Investigating *Brachypodium distachyon* as a Model System for Plant Biofuels Research

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INTRODUCTION

Renewable energy research is an important economic investment for a country like the United States. While global peak oil production has not been reached yet, the difficulty of estimating remaining oil reserves leaves energy security intangible.[1] The transition to new energy sources as well as upgrading infrastructure for their use will take a long time and a concerted effort. [2] Many renewable energy sources and technologies that are currently being investigated look promising. Solar and wind sources can provide renewable, clean energy, and are abundant, but current technologies do not allow for efficient storage and transport of the energy captured. The conversion of plant biomass to ethanol is an alternative energy source that provides a form of energy more suitable for transport, storage, and use in our existing infrastructure. Among many available biomass sources, grasses are an attractive option. Due to high efficiency of light, water and nitrogen use, species like Miscanthus and switchgrass are promising bioenergy crops.[3] The carbon stored in the cellulose and hemicellulose molecules of grasses can be broken down to simple sugars and fermented to make ethanol. Current lignocellulosic biofuel production is not as efficient at producing fermentable sugars for ethanol as is using sugarcane or corn starch for biomass.[4] This highlights the importance of improving the use of cellulose and hemicellulose to produce ethanol. To facilitate research on grasses as feedstocks for biofuel production, plant model systems are employed. While models such as rice and Arabidopsis are well established, they are not as well suited for grass genomics as the model grass *Brachypodium distachyon* (Brachypodium).[5] With favorable traits such as a small genome, short life cycle, and a variety of diploid ecotypes, Brachypodium is a powerful tool for investigating how we can make grasses better biofuel crops. Although Brachypodium is already being used for such research, a great deal of information still needs to be gathered on this grass. Variation in biofuel-related traits between Brachypodium genotypes can be used to investigate biofuel production. The first part of this project aimed to evaluate how well several genotypes of Brachypodium undergo the plant tissue culture process and the efficiency with which they form compact embryogenic callus (CEC) and regenerate to healthy fertile plants. The second component of this project examined how cold treatment affect Brachypodium plant height and mass, days to spike emergence, and the amount of fermentable sugars released from the stems of the mature plants.

MATERIALS AND METHODS

Comparative Evaluation of Tissue Culture Regeneration by Different Brachypodium Genotypes

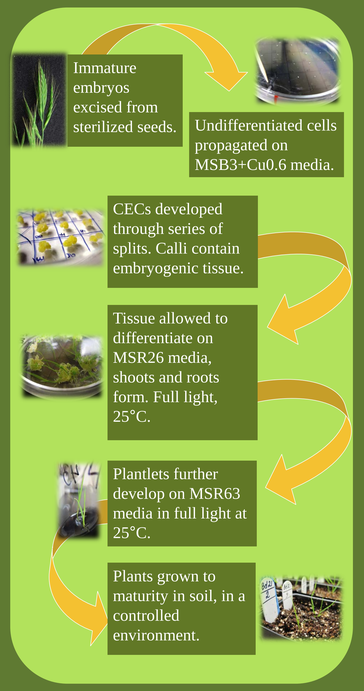

Figure 1. Overview of plant tissue culture method used.

Brachypodium Lines	Cold Treatment (4°C)	Growing Conditions
Bd 1-1	7 weeks	16 hour day length, 22°C
Bd 2-3	None	20 hour day length, 20°C
Bd 21	None	20 hour day length, 20°C
Bd 29-1	12 weeks	16 hour day length, 22°C
Bd 30-1	None	20 hour day length, 20°C

Table 1. Growth conditions for plants used to obtain embryos for tissue culture and regeneration.

• Plants were grown in a controlled environment growth chamber and watered with tap water, fertilized upon planting and as needed throughout growth.

• Lines investigated using plant tissue culture: Bd 1-1, Bd 2-3, Bd 29-1, and Bd 30-1. Bd 21 was used as an experimental control along with each genotype.

• Media and methods for plant tissue culture were followed as described previously (Alves, 2009)[6]

• 50 immature embryos were cultured from each genotype. While the CEC's were developing the tissue was transferred to fresh media twice, only a single piece of callus tissue forming from each original embryo was carried forward through the transfers. The third transfer was to regeneration media that stimulated differentiation of the CEC into plantlets.

• Regeneration media excluded selection drugs because the developing CEC's were not transformed.

Effect of Vernalization on Spike Emergence, Height, and Sugars Released From Brachypodium Genotypes

Vernalization (days)at 4°C	Bd 2-3	Bd 21	Bd 30-1
28	3 pots, 4 plants/pot	3 pots, 4 plants/pot	3 pots, 4 plants/pot
21	3 pots, 4 plants/pot	3 pots, 4 plants/pot	3 pots, 4 plants/pot
14	3 pots, 4 plants/pot	3 pots, 4 plants/pot	3 pots, 4 plants/pot
7	3 pots, 4 plants/pot	3 pots, 4 plants/pot	3 pots, 4 plants/pot
0	3 pots, 4 plants/pot	3 pots, 4 plants/pot	3 pots, 4 plants/pot

Table 2. Planting scheme for saccharification analysis. Vernalization was conducted in a deli cooler, and plants were then grown to maturity in a controlled environment growth chamber (20 hour day-length, 20°C) .

