METHOD OF POLLINATION AND HERITABILITY FOR SEEDLING VIGOR IN SWITCHGRASS

A Dissertation

by

HECTOR RAMIREZ DE LEON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Plant Breeding

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ABSTRACT

Method of Pollination and Heritability for Seedling Vigor in Switchgrass.

(May 2005)

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Switchgrass (*Panicum virgatum* L.) is a warm-season perennial bunchgrass native to North America. In addition to its importance as a forage grass, it has promise as a biofuel crop. However, its use is limited because the grass is difficult to establish. Improving seedling vigor is one approach for improving establishment. The objectives of this study were to: 1) select for increased seedling mass through half-sib family selection; 2) calculate an estimate of heritability for seedling mass; and 3) determine the mode of pollination of switchgrass. One cycle of selection was completed using a half-sib methodology. Seedling mass was determined in a series of growth chamber studies. The seed was produced in different space planted field nurseries in the College Station, TX area. Mean seedling weight of the base population (C_0) was 0.014 gm seedling^{-1}, while the mean seedling weight from the C_1 cycle of selection was 0.029 gm seedling^{-1}. Unfortunately, bulked seed from the base population was old and did not germinate well. Therefore, a new base population was recreated, and the C_0 seedlings from this population were heavier than the C_1 seedlings, 0.020 and 0.016 gm seedling^{-1},

respectively. The calculated heritability estimate was $H^2 = 0.6$. Since the C_0 and C_1 nurseries were not grown on the same soil type, the lack of a positive response for seedling weight may be due to the different soil types. However, it may require another cycle of selection to determine if seedling mass can be positively impacted via half-sib selection. The mode of pollination of the species was determined by 1) observing pollen germination and tube growth in the pistils using fluorescent microscopy and 2) determining seed set with selfed plants. When self-pollinated, the pollen tubes never grew into the ovaries but when cross-pollinated the tubes readily grew to the micropyle. Also, when switchgrass plants were self-pollinated, viable seed were not produced. These findings indicate that switchgrass is highly self-sterile because a self-incompatibility mechanism prevents the pollen tubes from growing into the ovary of the same genotype.

DEDICATION

To God.

To my parents: Héctor Ramírez García and Guillermina de León de Ramírez. Thanks for teaching me how to fight in life and to never give up no matter how hard life may be. As long as we work hard, we will find a way to success. Thanks for giving me one of the most valuable treasures a man can own: Education.

To my brother: Cesáreo Ramírez de León. Thanks for sharing with me some of the happiest times in my life. You can always count on me.

To my sister: Mayela Guadalupe Ramírez de León. Thanks for all the trust you have in me. I love you with all my heart.

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TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is an important warm-season bunchgrass that is grown throughout most of the United States, Mexico and Central America (Hitchcock, 1951). Switchgrass is valuable for forage, and erosion control in the central and southern Great Plains. It is most abundant on relatively moist, fertile soils and is coarse-stemmed with a vigorous root system. Under favorable conditions, switchgrass has high seed and forage yield potential. The forage quality of switchgrass is acceptable during periods of rapid growth, but is relatively low if fed as a standing winter feed (Alderson and Sharp, 1994).

Within the species, two ecotypes have been identified (Hulquist et al., 1996, 1997). The lowland ecotypes are tetraploid ($2n=4x=36$) while the upland ecotypes are either tetraploids ($2n=4x=36$) or octaploids ($2n=8x=72$) (Hopkins et al., 1996; Hulquist et al., 1996). The upland and lowland types are adapted to northern and southern latitudes, respectively (Casler et al., 2004). Upland ecotypes have semi-decumbent stems, pubescence on the upper surface of the leaf blades, short rhizomes and grows up to 1.5 m high while lowland plants have coarse stems and erect, glabrous leaves, and grows up to 3 m tall (Porter, 1966; Barnett and Carver, 1967). Examples of upland

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This dissertation follows the style and format of Crop Science.

switchgrass cultivars include ‘Blackwell’, ‘Caddo’, and ‘Cave-in-Rock’ while ‘Alamo’ and ‘Kanlow’ are cultivars of the lowland ecotype (Alderson and Sharp, 1994).

