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The Effects of Genotypic and Environmental Differences on Germination and Seed Yield in *Thinopyrum intermedium*

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ABSTRACT

Intermediate wheatgrass, *Thinopyrum intermedium,* commonly known as Kernza, is a perennial cool seasoned grass naturally found in the Kansas prairie. In recent decades there has been rising interest in the development of perennial crops to replace annual agriculture. Implementation of a perennial agriculture is thought to reduce soil erosion, nutrient run-off and contamination of waters by pesticides. One potential replacement of annual grain crops is intermediate wheatgrass. Due to a selective breeding program of the Land Institute located in Salina, Kansas, intermediate wheatgrass continues to have higher annual yields. This study looks at the effect of genotypic and environmental differences on the germination and seed yield of intermediate wheatgrass. Three repetitions of 70 different cultivars of Kernza were planted, totaling 210 Kernza plants. Data on plant height and yield were collected for the 2011 and 2012 growing seasons. Additionally, a germination study was conducted looking at the relative growth rate of 21 of the original 70 genotypes. Results of a two-way ANOVA showed that genotypic ($p=<.001$) as opposed to environmental difference ($p=.488$) had a greater effect on yield and seed mass. Specific genotypes produced higher yields in both years. Further linear regressions proved the relationship between seed mass and seedling size and growth rate to be statistically significant.

Keywords: *agricultural ecology, annual (crops), environment, genotypic selection, germination, intermediate wheatgrass, perennial (crops), plant breeding, relative growth rate, sustainable agriculture.*

INTRODUCTION

Annual grain crops—requiring planting each year—are the primary agricultural source of a large and essential portion of the human diet (DeHaan, 2010). These crops have negative consequences including soil erosion, nutrient loss and subsequent contamination of water, including pesticide contamination (DeHaan, 2010). Alternatively perennial grains have shown greater resilience to pests and drought, and help to maintain soil quality (Glover et al. 2007). One of the most promising of these grains is Intermediate Wheat Grass (IWG), trademarked as Kernza by The Land Institute of Salina, Kansas: a leading research organization not only in Kernza, but other perennial crops.

Intermediate Wheat Grass, *Thinopyrum intermedium*, is a cool-season grass that has been shown to survive in a variety of environments across the United States (USDA-NRCS, 2004). It has been found to be most fertile on moist, but well drained soil, but is also resilient to drought, episodic flooding, and poorer soil conditions without becoming weedy or invasive (USDA-NRCS, 2004). Its productivity and resilience has been credited to its remarkable capability of root growth (DeHaan, 2010). This deep root system sustains Kernza for several years, eliminating the need for annual tilling.

For my project, I worked with the Land Institute on their development of Kernza. Building on the work of two other McPherson College students, I focused on genotypic differences in three characteristics, plant height, seed mass, and overall yield, which have been identified as important traits (DeHaan, 2010). Additionally, we conducted a germination experiment examining two important determinants of seedling growth, seed mass and relative growth rate (Castro et al., 2008), to better understand the relationship between the mother plant and seedlings. The outcome of this project lead to increased knowledge regarding how to grow and breed IWG, expansion of the potential for IWG to be used as an agricultural crop, and increased scientific research on IWG in the central United States (DeHaan, 2010). My project was an extension of previous years' breeding of individuals with traits of large seed, short stalks and shatter resistance, as these characteristics are ideally combined into a single individual. Accomplishing this will dramatically increase seed-yield (DeHaan, 2010).

MATERIALS AND METHODS

The goal of my project is to determine the effect of different genotypes of Intermediate Wheat Grass (IWG) on three characteristics; plant height, seed mass and overall seed yield. The Intermediate Wheat Grass studied in my project was grown in a plot at the Land Institute in Salina, Kansas. The IWG was planted in the spring of 2011 for research conducted by two McPherson College students, Savannah Sievers (Sievers, 2012) and Brelynn Schoo, under project coordination at the Land Institute by Dr. Lee DeHaan. In addition, a germination experiment was

conducted examining the relationship between seed mass, relative growth rate, and characteristics of the mother plant.

As part of the Land Institute's research, two other plots were planted at Dordt College in northern Iowa, and at Prescott University in Arizona, with the intent that data would be shared with participating researchers for conclusions to be made about the effects of environmental conditions on the three characteristics of significance. The plot was planted with three plants of 70 different genotypes, totaling 210 IWG specimens per site. The plot in Salina was divided into three sections. One of each of the 70 different genotypes was planted in each of these three sections. These were planted randomly, to eliminate the confounding variable that a particular genotype might affect a neighboring plant. These plants are identified with a numerical genotypic indicator.

