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Genetic and Environmental Variation in the Growth and Germination of Intermediate Wheatgrass [*Thinopyrum intermedium*]

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ABSTRACT

The use of annual wheat crops has increased the amount of soil degradation, erosion, and nutrient availability. The Land Institute has been working on a perennial grain, Intermediate Wheatgrass *[Thinopyrum intermedium]* that would drastically reduce soil erosion, as well as be able to acquire more nutrients due to a longer root system. In a plot in Salina, KS, we looked at three different repetitions of 70 different varieties of Intermediate Wheatgrass. By looking at the yield of the 2011 harvest compared to the yield of the 2012 harvest, we found that all genetic varieties of Intermediate Wheatgrass have increased, with genotype 51 topping the highest yield per head for both years. We then took two repetitions of 21 selected genotypes and conducted a germination test to see if the mother plant had an effect on the seedling. We calculated the relative growth rate and found that the mothers' seed mass determined the seedling mass. This shows that the mothers' seed mass is related to the seedling mass.

Keywords: *Intermediate Wheatgrass, Thinopyrum intermedium, germination rate, yield, perennial grains, sustainable agriculture*

INTRODUCTION

Before the agricultural revolution and the use of annual crops, the Earth was filled with both annual and perennial plants. Over the past 150 years, our population has become increasingly reliant on annual grain crops, and the replanting and harvesting of these crops every year. Studies have shown that the expansion of these shallow-rooted annual crops has increased the amount of soil degradation around the world, as well as the inefficient use of the nutrients that the soil provides (Glover, 2003). There have been many attempts to decrease soil erosion. One would be "no-till" farming, which reduces soil loss, but also involves the use of chemicals to manage pests and weeds. These chemicals ultimately leach down into our groundwater. Growing organically tries to control the pests with non-chemical means, but sadly does nothing to halt soil erosion. The best solution that scientists can find is to use wild perennial plants and genetically alter them with annual grain crops to produce a perennial grain.

An experiment carried out over 100 years shows that the cultivation of annual crops has caused 50 times more soil erosion than perennial crops (Gantzer, 1990). Perennial crops do not need to be replanted each year, thus growing a larger root system. This root system is able to obtain more water and nutrients compared to annuals (Cox, 2010). Perennials also do not need any additional chemicals or additives to enhance the product. These perennials have been bred with their annual counterpart in order to produce a grain yield that can contend with the annuals' already high yield. The Land Institute in Salina, KS, is conducting research in domesticating intermediate wheatgrass [*Thinopyrum intermedium*] (trademarked Kernza) (Cox, 2010). By breeding the deep root system of a perennial grass into an annual grain crop, they are able to create a grain that will be sustainable for use in very diverse regions, such as the Pacific Northwest, where rolling hills are highly susceptible for soil erosion (Scheinost, 2001, DeHaan, 2010).

In this research, we will be looking at 70 different genetic variations of intermediate wheatgrass *[Thinopyrum intermedium]* and their distinct features in different environments. We will be conducting this research at the Land Institute in Salina, KS. We are hoping to indentify which of the 70 varieties of intermediate wheatgrass will produce the most grain yield, as well as grain size and mass in each environment. In addition to the initial yield and size of the Kernza, we conducted a germination of the daughter plants to see the relationship between seed mass and growth allocation of the Kernza.

Seed mass and relative growth rate are important factors of the growth of seedlings (Castro, 2008). In a study analyzing a family of Scots pine trees, researchers found that maternal plants were strongly correlated to seed mass (Castro, 2008). It was also found that seedling mass is also strongly related to seed mass and found that there was little relationship between seed mass and relative growth rate. These are important factors in determining the success of the Kernza seeds. Further study is needed in order to understand how Kernza seedlings allocate their seed mass.

MATERIALS AND METHODS

A plot has been set aside at the Land Institute in Salina, KS in order to conduct this research. In that plot, there are three plantings of 70 different genotypes, 210 in total. These crops were planted in the spring of 2010.The plot was randomized into a split plot design. This will ensure that there will not be two of the same genotype planted next to each other. After the 2011 season, the previous researchers found that there was not a 100 percent survival rate of the 210 plants. Over the summer, the plot was weeded, but not fertilized or watered.

My research partner, Emily James and I weeded weekly throughout the summer. Harvest took place July 22, 2012. We started by measuring the height of each plant to the tallest stalk. Afterwards, we collected up to 30 heads from each plant. The stalks were bagged in long, narrow paper bags and put in a humidity controlled room. Our next step was to thresh and sift through the seed. From there, we dehulled a three gram subsample of the seeds for 20 seconds at 110 PSI using the SeedTech seed blower.