In addition to its importance as a forage grass, the U.S. Department of Energy has identified switchgrass as a promising biofuel crop (Tietz, 1991; Samson, 1991; Vogel, 1996; McLaughlin et al., 1996; Sanderson et al., 1996). Switchgrass offers important advantages as an energy crop. Producing ethanol from corn requires almost as much energy to produce as it yields, while ethanol from switchgrass can produce about five times more energy than it takes to produce. When the energy required to make tractors, transport farm equipment, plant and harvest is factored in, the net energy output of switchgrass is about 20 times greater than that of corn. Switchgrass does a far better job of protecting soil, virtually eliminating erosion. It also removes considerable quantities of CO_2 from the air, storing it in soils and roots (McLaughlin et al., 1999). However, its potential is limited because it is difficult to establish desirable stands quickly.

Therefore, improving seedling vigor has been one of the objectives in improving switchgrass. Since measuring seedling vigor can be difficult and somewhat subjective, seed weight has been used as an indirect selection criterion for improving seedling vigor. Selecting for heavier seed, which is reported to be positively correlated with seedling vigor, allows greater germination levels to be achieved and generates a potentially larger seedling with a greater chance of stand establishment (Kneebone and Cremer, 1955; Aiken and Springer, 1995; Smart and Moser, 1999).

In addition to problems with stand establishment, relatively little is known about the mode of pollination of switchgrass. It has been considered a cross-pollinated species (Talbert et al., 1983; Taliaferro and Hopkins, 1996) but there are no reports of any systematic studies to confirm this assumption. The method of pollination is based on the observations by breeders working with the species. Primary from the amount of seed produced under self- and/or open-pollinated conditions. However, the best method to determine the mode of pollination in a plant is to examine pollen germination and pollen tube growth in the pistil of the female plant.

The objectives of this study were: 1) to conduct two cycles of half-sib family selection for seedling vigor and measure the progress, 2) to calculate an estimate of heritability for seedling vigor, and 3) to observe pollen germination and tube growth under self- and cross-pollinated conditions.

CHAPTER II

HERITABILITY FOR SEEDLING VIGOR IN SWITCHGRASS

Literature Review

Switchgrass is native to the North America where it grows primarily throughout the Great Plains from Canada to Central America (Hitchcock, 1951). Switchgrass was an important part of the native, highly productive Tallgrass Prairie (Weaver, 1968; Risser et al., 1981). It tolerates diverse growing conditions, ranging from arid sites in the shortgrass prairie to brackish marshes and open woods. Because of its growth habit, versatility, and survivability, switchgrass is used for range reseeding, pastureland, hay production, wildlife food and cover, re-vegetation, shoreline stabilization, and erosion control (Alderson and Sharp, 1994; Moser and Vogel, 1995).

The taxonomy of the species is as follows: Subfamily: Panicoideae, Tribe: Paniceae, Genus: *Panicum*, Species: *virgatum.* Some of the other *Panicum* species that are important forage plants include kleingrass (*Panicum coloratum* L.) and guinea grass (*Panicum maximum* Jacq.).

The species is usually found growing in large bunches; it is green in color and glaucous. Its culms are erect and the plants reach heights between 1 to 3 m tall. The leaf blades are 10 to 60 cm long, and 3 to 15 mm wide. In most cases the blades are glabrous, occasionally being pilose near the base. The panicles range from 15 to 50 cm long and are very open. One of the most distinctive characteristics of switchgrass is the deep, vigorous root system, which may extend to depths of more than 3.5 m (Weaver,

1968). Standing biomass in root systems may exceed that found aboveground (Shifflet and Darby, 1985), giving perennial grasses such as switchgrass, an advantage in water and nutrient acquisition even under stressful growing conditions. Within the panicle the spikelets are between 3.5 and 5 mm and they are long and acuminate. Each spikelet contains two florets; the upper floret is a perfect floret and is capable of producing seed, the lower floret is usually staminate (Hitchcock, 1951). It reproduces both by seeds and vegetatively, and a stand can last indefinitely once established.

While all switchgrass genotypes are perennials, Porter (1966) defined two distinct ecotypes (lowland and upland) based on growth morphology and habitat preference. Lowland ecotypes are tetraploids (2n=4x=36) that have an erect growth habit with coarse stems (Porter, 1966). Their leaves are glabrous and the plants are generally robust and often found in bunches standing 0.6 to 3 m tall. Upland ecotypes are either tetraploids (2n=4x=36) or octoploids (2n=8x=72) (Hopkins et al., 1996; Hulquist et al., 1996). Upland ecotypes have finer stems with varying amounts of pubescence on the leaf blade. They have a semi-decumbent growth habit, broad-base, and grow to a height of 90-150 cm (Hulquist et al., 1997). As expected, lowland ecotypes are best adapted to lowland and flood-prone regions; whereas, upland ecotypes are best adapted to drier regions where moisture is more limited. Porter (1966) also reported that lowland ecotypes have a lower nitrogen requirement than the upland ecotypes.