Given that IWG is perennial, no replanting was conducted between the 2011 and 2012 harvest. However, my research partner, Torey Fry and I weeded the plot weekly through the late Spring and Summer until harvesting the plants on the 22nd of July, 2012.

At the time of harvest, plant height was measured using a tape measure at the site. Staying consistent with the previous year's method, height was measured from the base of the plant to the tip of the tallest head. Following the measurement of plant height, up to 30 heads were collected from each plant. Samples were then placed in individual bags and labeled by genotype and repetition. While harvesting, we also recorded missing plants resulting both from a plant's inability to survive environmental conditions and some lost due to human error. In total we collected 170 different samples from the three plots, with at least one sample of each of the 70 genotypes present. Samples were then transported from the field to facilities at the Land Institute where they were placed for several weeks in a humidity-controlled room to dry.

Once the samples were amply dry, each sample was mechanically threshed. Threshed samples were then sifted to remove the larger amounts of unwanted stalk, already loose hulls, and weeds. After this preliminary cleaning, each sample was put through a SeedTech blower. Here, airflow is calibrated such that seeds fall into a canister and empty hulls float upwards, separating seed from unwanted biomass. Following this process, the whole hulled sample was weighed and recorded.

After all 170 samples had been threshed, a sub-sample of each sample was de-hulled. A three to four gram sample was weighed and then run through a de-hulling machine for 20 seconds. De-hulling for this duration removes most of the hulls without damaging the seeds. Given that samples were small, beads used for stuffing dolls were run through the machine with the samples. Then, samples were again sifted to separate de-hulled seed from the filler material, and again cleaned by being run through the SeedTech blower. At this point, with the samples processed, seeds were transported to McPherson College for further analysis.

In preparation for the germination component of our experiment; the average seed mass of each genotype was calculated. To do this, 20 seeds of each genotype were randomly selected and weighed and then divided by 20 and recorded. This information was then sent to our project coordinator, Dr. Lee DeHaan of the Land Institute. He analyzed the data and selected the two repetitions of 21 genotypes, totaling 42 samples used in the germination experiment.

To ensure undamaged seeds, 20 seeds of each of the 42 samples were de-hulled by hand. Each seed was weighed using an electronic balance with the weights recorded in conjunction with a seed number. Seeds from each genotype and repetition were germinated in their own petri dish. Petri dishes were lined with 90mm Whatman number 1 filter paper. Each filter paper circle was gridded with 1cm x1cm squares in a 5 x 4 grid. Each square was labeled 1-20 with the respective seed placed in each square. Filter paper was then moistened with 2mL of deionized water using pipets. The 42 petri dishes were then placed in a dark incubator at 4°C for five days to simulate winter and break dormancy. A 2mL aliquot of deionized water was added daily to the dishes. After a few days mold became present on some seeds. To combat the mold, seeds were treated with Fertilome brand "Broad Spectrum Liquid Fungicide." The active ingredient is Chlorothalonil (tetrachloroisophthalonitrite), which was at 12.5% concentration in the product as purchased. The concentrate was then diluted according to the manufacturer's recommendation at 3oz per gallon and was applied at 100microliters per seed using another auto pipette. The fungicide was only applied to those seeds with visible fungal growth. Following the five days of cold treatment, trays were removed from the incubator and placed at room temperature for eight hours per day (ie 16 hours at 4°C in the dark and 8 hours at 20°C in the light). After a few days, noticeable differences in germination became apparent, so some trays were left inside the cold treatment to slow germination to standardize germination of seeds at the time of planting.

After ten days of seed germination in the petri dishes, ten seeds of close to uniform germination of each genotype and repetition were selected and planted. Seeds were planted in cone-tainers previously labeled by genotype and repetition. These were filled 1cm below the lip with Turface MVP, an artificial growing medium that washes easily away from the plants' roots. The Turface was watered twice to saturation with a solution of MiracleGrow

prior to planting to ensure proper moisture and nutrient availability. Then 10 seeds of each genotype and repetition were placed on the surface of the Turface, each seed in its own container. As seeds were selected, cones were labeled with each seed's respective number. Seeds were then covered with Turface to fill the remaining 1cm of space and watered again with the MiracleGrow solution.