After dehulling, we counted and weighed 20 seeds to the 0.001g. These results were then entered into an Excel spreadsheet and sent to Lee DeHaan at the Land Institute. DeHaan selected 21 genotypes based on the seed mass in order to get a wide range and even distribution. He then chose two repetitions of each genotype, so that 42 plants total would be candidates for the germination project.

For the germination project, we hand dehulled and individually weighed 20 seeds from each plant. We then lined sterilized Petri dishes with 20 gridded 1 cm x 1 cm squares of 90mm Whatman filter paper. The filter paper was wetted with 2mL of dionized water using an auto pipettor. We then placed one seed in each square and placed the Petri dish in an incubator set at four degrees Celsius for five days straight. We moistened them daily with 2mL of dionized water. Fertilome Liquid Fungicide, prepared according to the manufacturer's directions, was applied at 0.1 mL using an auto pipettor to seeds that had visible fungal growth.

After five days in the incubator, we allowed the Petri dishes to be left out for eight hours a day. It took five more days, ten days total, until we observed that the seeds had germinated and were ready to be planted. Of the 20 seeds from each plant, we chose ten seeds that were similarly germinated to be planted. We used Ray Leach Single Cell Cone-tainer ™, product SC 10 super cone-tainers, filled with Turface Atheletics™ MVP Calcined Clay and moistened before planting the seeds. We labeled the cone-tainers with the genotype, repetition, and seed number. Turface MVP was chosen so that the soil would not stick to the seed. We then randomized the cone-tainers so that there would be no environmental advantage.

We planted the seeds 1cm deep in the cone-tainers and watered the seeds. We used two GE Lucalox® High Pressure Sodium ED18 light bulbs. Measured at soil level, the light produced 550-750 umol s^{-1} m^{-2} amount of light during the day and 400-450 umol s^{-1} m^{-2} of light for the plants. To achieve this amount of light, we hung the lights 70 cm from the soil level and the two lights were 80 cm apart. The plants were placed in a greenhouse and were lit for 18 hours with the GE Lucalox® High Pressure Sodium ED18 lights. The plants are watered everyday with a mister, enough so that water starts to drip out of the cone-tainer, about 8-10 seconds. We used Miracle Grow as instructed on the container.

We recorded when each leaf sprouted from the shoot. After 22 days, we took the plants out of their cone-tainers, separated the shoot and root, measured the length of the shoot and root, and put them in separate envelopes. These envelopes where then put in a drying oven set at 105 degrees Celsius for 3 days. After drying, we weighed the shoots and roots. We entered the data into an Excel spreadsheet. Data analysis was done using an analysis of variance (ANOVA) and linear regression tests.

RESULTS

First we determined the yield of the Intermediate Wheatgrass. Table 1 shows the differences between the 2011 and 2012 harvest seasons.

Table 1. Top ten average genotypic yield per plant and yield per head of the a) 2011 and b) 2012 harvest seasons.

Genotype	Av # Heads Harvested	Yield/Plant	Yield/Head
a) 2011 Harvest			
51	4.67	0.3529	0.0756
6	4.67	0.2806	0.0601
22	6.5	0.2180	0.0335
20	10.33	0.2169	0.0210
64	4.67	0.2047	0.0439
19	6.5	0.1695	0.0261
50	3.33	0.1644	0.0493
61	1	0.1487	0.1487
31	4.33	0.1485	0.0343
25	3.5	0.1469	0.0420
b) 2012 Harvest			
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

In order to determine if the repetition of the genotypes affected the growth and yield of the maternal plants, we used a two-way analysis of variance (ANOVA).

We found that the repetition of the genotypes was not significant (P=0.488), and therefore meaning that the environment had no effect on the growth and yield of the parent plant. We found that there was a statistically significant difference between the genotypes (P=<0.001). Since the repetition of the genotypes was not significant, we used a linear regression test to see if there was a correlation between seed mass and the following: shoot length, root length, root mass and shoot mass (Table 2, Figures 2-5). As shown in Table 2, we listed the results of the genotypes that showed statistically significant relationships with seed mass.

Table 2. A summary of the slopes, r^2, and P values of the linear regression tests comparing seed mass to: a) Shoot Length (cm), b) Shoot Mass (g), and c) Root Mass (g) of Intermediate Wheatgrass.

Genotype	Slope	r^2	P
a) *Shoot Length*			
4	1021.788	0.405	0.003
6	1469.214	0.474	<0.001
12	1029.950	0.249	0.030
b) *Shoot Mass*			
4	3.908	0.504	<0.001
6	3.672	0.487	<0.001
17	5.217	0.449	0.002
43	3.491	0.243	0.032
48	1.551	0.223	0.048
55	3.423	0.222	0.036
57	3.120	0.534	<0.001
c) *Root Mass*			
4	1.865	0.300	0.015
6	1.931	0.298	0.013
17	2.131	0.367	0.008
46	2.884	0.256	0.027
55	2.376	0.200	0.048
57	1.912	0.330	0.013

There was a statistically significant correlation between genotype and seed mass. There is also a strong relationship between seed size and the growth of the seedling, with a slope of 0.022, r2 value of 0.0415 and P<0.001.