Switchgrass is considered to be a cross-pollinated species that produces little or no seed when self-pollinated, indicating the presence of self-incompatibility mechanisms

(Taliaferro and Hopkins, 1996). However, little is known about the reasons why very little or no seed are produced when self-pollinated. Seed development and set are strongly influenced by the environment with specific stresses known to induce or delay anthesis. An understanding of self-incompatibility mechanisms is necessary to effectively utilize the available germplasm of a species in a breeding program. Also, the commercial mode of reproduction and distribution of switchgrass is by seed, therefore knowledge of incompatibility systems in this crop would be helpful in trying to answer some questions about seed production.

Physiologically, switchgrass is a C_4 species, fixing carbon by multiple metabolic pathways. Switchgrass has high water-use efficiency (Koshi et al., 1982). In general C_4 plants such as warm-season grasses will produce 30% more plant mass per unit of water than C_3 species such as trees and broadleaved crops and cool-season grasses (Samson and Knopf, 1994).

Chromosome number of switchgrass range from 2n = 2x = 18 to 2n = 12x = 108 (McMillan and Weiler, 1959). Lowland ecotypes tend to be tetraploids while upland ecotypes are tetraploids and octaploids. Several studies have tried to determine how the genetics of the crop can influence its breeding. Crosses between tetraploids and octaploids have been attempted by Taliaferro and Hopkins (1996) and they reported a crossability of 0.06% for the octaploid (female) by tetraploid (male) cross but no hybrids were recovered from the reciprocal cross. These results suggest a genetic barrier that prevents gene flow between ploidy levels of switchgrass because tetraploid and

octaploids are often found in the same native prairies while hexaploids are rare (Hulkist et al., 1997).

Breeding and Improvement in Switchgrass

Like most forage species, long-term and systematic genetic improvement of switchgrass is limited because of funding and a general lack of knowledge of basic genetic and cytogenetic characteristics. However, breeders have identified several traits of importance in the species and they have made significant progress in improving these traits. The traits that have been emphasized in breeding programs include (1) increasing *in vitro* dry matter digestibility (IVDMD), (2) total biomass production, and (3) several seed related traits such as seed mass, crown node placement, and seed dormancy. These traits were selected because of the importance of forage quality, yield and stand establishment (Vogel et al., 1981; 2002; Tischler et al. 1994).

IVDMD

Vogel et al. (1981) reported a strong positive relationship between IVDMD and forage quality. Improving IVDMD has been an important breeding objective in most forage improvement programs. Switchgrass is no different, and selection for IVDMD has been ongoing for over 20 years (Vogel et al., 2002). They conducted a series of experiments evaluating divergent selection for increased IVDMD in switchgrass and the associated changes in the biomass composition of switchgrass (Vogel et al., 1981, 1984;

Anderson et al., 1988). This led to the release of two switchgrass cultivars, Trailblazer and Shawnee, with improved IVDMD (Vogel et al., 1991, 1996).

Genotypes with improved IVDMD have been used to determine the effect of high IVDMD on plant yield and survival. Casler et al. (2002) reported that the winter survival of switchgrass was negatively correlated with IVDMD, indicating that increased IVDMD reduces winter hardiness. Vogel et al. (2002) reported similar findings when they evaluated winter survival in different switchgrass populations selected for high IVDMD.

Godshalk et al. (1988) used several half-sib lines of switchgrass to estimate the feasibility of using cell wall monomers as selection criteria to increase IVDMD of switchgrass. Thirty three parents were selected from a population of 660 plants on the basis of forage yield, IVDMD, and N concentration. Plant samples of the parents and the offspring were assayed for IVDMD, acid detergent fiber (ADF), neutral detergent fiber (NDF), concentration of arabinose (ARA), galactose (GAL), glucose (GLU), xylose (XYL), and ratios of the monosaccharides. Broad-sense heritability for IVDMD was 0.89 and it was concluded that most effective method to improve IVDMD was to use clonal selection or modified ear to row selection for reductions in the arabinose:glucose or xylose:glucose ratios.