The cones were than randomized to prevent environmental advantage for certain genotypes. Plants received 18 hours of supplementary light at 450 µmol m^{-2} s^{-1} measured with LI-Model 189 PAR sensor light meter. Each cone was watered daily with a nutrient solution of Miracle Grow and water with one teaspoon of Miracle Grow per gallon of water. This is mixed according to the manufactures instructions for daily watering of indoor plants. Cones were watered for eight seconds with a mister. This duration of time saturated the Turface such that the water solution began to drain from the cones.

After the growing period, plants were removed from the cones. Both root and shoot length were measured and cut apart. These were then dried for each plant and the weight of both recorded to find the growth rate relative to the seed's mass.

RESULTS

Genotypic Variation in Yields

In 2011, all heads were harvested from each plant with every seed counted and weighed. The yields of each plant were then averaged by genotype to give an average genotypic yield. The top ten yielding genotypes and their yields are presented in Table 1.

Due to rapid plant growth between 2011 and 2012, only 30 heads were harvested from each plant. Given the need to preserve seed for the germination component of this experiment it was not possible to de-hull entire samples, due to potential damage of seed in the de-hulling machine. Thus, 2012 yields were estimated. This was done by de-hulling a sub-sample and finding the weight of the cleaned sub-sample. Hence, the weight of the threshed but not de-hulled sample was multiplied by the ratio of the weight of de-hulled sub-sample to the weight of the not de-hulled sub sample. Similar to the data from 2011 this estimated yield was averaged by genotype with the top ten yielding genotypes from 2012 also presented in Table 1. Genotype 51 had the highest genotypic yield in both the 2011 and 2012 growing seasons.

Figure 1 plots the weights of 40 seeds that were hand de-hulled for each of the 21 selected genotypes. Genotypes 10, 30, 65, and 3518 demonstrated highest average seed masses, indicative of producing bigger seeds than the other selected genotypes from the 2012-growing season.

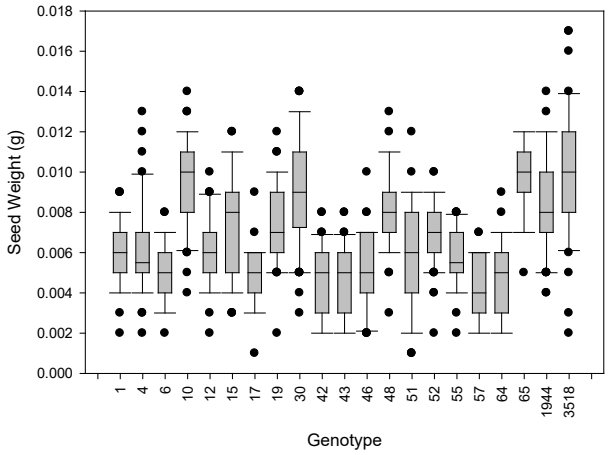

Figure 1. Box and whisker plot of the seed masses of the 21 selected genotypes from the 2012-growing season.

Relative Growth of Seedlings

Data were analyzed by linear regression and analysis of variance (ANOVA). Given the possibility of multiple variables (genotype and location) affecting the growth and yield of parent plants, two-way analysis of various was first run.

Table 1. Ten highest yielding genotypes for 2011 and 2012 growing season with seed yield weights (g) and number of heads harvested.

Genotype	# of Heads	Yield (g)/ Plant	Yield (g)/ Head
(a) 2011 Yields			
51	4	0.3528	0.0756
6	5	0.2806	0.0601
22	10	0.2180	0.0335
20	13	0.2169	0.0210
64	6	0.2047	0.0439
19	5	0.1695	0.0261
50	3	0.1644	0.0493
61	1	0.1487	0.1487
31	5	0.1485	0.0343
25	5	0.1469	0.0420
(b) 2012 Yields			
51	30	20.3702	0.6790
48	30	19.0802	0.6360
16	30	18.2298	0.6077
39	30	15.7139	0.5238
493	30	15.2967	0.5099
899	30	12.6545	0.4218
1530	30	12.4919	0.4164
20	30	12.2818	0.4094
64	30	11.9685	0.3990
4	30	11.3511	0.3784

The difference in the mean values among the different levels of genotype is greater was greater than would be expected by chance after allowing for the difference in repetition with p= <.001. The difference in the mean values among different levels of repetition was not statistically significant with p=.488. Because of this, linear regressions were performed as one data set with both repetitions of each genotype (both 1A and 1B). Regressions comparing (a) shoot length, (b) shoot weight, (c) root weight and (d) root length to seed mass were completed for all 21 genotypes. The results of those that were statistically significant are presented in the Table 2.

