it matures. However, if the haploid plant is treated with colchicine, a chemical that induces doubling, then it is called a doubled haploid, dihaploid or diploid (Tayeng et al 2012). In this case, the single set of chromosomes is doubled, and the plant would have the same genetic qualities as passed on from the mother plant (Tayeng et al 2012). The doubled haploids are used for genetic research and plant breeding. Very rarely a doubled haploid plant is found naturally.

There have been no known studies of the frequency of haploid and dihaploid twins in intermediate wheatgrass. There have been numerous studies of haploids in wheat, barley, and other cereal plants for over one hundred years (Dunwell, 2010), and haploid twins have been studied in annual wheat, oil palm trees, cotton, and several other flowering plants. It is known that some plant species are more likely than others to produce twins (Dunwell, 2010). Haploid twins could be very useful with the plant breeding process for intermediate wheatgrass. Haploids can be used to improve plant breeding with doubled haploid production, which can produce true-breeding of homozygous offspring (Dunwell, 2010). The plants that are produced from doubled haploids are sure to be truly homozygous. They will be genetically the same as the parent plant. Breeding haploid plants could save time and money because several plant generations could be skipped as plant breeders try to develop advantageous traits. If the haploid twin saves five years of plant breeding, that also means it saves five years of scientist salaries, farmhand salaries, operating costs, water, and fertilizer. It could also give a competitive advantage over other seed companies.

This research included the germination of wheatgrass seedlings and analysis of the twins to determine the frequency of haploid and dihaploid twins in Intermediate wheatgrass. I predicted that haploid twins would be more prevalent in some genotypes than others.

MATERIALS AND METHODS

The goal of my project was to research the frequency of twins in intermediate wheatgrass. I used the wheatgrass plants and seed from the plot at The Land Institute and worked under the direction of Dr. Lee DeHaan. After some discussions, it was decided that Dordt College students would join me in a study of the frequency of haploid and dihaploid twins from the seeds harvested from the successful genotypes. It was decided that we would only use genotypes that were successful in both Iowa and Kansas, so we only harvested and studied twelve of the original seventy genotypes.

The first step to acquiring wheatgrass seeds, therefore was to weed the wheatgrass plots about once per week during the late spring and early summer. On the day before harvesting, the plants that were used in the study were labeled. The wheatgrass was harvested on July 24 and July 25. The wheatgrass stems were cut about eighteen inches below the seed heads. All of the stems from a particular genotype were harvested. The bundles of wheatgrass were placed in the climate-controlled dryer room for five days. The climate-controlled dryer removed the moisture from the bundles of wheatgrass to prevent mold. Then each separate bundle of wheatgrass was threshed and cleaned.

The seeds were weighed (in hulls) using a Denver Instrument Company Scale TR-603D. Each of twenty genotypes was weighed separately by Plot A, B, or C. These data were put into an Excel document to give a sum total seed yield by genotype. Although this is not as accurate as the de-hulling method used by James and Fry last year (James, 2013), it did provide a comparison of seed yield. Dr. DeHaan recommended that the seeds would germinate better if they were still protected in the hull. The results of the seed yield allow a comparison by genotype with the seed yield from the past two years, and provided a comparison with the Dordt college wheatgrass seed yield.

The seeds were counted with a seed counter machine. Six sets of 1000 seeds were put into seed packets and labeled by genotype and place of harvest (Kansas or Iowa). Then I kept two of the sets of each genotype to be used for my twin study, and four of the sets were sent to Dordt College.

The seeds were germinated in sets of 1000 seeds in order to search for twins, either haploid twins or dihaploid twins.

The procedure to germinate seeds was very simple. The set of 1000 seeds was placed on a moist brown paper towel. This was put into a plastic sandwich bag. Then 6mL of deionized water was added, and the bag was zipped closed and put into the refrigerator for about one week to simulate winter and break dormancy.

After one week in the refrigerator, the seeds were placed on moist paper towels in large covered trays, and 120mL of deoinized water was added before the seeds were spread evenly around on the paper towels. These trays are then placed in dark room at room temperature for 3-4 days, and then moved near a window when the seedlings were about two inches tall. If mold developed on a few seeds, then a drop of fungicide (diluted Fertilome Broad Spectrum Liquid Fungicide) was put on the mold. When the seedlings were two to three inches tall, they were sorted to look for twins.

If a twin seedling was found or a possible twin was found, it was planted in Miracle Grow potting soil, watered, and labeled. The twins were kept moist and then allowed to dry out so they wouldn't die from damping off.

The twin seedlings were allowed to grow for several weeks at McPherson College, and then they were transported to The Land Institute greenhouse. The seedlings were grown to maturity. If a plant is haploid, then it will not produce pollen and it will be sterile. This procedure was conducted at The Land Institute.