Biomass Yield

Production of transportation fuels, such as ethanol, and generation of electrical power are the two primary markets for bioenergy. The biofuels industry in the US is

based almost entirely (98%) on the conversion of corn (*Zea mays* L.) to ethanol (Petrulis et al. 1993). There are three principal technological endpoints for bioenergy crops: 1) conversion to liquid fuels; 2) combustion alone or in combination with fossil fuels to produce heat, steam, or electricity; and 3) gasification to simpler gas products that can be used in a variety of endpoint processes. Switchgrass is classified as a lignocellulosic crop because it is primarily the cell walls that are digested to form sugars, which can subsequently be fermented to produce liquid fuels. The rationale for developing lignocellulosic crops for energy is that less intensive production techniques and poorer quality land can be used for these crops, thereby avoiding competition with food production on better quality land (McLaughlin et al., 1999).

Switchgrass biomass can be converted into energy by fermentation, gasification, or combustion as previously mentioned. High fiber content, consisting largely of fermentable or combustible sugars, would be beneficial for each of the processes. Lignin is detrimental for fermentation, because it can not be broken down during the process. Ash is detrimental for combustion, causing slagging and fouling of biomass boilers, increasing maintenance costs, and creating a disposal problem for fly ash (Miles and Miles, 1994). Switchgrass cultivars with high biomass production, combined with a high fiber concentration, low lignin, and low ash would be most desirable as general purpose bioenergy feedstock.

Casler and Boe (2003) conducted a study to estimate the effect of harvest date on switchgrass cultivars at two locations in the north central USA and to determine the cultivar x environment for agronomic and biofuel traits in switchgrass. Six cultivars

were used and they were harvested three times (August, September, and October). Biomass yield did not respond consistently to harvest date, varying with cultivar, location, and year. Mean dry matter, forage fiber, and lignin concentration also varied among cultivars, consistently across locations and years. These three traits increased with later harvest consistently across locations and years, but inconsistently among cultivars. They concluded that it should be possible, through selection and breeding, to develop switchgrass germplasm with increased fiber and decreased lignin and ash, increasing the availability of fermentable sugars and decreasing the unfermentable and/or incombustible residues.

The increase of atmospheric CO_2 levels has placed emphasis on better understanding the role of agriculture in slowing down this increase. Switchgrass is a perennial grass that is being proposed as a biofuels crop and to help mitigate the atmospheric CO_2 gains (Zan et al., 1997). Switchgrass has many traits that make it an attractive crop for sequestration of atmospheric CO_2. It has an extensive deep root system, about 50% greater water use efficiency than cool season forage grasses, a relative low nutrient requirement, and the potential to produce large amounts of biomass (Bransby et al., 1998).

Frank et al. (2004) determined biomass and C partitioning in aboveground and belowground plant components. At the ripe-seed harvest stage, stem biomass accounted for 46% of total aboveground biomass, leaves 7%, senescence plant parts 43%, and litter 4%. Excluding crowns, root biomass averaged 27% of the total plant biomass and 84% when crown tissue was included in root biomass. Carbon partitioning among

aboveground, crown and root biomass showed that crown tissue contained 50% of the total biomass C. They concluded that switchgrass has potential for storing a significant quantity of soil C.

Switchgrass production practices are different for biomass than for forage. Yield and quality are related because the goal is to maximize production of lignocellulose (Sanderson et al., 1999). Muir et al. (2001) conducted a study to determine the yield and stand responses of Alamo switchgrass to N and P fertilizations affected by row spacing in two different locations. They used five rates of N (0, 5, 112, 168, 224 kg ha^{-1}) and five for P (0. 9.8, 19.6, 29.4, 39.2 kg ha^{-1}). The row spacing was 18, 36, and 54 cm. at Stephenville, TX and 25, 51, and 102 cm at Beeville TX. A maximum yield of 22.5 Mg ha^{-1} occurred when using 168 kg N ha^{-1}, and lodging occurred when 224 kg N ha^{-1} were applied. Biomass was not influenced by the addition of P. They concluded that switchgrass biomass production was sustainable at Stephenville only with the application of at least 168 kg N ha^{-1} yr^{-1}, but P application and row spacing are not crucial. Also, at Beeville the conclusions were that biomass production decreased as row spacing increased in two out of three years of the study and lodging occurred when 224 hg N ha^{-1} were applied (as at Stephenville).