• Brachypodium genotypes were planted into SB500 soil medium that was saturated with fertilizer/water solution and vernalized at 4°C. Vernalization periods are listed in Table 2.

• Plants were checked for spike emergence daily, and watered with room temperature tap water.

• Once the plants had matured, each plant's height and mass recorded. For each of the four plants in a pot, the seeds were removed and stems of each individual plant were packed together, then all four packed plants were wrapped together for shipping.

• The stems were sent to the Sedbrook lab at the University of Illinois for saccharification analysis. Data from each treatment will be used to analyze the effect of vernalization on sugar release along with height , plant mass, and days to spike emergence of Brachypodium genotypes.

RESULTS

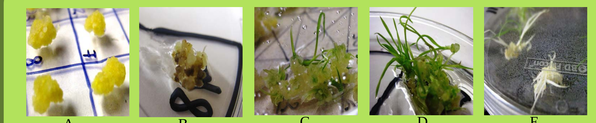

Figure 2. A) Compact embryogenic callus (CEC), B) Non-regenerating callus tissue, C) and D) Green shoots regenerating from callus tissue, E) Albino shoots regenerating from callus tissue.

Brachypodium distachyon Genotype (diploid)	Growth Habit*	% Embryos Forming Embryogenic Callus	% Embryos Forming Green Shoots Upon Regeneration	% Embryos Forming Albino Shoots Upon Regeneration	% Embryos Regenerating Into Healthy Fertile Plants[y]
Bd 1-1	Winter	48	40	0	---
Bd 2-3	Spring	86	64	0	---
Bd 29-1	Winter	22	22	0	---
Bd 30-1	Winter	44	32	4	---
Bd 21 (average of 3)	Spring	79	63	3.3	---

Table 3.*Winter growth habit genotypes require several weeks of cold temperature to induce flowering. [y] Data will be gathered once plants fully develop and produce seed.

Figure 3. A) Spike emerging on Bd 30-1 plant, B) Bd 21 plants showing height reduction with longer vernalization period, left to right 0, 7, 14, 21, 28 days.

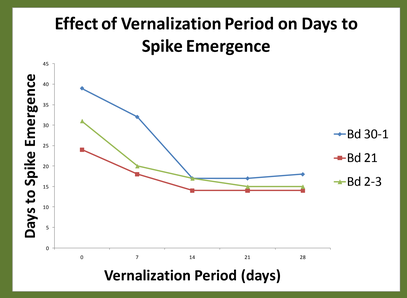

Figure 4. Comparison of days to spike emergence for three Brachypodium genotypes. Data from first replication of the experiment.

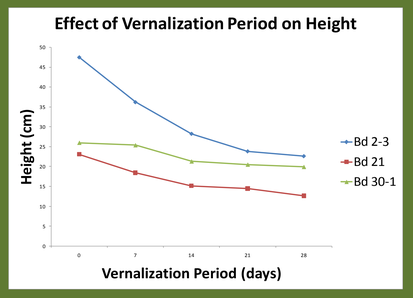

Figure 5. Comparison of plant height for three Brachypodium genotypes. Data from first replication of the experiment.

CONCLUSIONS TO DATE

Comparative Evaluation of Tissue Culture Regeneration by Different Brachypodium Genotypes

• The data collected from the first replication of plant tissue culture of different Brachypodium genotypes (Table 3) shows that immature embryos of lines Bd 1-1, Bd 2-3, Bd 29-1, and Bd 30-1 can all produce compact embryogenic callus and regenerate green shoots from callus tissue at different efficiencies.

• The data also suggests that spring habit genotypes like Bd 2-3 and Bd 21 form compact embryogenic callus (CEC) at higher frequencies than do winter types. The formation of CECs is essential for transformation with *Agrobacterium* and for regenerating transgenic plants.

• Winter habit genotypes regenerated a larger number of green shoots from each callus piece on MSR63 media than did spring types. Those green shoots were also larger in size, which is favorable because larger green shoots appear to have a better chance of growing into mature plants than do small green shoots.

• Not every genotype produced albino shoots upon regeneration. However, since the occurrence of albinos is infrequent, it is possible that the sample size was not large enough to see any albino development in some lines. When transplanted to soil medium, albino plantlets did not survive. Callus pieces that formed clear shoots did not develop well into plantlets. A final count of fertile healthy plants regenerating from green shoots is not yet available.

Effect of Vernalization on Spike Emergence, Height, and Sugars Released From Brachypodium Genotypes

• The relationship between vernalization period and days to spike emergence is evident in Figure 4, with dramatic acceleration in spike emergence seen between the zero and two week vernalization period. Plants with two or more weeks of vernalization time did not have any further significant reduction in days to spike emergence.

• Longer vernalization time reduced plant height as seen in Figure 5. This is supported by the data on spike emergence. As development is accelerated by vernalization, less time is available for plants to uptake nutrients and grow.

• Data showing how much fermentable sugars were released by the different Brachypodium genotypes is not yet available. This data will be used to determine whether there is a relationship between the rate of plant development and the amount of fermentable sugars that can be released from the stems.

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ACKNOWLEDGMENTS

I would like to thank my PI, David Garvin, for his help and insight on designing this project. I would also like to thank John Sedbrook for conducting the saccharification analysis of plant stems, and Zachary Blankenheim for assistance and instruction on tissue culture methods.