The Kansas plot of wheatgrass and the Iowa plot of wheatgrass were compared for the total seed weight of each genotype. The proportion of twins germinated at each site was also calculated with a Paired t-test (t_{30}=-3.57, p=0.724), and a Two-Sample z-test (z value= .52, p value =0.3). The results are shown below, and they indicate that the two plots were not significantly different for seed weight or twins produced.

RESULTS

The original intermediate wheatgrass plants were all from The Land Institute. The wheatgrass plants had been growing for three summers when these seeds were harvested. Mold was observed on many of the Iowa seedlings and presented a problem, and it may have damaged some possible twins.

Below you see a comparison of total plant seed weight for genotypes grown in Kansas and Iowa.

Table 1. Paired Samples Test (KS-IA)

Mean Difference		-0.129
Std. Dev.		2.0123
Std. Error		0.3614
95% Confidence Interval		
	Lower	-0.8672
	Upper	0.6901
t-stat		-0.357
df		30
P		0.724

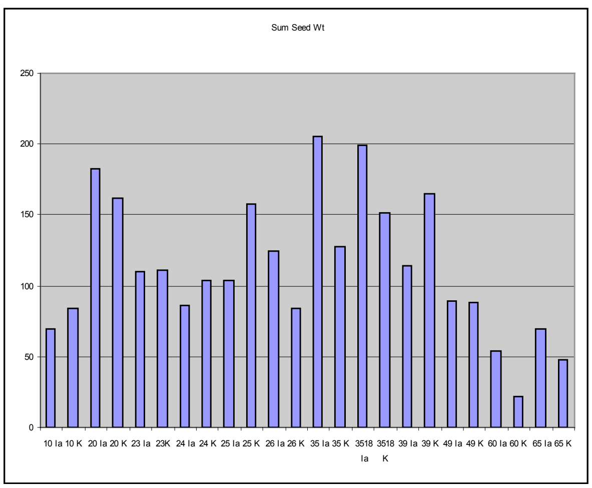

Figure 1. Total seed weight for genotypes

In Figure 2 below, you see the initial data that was collected from the seedlings. Genotype 25 and 39 had the most twins. Many of the genotypes (65, Clarke K, Luna K, Manska K) did not have any twins. So, there was variability between the twins in a genotype, but no significant values were indicated, because the numbers are so low.

There were no statistically significant differences in the proportion of twins among the 12 genotypes or between the two geographic sites.

The genotypes of the intermediate wheatgrass plants does not appear to be a significant factor in the probability of producing twins for these particular genotypes studied. The twin seedlings were very fragile, and it was difficult to keep them alive for the duration of the study. Many of the twins died before it could be determined if they were haploid or dihaploid.

Figure 2. Total number of twins/genotype.

Six living twin sets were taken to The Land Institute in December from McPherson College at the end of the study. As of April, only four of the twin sets have survived. All of the twin sets from Dordt College died, probably from too much water. The Kansas twin plants will be allowed to grow to maturity. The twin plants that appear to be sterile will be assumed to be haploids. Dr.Lee DeHaan at The Land Institute has confirmed one known haploid at this time. The final results of this study are not known at this time.

DISCUSSION

The frequency of twins in intermediate wheatgrass is very low. In a twin study by Dewey, he found 0.00035-2.0% twins in various varieties of crested wheatgrass (Dewey 1964). Out of 118,000 total seedlings of intermediate wheatgrass genotypes germinated at both McPherson College (27 twins) and Dordt College (31 twins), there were 58 twins total, or 0.00049%. There was not a significant difference between the proportion of twins at the Kansas or Iowa locations of intermediate wheatgrass plots or germination sites. Of the 27 twin sets grown at McPherson College, only six twin sets were healthy enough to continue with the haploid tests. One problem was the lack of natural sunlight in the growing conditions (in November and December), and another was the length of the time for the study. If this study were to be replicated, it would be recommended that the wheatgrass plants should have access to bright sunlight (summer conditions) or utilize a sunlamp in a greenhouse.

The four sets of haploid twins are being grown at The Land Institute to maturity. They were in vernalization for several weeks, and when they flower and grow seed heads in a few months, we will know if they are haploid. If they are not haploid, then they will produce pollen like normal wheatgrass plants. If they are haploid, then the sterile heads (with no pollen) will indicate they are haploid, and if they have pollen in the seed heads, then they would be normal twins. (DeHaan personal comment) The twins produced at The Land Institute had inconclusive molecular markers.

If any of the twins are haploid, it will provide scientists at The Land Institute with possibilities of further haploid breeding studies. The haploid plants would need to be treated with colchicine and then self-pollinated to produce viable seeds. The haploid plants can be used for plant breeding that may offer clues for breeding other species of perennial plants, and it could save time and money for future research.

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