Table 2. Summary of the slopes, r^2 and P values for the linear regressions of: (a) shoot length vs. seed mass, (b) shoot weight vs. seed mass and (c) root weight vs. seed mass for differing genotypes of *Thinopyrum intermedium.*

Genotype	Slope	r^2	p
(a) Shoot length (cm) vs. seed mass (mg)			
12	1.029	0.249	0.030
6	1.469	0.474	< .001
4	1.021	0.405	0.003
(b) Shoot weight (g) vs. seed mass (g)			
57	3.120	0.534	< .001
55	3.423	0.222	0.036
48	1.551	0.223	0.048
43	3.491	0.243	0.032
17	5.217	0.449	0.002
6	3.672	0.487	< .001
4	3.908	0.504	< .001
(c) Root weight (g) vs. seed mass (g)			
57	1.912	0.330	0.013
55	2.376	0.200	0.048
46	2.884	0.256	0.027
17	2.131	0.367	0.013
6	1.931	0.298	0.013
4	1.865	0.300	0.015

Additionally, linear regressions were performed for all seed masses relative to all shoot lengths (Figure 2), shoot weights (Figure 3), root weights (Figure 4) and root lengths (Figure 5) as opposed to examining these relationships in a single genotype. With the exception of root length, all proved statistically significant.

A linear regression of seedling mass as a function of seed mass was performed and proved statistically significant with the slope=2.874, r^2=0.134, and P <.001. Thus, a correlation is seen between seed and seedling size. Additionally, a linear regression was performed of seedling mass as a function of the seed to seedling ratio. This proved to be statistically significant with a slope=.022, r^2= .0415, and P <.001, demonstrating a strong positive relationship between seed mass and the rate of seedling growth.

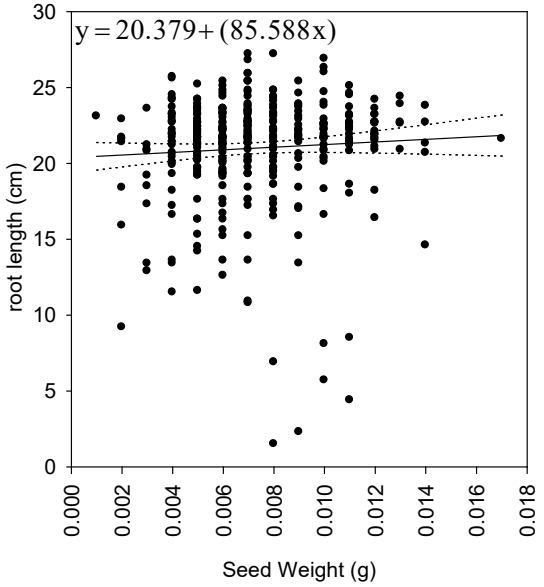

Figure 2. Graph of linear regression of seed mass to root length for all 21 genotypes. r^2=0.00415 and P=0.209.

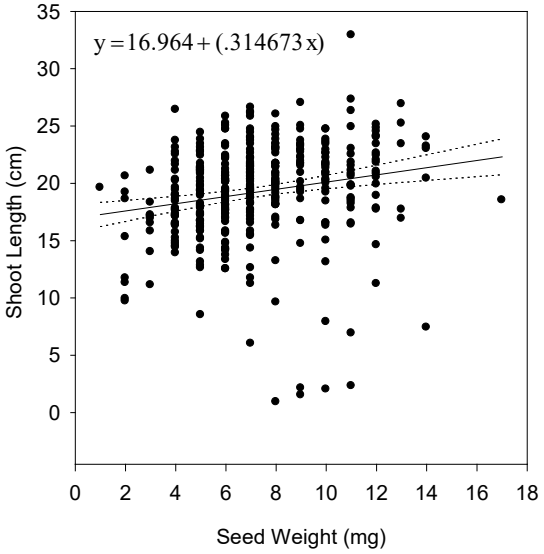

Figure 3. Graph of linear regression of seed mass to shoot length for all 21 genotypes. r^2=0.0402 and P=<0.001.

