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The Genetic versus Environmental Effects on Seed Yield Traits in Intermediate Wheatgrass (*Thinopyrum intermedium*)

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ABSTRACT

Intermediate wheatgrass (IWG) is a perennial grain crop that has been genetically altered in order to produce a higher sustainable yield. In the past decades, there has been a rising interest in perennial crop as a replacement for annual crops. Currently, all commonly grown grain crops are annuals; however, annual crops have contributed negative impacts on the environment such as soil erosion, nutrient run-off and contamination of waters by pesticides. Widespread planting of perennial crops has the potential to alleviate the pressures imposed on the environment from annual cropping systems, however, seed yield for IWG is not currently competitive. There is minimal research regarding the sustainability and environmental effectiveness of IWG, therefore, this study examines two components contributing to increased yield and success of IWG: desirable genetic traits and the effects of differing environments on these traits. In this study, 70 different cultivars of IWG were planted. Three plots were used, so each cultivar was represented three times resulting in a total of 210 IWG plants. Seed yield was measured by weighing and counting the seeds associated with each plant. Upon obtaining seed yield, a two-way analysis of variance (ANOVA) was run determining if variation among environments and genotypes was due to random sampling. The results showed that the variation among plots was too low to rule out random sampling variability. The variation among genotypes, however, proved to be significant. In summation, it was shown that environment did not play a role in seed yield, and that genotypes did have a significant role in seed yield for this study. A multiple comparisons test was run, and it found that one cultivar, #51, outperformed the others in terms of seed yield.

Keywords: *agricultural ecology, annuals (crops), environment, gene selection, intermediate wheatgrass, perennials (crops), plant breeding, soil conservation, sustainable agriculture.*

INTRODUCTION

In October 2011 the world's population reached 7 billion. Global population is growing, demand for food is increasing (Glover et al., 2010) and, even more striking, agriculture has been identified as the greatest threat to biodiversity and ecosystem function of any human activity (Glover and Reganold, 2010). Due to factors such as climate change, rising energy costs, and land degradation, the number of "urgently hungry" people is at its highest level ever (Glover and Reganold, 2010). Gomiero, et al. (2011) report that by 2050 the world will need 70 to 100% more food, As of now, we are depleting our soil and natural resources so that by 2050, feeding and sustaining our society (of possibly 9 billion) may not be possible unless agriculture can be reshaped.

Currently, all commonly grown grain crops are annuals (DeHaan, 2010). Global food security depends on annual grains- cereals, oilseeds, and legumes- planted on almost 70% of croplands, which combined supply a similar portion of human calories (Glover et al., 2010). The large-scale production of grains required to meet human food needs inevitably results in soil erosion, nutrient loss, contamination of waters, and pesticide contamination (DeHaan, 2010). Biodiversity loss due to land use and emission of greenhouse gases from agricultural activities are also a cause for concern (Gomiero, et al., 2011).

Perennial grain crop development could expand options to ensure food and ecosystem security (Glover, 2010). Perennial crops will increase sustainability by reducing soil erosion below replacement levels, reducing nitrate loss to ground and surface waters by more than 90% and reducing herbicide contamination (DeHaan, 2010). Sustainability will also be reached with perennial crops by limiting pesticide use and increasing farmer incomes through a decrease in inputs. Greater soil carbon storage and reduced input requirements mean that perennials have the potential to mitigate global warming (Glover and Reganold, 2010). Also, increased use of perennials could slow, reverse, or prevent the increased planting of annuals on marginal lands, which now support more than half the world's population (Glover and Reganold, 2010).

With the assistance of Dr. Jonathan Frye and those at the Land Institute, I experimented with intermediate wheatgrass (IWG), which is a type of perennial grain crop. In fact, it has the potential to become the first widely grown perennial grain crop (DeHaan, 2010). Success in breeding, growing, processing, and marketing IWG will prove the concept of perennial grains. Intermediate wheatgrass can tolerate a wide range of conditions and is considered to be tolerant to drought, episodic flooding, acid soil, saline soil, fire, and cold (USDA-NRCS, 2004). The IWG plant is easy to establish because it has good germination and the seedlings are vigorous (USDA-NRCS, 2004). Among cool season grasses, IWG frequently has the highest yields (Sleugh et al., 2000; USDA-NRCS, 2004). One explanation for its productivity and stress-tolerance is its remarkable capacity for root growth (DeHaan, 2010). In addition, Cox, et al. (2005) found that IWG was highly resistant to three important diseases characterized in the Great Plains. It's resistance to multiple pathogens makes IWG important in disease management (Cox, et al., 2005).

There are number of short-term project outcomes including increased optimism for the potential of IWG as a crop in the food and agriculture community, and increased scientific research with IWG. There has been an increased interest in perennials the last two decades, specifically with IWG, however, there is a major lack of funding. Whelchel and Berman (2011) shared that because success of perennials is long term, there is limited resources to financially support research in this field. As a result, it is crucial for information regarding the impacts of IWG to be shared.