Interest in selecting for yield in switchgrass is relatively new, prompted by the search for durable and sustaining sources of biofuel. Switchgrass was identified as a promising crop for biofuel because of its: high biomass production; survivability and durability; and range of adaptation (Vogel, 1996). However, breeding methodologies commonly used for more traditional crop species may not necessarily apply due to basic

characteristics of the species. In addition, measuring and selection based on total biomass is difficult because the duration of tests and the need to continually harvest and weigh. Therefore, breeding programs for switchgrass biomass have focused on using different tools to measure the relationships among traits. Correlation coefficients have been used to provide limited information on the relationship between measurable traits and biomass. However, that approach is unreliable because it often disregards or masks complex interrelationships among traits; therefore, they must be used with caution when making decisions regarding indirect selections (Kang, 1994).

Partitioning correlation coefficients into direct and indirect effects provides more useful information. Redfearn et al. (1997) studied associations among several morphological traits and forage yield in switchgrass. They reported that forage yield of switchgrass populations were affected primarily by tiller growth and development. Das et al. (2004) studied the importance of direct and indirect effects of biomass yield components on biomass yield in switchgrass and they concluded that selection for increased number of tillers per plant was the most effective means of indirectly increasing biomass yield. Alexander et al. (2003) reported that the realized heritability for seedling tiller number in big bluestem (*Andropogon gerardii* Vitman) and switchgrass and was 0.26 and 0.23, respectively. Agronomic evaluation and the development of systems for biomass production have revealed some limitations to the production of this species. Two of the most important problems are seed dormancy and stand establishment. In the case of seed dormancy, several approaches may be useful in addressing the problem. These could range from seed treatments to breeding. However,

seed dormancy can be reduced with proper storage conditions (Kalmbacher et al., 1999), but it does cause some financial hardship with seed producers having to hold inventory to allow for dormancy to decline (Dr. W.R. Ocumpaugh, personal communication). Stand establishment is more complex, with many factors influencing the trait ranging from seed size to seedling vigor. The best approach to address this issue is through breeding and selection. If switchgrass is to be cultivated on a large scale, it will be necessary to resolve these issues. In each case, there is a need to develop a basic understanding of the problem specific for switchgrass.

Seed Dormancy

Seed dormancy is a condition in which the seed do not germinate because of physical or chemical factors that delay germination. In nature, seed dormancy is likely a method of survival. Species that avoid rapid germination after seed maturation are more likely to survive to reproduce than those that germinate immediate after maturation. Seed dormancy is complex because numerous factors interact to produce the condition. Factors that are known to influence dormancy include water, light, temperature, gasses, physical or structural factors, and hormones (Cardwell, 1944; Loch et al., 2004). While all of these factors influence dormancy, not all of these factors affect dormancy in every species in which it occurs.

Seed dormancy and slow seedling development are common in warm-season grasses. While this characteristic may be beneficial in nature, it limits the success of warm-season grass stands in a cultivated setting. Beckman et al. (1993) used two seed

priming treatments to increase the establishment of big bluestem and switchgrass. These were 2-d moistened (17° C) and 14-d wet-chill (4° C) treatments. In a greenhouse, emergence of both species was increased by the treatments, but under field conditions emergence from dry untreated seed was greater than either treatment.

Zhen-Xing et al. (2001) investigated the influences of prolonged stratification, post-stratification drying, restratification, and after ripening on germinability of Cave-in-Rock switchgrass seed. Germination was increased up to ≥80% with 14 d of stratification, if the seed are germinated without drying. However, they found that germinability (but not viability) may decrease by half or more if the stratified seed are first dried and then rehydrated for germination. The reoccurrence of dormancy (secondary dormancy) during post-stratification drying is herein referred to as *reversion*. During post-stratification drying, dormancy reversion increased as the degree of desiccation increased. Extended stratification (≥42 d) prevented reversion. After-ripening also reduced the potential for reversion. Stratification and after-ripening appeared to work additively to block reversion. Restratifying dried seed revealed that, while drying interruption caused reversion, it also decreased dormancy variability within a seedlot and reduced the total stratification time needed to obtain maximum germination compared with continuous stratification. Stratification will increase germinability of switchgrass seed, but drying following insufficient stratification can cause dormancy reversion.