DISCUSSION

The results of this study provide two important points of discussion. First, results give important information on the relationship between environmental and genotypic contributions to yield as well as providing data on the changes in yield of different genotypes between multiple growing seasons. Results suggest

success in recurrent selection programs for improved

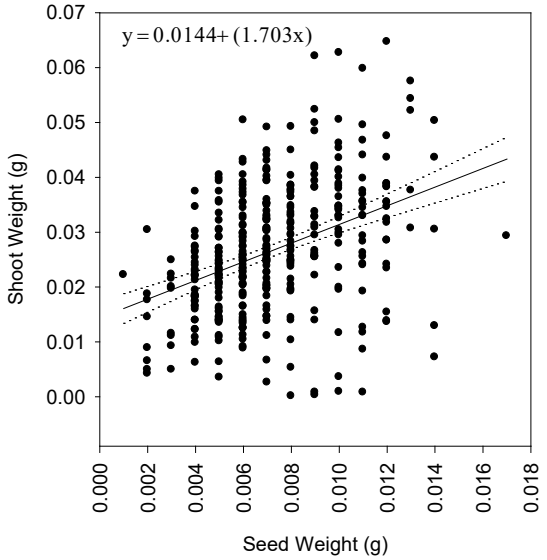

Figure 4. Graph of linear regression of seed mass to shoot weight for all 21 genotypes. r^2=0.158 and P=<0.001.

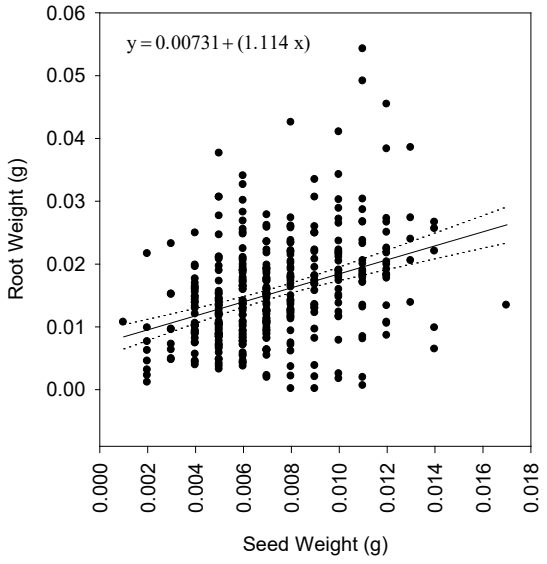

Figure 5. Graph of linear regression of seed mass to root weight for all 21 genotypes. r^2=0.136 and P=<0.001.

kernel size, yield per unit area and threshability of intermediate wheatgrass (DeHann, 2005). Identification of multiple genotypes that recurrently generated higher yields is an important step in the genetic narrowing of existing cultivars and the future breeding of intermediate wheatgrass as a grain crop (Cox et al., 2006).

Results form this study showed that two genotypes 51 and 64, both had average genotypic yields within the top ten yielding genotypes in both the 2011 and 2012 growing seasons. Genotype 51 was the top yielding genotype in both 2011 and 2012. A high yield can be obtained by a plant either producing either a great number of seeds, or in producing larger seeds. This difference can be seen in comparing results from Figure 1 and Table 1. The genotypes with the highest averaged seed masses (10, 30, 65, 3518) suggesting bigger seed size were not the genotypes that had the greatest yield. This is an important distinction when considering selection for plant breeding. A possible future step would be to breed genotypes such as 51 that have demonstrated high yields, with the genotypes identified in having larger seeds, to further increase yield in future years.

Second, information regarding the relationship between seed mass and relative growth rate within a species—a relationship that has been examined in relatively few studies—has typically demonstrated a weak relationship despite a positive correlation between seed and seedling masses (Castro, 2010). This study provides another examination of this relationship and confirms the importance of seed mass in seedling and plant growth. Given that certain genotypes produce larger seeds and seed mass was linked to seedling size and growth rate, this study affirms the importance of genetic selection in striving for higher yielding perennial grain crops as thought by previous plant breeders. Clearly larger seed mass is a characteristic to strive for in further plant breeding.

This study found a positive relationship between seed mass, shoot length, shoot weight, root length and root weight. All proved statistically significant with the exception of root length. However, seed mass had the strongest relationship in increasing shoot weight with r^2=0.158. The next strongest relationship was with root weight with r^2=0.136.

Originally, this study sought to examine the performance of various genotypes in two additional locations, Prescott College in Arizona and Dordt College in Iowa, in addition to the Salina site. Due either to death of plants (Prescott College) or lack of access to threshing and de-hulling equipment (Dordt College) no comparison was made between locations. Though the germination experiment was not completed with seed obtained from cultivars at the Iowa site, there is still opportunity for this seed to be used to replicate this study, as well as for yield data to be calculated in regards to cultivars at Dordt College. Collection and analysis of this data could provide important insights on the influence of environmental differences and conditions on the performance of various genotypes. This is a logical next step in expanding this study.

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