Over the past four years, the project coordinator, Dr. Lee DeHaan, and others have identified and studied three rare plant types: those with short stalks, some with tough, shatter-proof heads, and others with large seeds. The aim in the project is to combine these traits into a single individual to increase grain yield and to compare yields among different locations to determine environmental effects. I measured seed yield using the number and weights of the seeds.

MATERIALS AND METHODS

On April 27, 2011, in coordination with Dr. Lee DeHaan at the Land Institute as well as my partner, Brelynn Schloo and my advisor, Dr. Jonathan Frye, 210 ramets of IWG were planted just outside of Salina, Kansas. These 210 plants consisted of 70 different cultivated genotypes (cultivars) and each cultivar was planted three times. Coordinators at the Land Institute genetically determined each of the plants prior to planting.

Each plant was labeled with its cultivar number. Permanent markers were used on plastic tags, and then these tags were placed in each pot. Once labeled, one plant of each genotype was placed into three groups (A, B, and C). These groups were taken to the plot near the Land Institute designated for our use. The plot had been rotary-tilled, and 210 holes had been dug out. Each hole was spaced at an equal distance of approximately 1 meter from the next hole. We randomly placed the 70 plants designated for group A in the group A plot section, the group B plants in the group B plot section, and the same for group C. Record of the location of each plant was kept in our lab notebooks.

Throughout the summer of 2011, Brelynn and I visited the plot 1-2 times a week to remove weeds. This was vital to the survival of the plant. A component of the project outcome and success of IWG is to prove that it has the potential to lower pesticide use. As a result, pesticides were not used to control the weed population. It was possible for the weeds to overtake the IWG. In fact, this occurred in several plants. The weed's roots grew into the roots of the plants, so when pulling the weeds, there were times when the plant would also be uprooted.

Harvest took place on September 19, 2011 and threshing began thereafter. Harvesting consisted of Brelynn and I counting the number of stems, and once counted, the stems would be snipped from the plant using scissors. The stems from each plant were bundled together using masking tape. The masking tape was also used to label the stems according to their plant's cultivar number. Each cultivar number was labeled in our lab notebooks by corresponding location to the plant cultivar number previously recorded. At this point, we also recorded the number of stems associated with each plant.

Threshing involved removing all the seeds from the hulls of the stems. The Land Institute provided wooden boxes with a rough lining coating the inside/bottom portion of the box. The same lining was on a square, wooden block that was about the size of an average hand, making it easy to hold. Threshing was made possible by placing the stem on the bottom of the box and rubbing the block back and forth over the stem. Seeds were removed easily with this method and time was saved because we no longer needed to do this process by hand.

Seeds and hulls alike were then swept into small, manila envelopes and labeled according to their section and plant number (e.g. A65). On November 22, 2011, I visited the Land Institute and used their seed blower to separate the seeds from the chaff. The next step was to count and weigh the seeds. The seeds were weighed using scales provided by the Natural Science Department at McPherson College.

After all data collection was complete, weights were recorded and transferred to an Excel® spreadsheet and then to SigmaStat® to be analyzed. Variation between cultivars, as well as plots, was analyzed using a two-way analysis of variance and the Holm-Sidak method for multiple comparisons.

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RESULTS

Focusing on seed weight, variation among the 70 different cultivars was analyzed. There was a single site used, but this site was separated into three plots, so, in addition to analysis of cultivars, there was also an analysis of variation between the three plots.

A two-way analysis of variance (ANOVA) was run. The assumptions for ANOVA are that data are obtained in a randomized, unbiased manner and that the populations have equal variance and are normally distributed. The Normality and equal variance test both failed for my data set. The main reason for this is that we only have three sets of data, and in any statistical analysis, a data set of three will fail the Normality and equal variance test. However, the power of the two-way ANOVA was the greatest of statistical tests pertinent to my data. To be a significant power, a result of 0.8 is needed. Power is a measure of the probability that you'll find a difference if a difference really exists. The power of the performed test for genotypes was 0.923 indicating that there's a high likelihood that the test will find a real difference.

ANOVA uses an overall critical value, or α-value, of 0.050, meaning that P-values under 0.050 are considered statistically significant. My results showed that there was no statistically significant variation among the plots because the P-value was 0.625. This means that the difference in the mean values among the different plots is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in genotypes.

However, the variation in seed weights among the different genotypes was statistically significant because the P-value was 0.001, meaning the difference in mean values among genotypes is greater than would be expected by chance. Results are found in Table 1.

Table 1. Two Way Analysis of Variance of IWG

Source	DF	SS	MS	F	P
Plot	2	0.00743	0.00372	0.471	0.625
Genotype	66	0.976	0.0148	1.875	0.001
Residual	132	1.042	0.00789		
Total	200	2.026	0.0101		

In order to isolate which cultivars differed from the others, I ran a multiple comparison test. This was necessary because each and every plant cultivar number needed to be compared to one another. For this, the Holm-Sidak method was performed. A critical t-value of 4.500 was used to determine if the difference in seed weight among plant cultivars was statistically significant. Any value greater than the critical t-value was significant and any value less than the critical t-value was not significant.