Tischler and Young (1983) investigated the effects of chemical and physical treatments on germination of freshly harvested kleingrass seed. They used sulphuric

acid and found that a 5 minute sulphuric acid treatment was the most effective at promoting germination. Tischler et al. (1994) investigated chemical treatments to reduce seed dormancy in switchgrass. They treated switchgrass seed with sulphuric acid and chloroethanol treatments at 5, 10, 15, and 20 minute intervals. A 10 minute sulphuric acid treatment was the most effective at increasing germination, and a 15 minute chloroethanol treatment gave similar results. Because chloroethanol was no more effective than sulphuric acid in improving germination, and the compound is highly toxic, chloroethanol was not recommended as a treatment for dormancy in switchgrass. Tischler and Young (1983) hypothesized that the acid treatment either destroy a germination inhibitor or modify the kleingrass lemma and palea to allow inhibitor(s) to diffuse out of the caryopsis. This could be the reason why sulphuric acid is also effective in increasing germination in switchgrass.

Tischler et al. (2001b) performed four cycles of selection for low dormancy in Alamo switchgrass and unselected Alamo plants were used as a source of control seed. The treatments were alternating (30°C, 15°C) and constant (30°C) germination temperatures. Germination was much higher in alternating temperature environment, but overall results were comparable. Differences between control and cycle 4 plants were more pronounced at constant temperature. Although germination of seed from some cycle 4 plants was no greater than the control, one third of the selected plants had significantly higher germination than Alamo. They concluded that this germplasm should exhibit more rapid and complete germination in field plantings and consequently result in better stand establishment.

Seed Mass

In many cases where stand establishment is a problem, the first approach is to determine if seed size influences stand establishment. In tobacco (*Nicotiana tabacum* L.), Kasperbauer and Sutton (1977) reported that seed weight was positively associated with stand establishment, especially when seed were directly planted in the field. Past efforts to enhance legume seedling growth have focused on seed size because of its strong correlation with seedling vigor (Black, 1957; Townsend and Wilson, 1981; Knight, 1985). Evers (1999), compared the morphological seedling traits of arrowleaf (*Trifolium vesiculosum* Savi.), crimson (*T. incarnatum* L.), rose (*T. hirtum* All.), and subterranean (*T. subterraneum* L.) clovers to identify which factors are most limiting for seedling growth. In crimson clover, greater seedling vigor was due in part to its larger and faster-expanding leaf area index and tolerance to cooler temperatures. The slower seedling vigor of arrowleaf clover was due to its smaller seed size. He concluded that good seedling vigor of cool-season annual clovers was primarily dependent on large seed size, rapid leaf area expansion, and early nodulation.

N'Diaga and Ejeta (2003) calculated the broad-sense heritability for seedling weight in sorghum [*Sorghum bicolor* (L.) Moench.] as 0.40 and 0.46 at two and three weeks after emergence, respectively. They concluded that the breeding progress using this trait would be slow as suggested by the low heritability estimate. Berdahl and Frank (1998) used four cool-season forage grasses to assess seed quality at different stages of development by germination percentage and emergence of seedlings. Seed mass was closely associated with seedling vigor in all four species, as evidence by correlation

coefficients between seed mass and seedling emergence of 0.88 for crested wheatgrass [*Agropyron desetorum* (Fisch ex Link) Schultes], 0.93 for Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski], 0.92 for intermediate wheatgrass [*Thynopyrum intermedium* (Host) Barkw. & D.R. Dewey], and 0.77 for western wheatgrass [*Pascopyrum smithii* (Rydb.) A.Löve]. Prairiegrass (*Bromus willdenowii* Kunth = *B. catharticus* Vahl) has good emergence and establishment, which has been attributed to its relatively high seed mass (Andrews et al., 1997). Sangakkara et al. (1985) also noted greater seedling growth in 'Matua' prairiegrass compared with perennial ryegrass (*Lolium perenne* L.) and orchardgrass (*Dactylis glomerata* L.), and they attributed these differences to seed mass.

These trends do not necessarily apply to switchgrass. Smart and Moser (1999) conducted a study using switchgrass to determine the effect of seed size on seedling and plant development over a period of 60 days under field conditions. Seed from two cultivars, Blackwell and Trailblazer, were divided into two groups, heavy (HS) (0.19 to 0.21 g 100 seed^{-1}) and light (LS) (0.13 to 0.16 g 100 seed^{-1}). Seed size differences produced only slight differences in morphological development of shoot and root systems, leaf area, shoot weight, and adventitious root weight from seedling emergence to six weeks of growth. By 8 to 10 weeks after emergence, growth and development of LS seedlings were similar to HS seedlings. They concluded that seed size in switchgrass appears to have minimal long-term effect on growth and development of seedlings and had relatively little influence on stand establishment. Contrary to the findings of Smart and Moser (1999), Aiken and Springer (1995) investigated the germination and

emergence for six switchgrass cultivars (Alamo, Blackwell, Cave in Rock, Kanlow, Pathfinder, and Trailblazer) and reported that both germination and emergence increased with seed size. This indicates that improvement for seedling mass and establishment in switchgrass may be possible using heavy seed.