DISCUSSION

When it comes to the yield of the Kernza grains, there was a significant increase in the yields from the 2011 to the 2012 harvest seasons for all the genotypes. It is important to note that genotype 51 was the highest yield per plant for both 2011 and 2012 harvests. This could push plant breeders to finally find a consistent plant that performs well in an environment similar to central Kansas. We also noted that there was a difference between the yield per plant and the yield per head in the 2011 harvest. Genotype 51 was the highest performing yield per plant. However, genotype 61 was considered the highest yield per head. This could be a potential error in the data collection of the 2011 harvest, as there was only one head harvested.

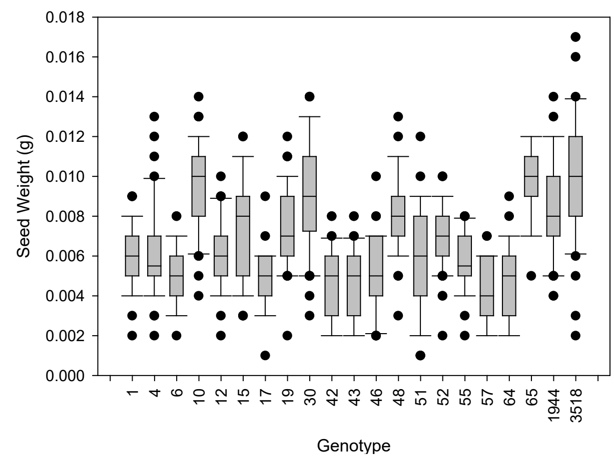

Figure 1. A box and whiskers plot to show the variation in average seed mass for the genotypes that were chosen for the germination experiment.

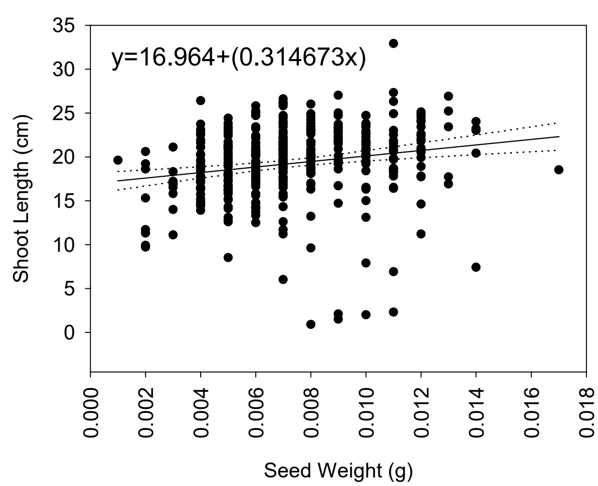

Figure 2. A linear regression between seed mass (g) and shoot length (cm) of all tested seedlings with a r^2=0.0402 and P=<0.001.

Additionally, there were only two genotypes that were repeated in the 2011 and 2012 harvests. Those genotypes, 64 and 51, are the only two that held high yields for the two consecutive years. One thing to note though is that both genotypes 51 and 64 were not high on the average seed mass. This could be because some genotypes put more emphasis on producing many heads and many seeds, while other genotypes put more energy on the mass of the seed.

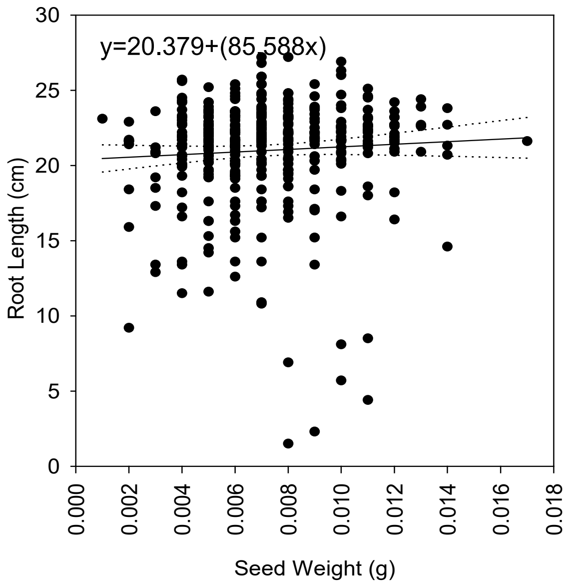

Figure 3. A linear regression between seed mass (g) and root length (cm) of all tested seedlings with a r^2=0.00415 and P=0.209.