The results showed that one cultivar in particular, #51, had seed weights that differed significantly from several other plants. Cultivar #51 had seed production that was favorable to the desired outcomes of this project. I am looking for plants with a high yield and that have the ability to grow in different environments.

DISCUSSION

The main objective of this study is to determine how genetics and environment affect the seed yield of intermediate wheatgrass. At this time, it is not commonly known that perennial grains have the potential to become a viable crop. There is minimal research that focuses on the long term productivity of perennial crops, especially IWG. As a result, it is difficult to compare my results to previous investigations.

At the beginning of our project, there were three schools, each from different states, who would also be performing the same study. The plan was to compare our results to theirs. This would determine environmental effects on the IWG. However, we have no data to compare among states.

As a result, we ran tests comparing the seed yield among the three plots at our own site. The issue with this is that the plots are next to each other, so as expected, there was no significant difference in seed yield of these cultivars from plot to plot.

The cultivars, as mentioned previously, have been genetically determined, therefore, each one has different characteristics, or genes. Researchers in the field of perennial crops are looking for cultivars that can grow well in a variety of environments, or in other words, with desirable traits.

My results showed that, in this central Kansas environment, there is a significant difference in genotypes meaning that some cultivars are genetically more desirable than others because they produced the highest seed yield. When the multiple comparisons test was run, cultivar #51 was seen to have significantly higher yields than the other plants.

It is important to note here that other plants also had high seed yields, the trouble with the data set, however, is that if plants of a cultivar did not survive in all three plots, then the data for this cultivar was not properly represented. To clarify, if cultivar #1 had results from plots A and B, but no results from plot C, then it did not receive proper representation in the data. A possible reason as to why this happened is our own error, for example, when weeding, some plants were uprooted and had to be transplanted.

In the future, comparisons could be made among various environments. Samples from different environments allow for a greater understanding of how to improve growth and production of IWG. It is important to test various genotypes in these different environments. By doing so, it will be possible to observe which genotypes are more favorable in certain environments.

The genetic aspect of IWG is equally important. Continued research of the genetic material that makes up IWG could be an invaluable source of information for wheat improvement. Further identification of IWG's genomic constitution is still being unraveled. In a recent study, IWG was shown to have higher genomic heterogeneity than was previously assumed (Mahelka, et al., 2011). This is an important component for wheat breeders because it shows the gene pool for IWG is larger than expected. Mahelka, et al. (2011) researched within a small geographic region and stressed the importance of environment affecting genes. This confirms that genes, as well as environment, play an important role in seed yield.

At this point, IWG has the potential to become a viable crop for our agricultural community, and with all this knowledge taken into consideration, it will be possible to gain a higher yield for IWG overall. With a higher yield, it's possible that IWG can begin replacing annual crops. If this is accomplished then ultimately, we are conserving the very soil that sustains us.

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LITERATURE CITED

Cox, CM, KA Garrett, TS Cox, WW Bockus, and T Peters. 2005. Reaction of perennial grain accessions to four major cereal pathogens of the Great Plains. Plant Disease 89:1235-1240.

DeHaan, LR, 2010 Participatory plant breeding and agroecology to develop intermediate wheatgrass for sustainable grain production. Research Proposal to North Central Region-Sustainable Agriculture Research and Education (NCR-SARE).

Glover, JD and JP Reganold. 2010. Perennial grains: food security for the future. Issues in Science and Technology 26:41-47.

Glover, JD, JP Reganold, L Bell, J Borevitz, EC Brummer, ES Buckler, CM Cox, TS Cox, TE Crews, and et al. 2010. Increased food and ecosystem security via perennial grains. Science 328:1638-1639.

Gomiero, T, D Pimentel, and MG Paoletti. 2011. Is there a need for a more sustainable agriculture? Critical Reviews in Plant Sciences 30:6-23.

Mahelka, V, D Kopecky, and L Pastova. 2011. On the genome constitution and evolution of intermediate wheatgrass (*Thinopyrum intermedium*: Poaceae, Triticeae). BMC Evolutionary Biology 11:127-143.

Sleugh, B, KJ Moore, JR George, and EC Brummer. 2000. Binary legume-grass mixtures improve forage yield, quality and seasonal distribution. Agron. J. 92:24-29.

USDA-NRCS. 2004. Plant guide for intermediate wheatgrass, *Thinopyrum intermedium* (Host) Barkworth & DR Dewey. The PLANTS Database, version 3.5. National Plants Data Center, Baton Rouge.

Whelchel, S and EP Berman. 2011. Paying for perennialism: a quest for food and funding. Issues in Science & Technology 28:63-76.