Boe and Johnson (1987) considered that selection for seed size is important in native, warm-season grass improvement programs because of the positive correlation between seed size and seedling vigor. Bulk seed from a switchgrass population of 750 spaced plants from a field nursery was separated into four weight classes (174.1, 190.5, 209.5, and 225.8 mg 100 seed^{-1}) utilizing a South Dakota Seed Blower. A highly significant ($P < 0.01$) linear relationship was detected between mean 100-seed weights of weight classes and mean seed weights of open-pollinated, spaced planted progeny produced from these classes. Highly significant differences in mean seed weight also existed among plants within weight classes. The authors concluded that in switchgrass, high seed weight was an effective selection criterion for increasing the seed weight of progeny from a bulk seed lot.

Genetic improvement of seed weight appears to be possible in many species. Boe (2003) estimated narrow sense heritability for seed weight to be 0.88 in the cultivar Sunburst and 0.58 in the cultivar Summer. Both Summer and Sunburst are upland ecotype cultivars of switchgrass. Selection for seedling weight has been successful in improving seedling vigor in legumes (Twanley, 1974; Simons, 1990; Xie and Mosjidis, 1995). However, McLean and Nowak (1997) indicated that while seed size is often

highly correlated with seedling vigor, selection only for seed size is not likely to improve vigor.

Hussey and Holt (1986) investigated the selection for increased seed weight as a potential method for improving seedling vigor and stand establishment characteristics in kleingrass. Three cycles of mass selection for increased seed weight were conducted. Selection resulted in a total gain of 53.3% of the mean seed weight of the base population with gains of 27.1, 1.8, and 18.5% in cycles 1, 2, and 3, respectively. The increased seed weight for cycles 1 and 2 was attributed to the elimination of light seed weight classes, while increased seed weight in cycle 3 was due to both the elimination of light seed weight classes and an increase in maximum seed weight. Broad-sense heritability estimates declined with selection from 0.73 in cycle 0 to 0.41 in cycle 3. This research resulted in the release of the cultivar 'Verde' with a larger seed mass (Alderson and Sharp, 1994).

Seedling Development and Morphology

Upon germination, a seedling goes through three stages of development: 1) *heterotrophic*, from germination to emergence and commencement of photosynthesis in cotyledons; 2) *transitional*, when cotyledons begin to photosynthesize but before the exhaustion of seed reserves; and 3) *autotrophic*, when the seedling is dependent on photosynthesis (Cooper, 1977). An established plant should have adequate root penetration in the soil to maintain favorable water potential in the shoot. Perennial

grasses are considered established when four to six leaves and at least two adventitious roots have developed on the seedling (Ries and Svejcar, 1991).

However, some morphological and anatomical features that influence establishment can be changed through breeding and selection. Because seedling vigor influences the rate at which a plant reaches a given level of morphological development, it can influence plant morphology (Tischler and Voigt, 1987).

For some Panicoid grasses, the development of the coleoptilar node (CN) relative to the soil surface is critical for establishment. The CN is the site where adventitious roots originate. These roots are essential because they replace the roots derived from the embryo; therefore, seedlings become established plants only after the adventitious roots have penetrated the soil (Olmsted, 1941; Hyder et al., 1971; Tischler and Voigt, 1987).

In monocots, the radicle gives rise to the seminal root system. This root system is generally short-lived, and is replaced by nodal or adventitious roots arising from the coleoptile or higher nodes. Because the seminal root system is temporary, this is a major reason why it is difficult to establish warm season grasses (Tischler and Voigt, 1987, 1993; Tischler et al., 1997).