We found that there was no effect of the environment on yield, therefore finding that there was no difference between the environment and the genotypes. So when we did the linear regressions, we included both repetitions in the tests. In Figure 1, you could see the variation in seed mass of the different genotypes. The three genotypes with the highest average seed mass were 10, 65 and 3518.

Results of the germination study are similar to the study done on Scots pine (Castro, 2008). Just like seedling mass was strongly determined by the maternal seed mass. This is an important finding that plant breeders can use to attempt to breed bigger and better-yielding plants. By looking back at Table 2, you can see the genotypes that have showed statistically significant relationships between the seed mass and shoot length, shoot mass and root mass. Genotypes 4 and 6 show the most significant relationships between all of the factors. It is important to note that genotype 4 was also on the top 10 yields for the 2012 harvest season.

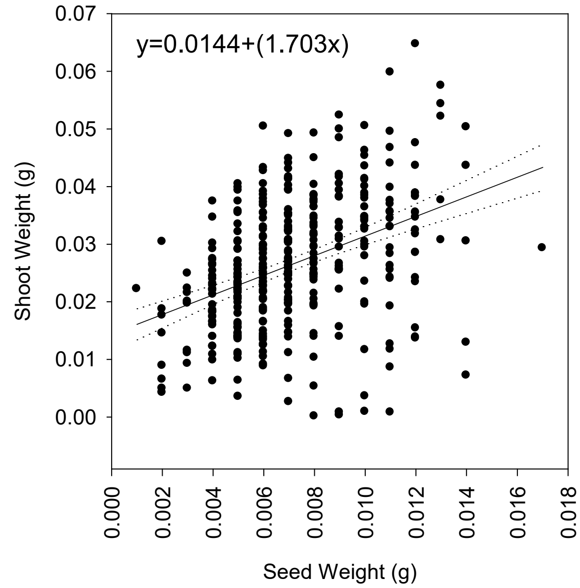

Figure 4. A linear regression between seed mass (g) and shoot mass (g) of all tested seedlings with a r^2=0.158 and P=<0.001.

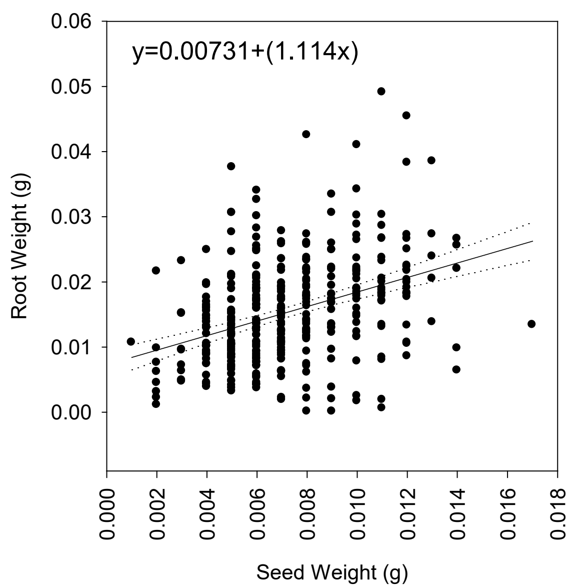

Figure 5. A linear regression of seed mass (g) and root mass (g) of all tested seedlings with a r^2=0.136 and P=<0.001.

There were significant relationships between seed mass and shoot length, shoot mass, and root mass. However, root length did not show a significant relationship with seed mass. This can show plant breeders that seed mass is a good determinant of shoot length, shoot mass and root mass, but not as strong of a correlation with root length. Seed mass has the strongest correlation with shoot mass (Figure 4). Plant breeders will be able to early determine the mass of the shoot simply based on the mass of the seed before it is planted. This will be an important finding for the future of Intermediate Wheatgrass.

What is surprising about this finding is that none of the three genotypes that had the highest average seed mass (genotypes 10, 65 and 3518) were statically significant between their seed mass and shoot length, root length or root mass. If you look back at Figure 1, there are some outliers than did not fit within the box. This shows that although the average seed mass was high, there was too much variation within the genotypes to produce a statically significant value.

Some further studies could be done to see if the yields continue to increase and to determine if there is a specific rate these yields are raising. It would also be interesting to see if there are any more differences between the top ten highest yields per head within the past three years. Another future study could include more on the seed mass in relation to yield as well as shoot and root mass.

In the original proposal for this project, we were to look at the yields of Intermediate Wheatgrass in three different environments; one being in Salina, KS, with the other two being in Arizona and Iowa. Due to loss of plant in Arizona and lack of proper equipment in Iowa, we were unable to receive completed data on those two environments. What would be interesting to study is if there is an environmental factor in the productivity of Intermediate Wheatgrass in different climate regions. This could give The Land Institute more insight on what genotypes are advantageous in different regions.

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