Some festucoid grasses have the capacity to produce tillers in the axis of the coleoptile. These structures have been observed in tall fescue (*Festuca arundinacea* Schreb.) and it was concluded that coleoptile tillers are associated with higher seedling vigor. Therefore, selection for the presence of coleoptile tillers could be a method for improving seedling vigor in the species (Lewis and Garcia, 1978). The development of coleoptile tillers occur most frequently in seedlings arising from large seed, suggesting

that in wheat (*Triticum aestivum* L.), the development of coleoptile tillers is not itself a morphological characteristic which confers seedling vigor, but rather more a manifestation of seedling vigor (Peterson et al., 1982). Faulkner et al. (1982) increased seedling vigor in tall fescue by selecting for larger coleoptile tillers over two cycles.

Efforts have been made to increase seedling establishment in both switchgrass and kleingrass through breeding for crown node placement. In seedlings of *Panicoid* grasses, elongation of the subcoleoptile internode (SCI) stops shortly after the coleoptile tip receives a red light stimulus. This results in final placement of CN near the soil surface. Because adventitious roots originate from the CN, excessive elongation of the SCI places the CN above the soil surface, negatively impacting seedling establishment. Tischler and Voigt (1995) with kleingrass and Elbersen et al. (1998, 1999) with switchgrass made some progress using recurrent selection for divergent CN placement. This selection progress resulted in the release of germplasm with a low crown and elevated crown placement in kleingrass (Tischler et al., 1996) and switchgrass (Tischler et al., 2001a). Even though selection for low crown was successful, the authors concluded that this trait may not be directly responsible for greater field establishment success (Elbersen et al., 1999).

Although selection for larger seed improved seedling growth rate and resulted in the cultivar Verde, selection for increased seedling growth rate in kleingrass had not been attempted. TEM-SV1 was derived from three cycles of recurrent selection for increased seedling shoot growth using Selection 75 as the initial germplasm source (Young and Tischler, 1994). Seedlings of TEM-SV1 had a faster growth rate and

accumulated significantly more forage dry matter in both controlled environment and field tests (87% more at 14 days after emergence in a growth chamber and 33 and 16% more at 16 and 30 days after emergence in the field, respectively). Although TEM-SV1 was selected for greater shoot growth, there was a significant increase in root growth rate.

The amount of endosperm present in a caryopsis is often related to seed size and weight, and many reports show the importance of more endosperm as food reserves in relation to seedling growth (Demivlicakmak et al., 1963; Kneebone and Cremer, 1955; Bremner et al., 1963). Root growth has also been correlated to seed size (Kittock and Patterson, 1962; Tador and Cohen, 1968). Seed size or mass are also associated with seedling vigor. Rate of growth is often regarded as evidence of seedling vigor because rapid top growth and root growth can be an advantage in seedling establishment (McKell, 1972; Davies, 1967).

Tischler and Voigt (1993) conducted a study to determine why wilman lovegrass (*Eragrostis superba* Peyr.) has a greater shoot mass at 14 days post emergence than does kleingrass. Caryopses of both species contain the same amount of starch, but wilman lovegrass caryopses contain more protein and phosphorous (P). Wilman lovegrass had a greater shoot mass than kleingrass at 3 and 7 days post emergence. At 3 days after germination, wilman lovegrass caryopses contained less starch than kleingrass. At 6 days after planting, starch reserves were depleted in both species and protein and P contents of the caryopses residues were similar in both species, thus indicating that wilman lovegrass caryopses supplied more protein (or amino acids) and P to the growing

shoot than did corresponding kleingrass caryopses. The authors concluded that selection for greater seedling vigor in kleingrass may result in genotypes with a) a faster rate of starch mobilization and utilization, and b) a higher starch, protein, and P content per caryopsis.

Heritability: Methods of Estimating

Methods for estimating heritability are based on the partitioning of observed variation for a quantitative character into specific family units with a known genetic relationship and controlling variation that is due to the environment and error. The total variance calculated from the observed population constitutes the phenotypic variance, V_P. The phenotypic variance may be divided into three components: genetic variance, V_G, non-genetic or environmental variance, V_E, and variance due to interactions between genotype and environment, V_{GE}. Thus, phenotypic variance can be expanded as:

$$V_P = V_G + V_E + V_{GE}$$

The genetic variance, V_G, is composed of three major components: additive genetic variance, V_A, dominance variance, V_D, and non allelic interactions or epistasis variance, V_I. This may be written as:

$$V_G = V_A + V_D + V_I$$

The additive component of the genetic variance contributed is the variance contributed by genes having linear additive effects. The resemblance between parents and offspring is the result of additive genetic effects, which largely determines the response of a population to selection. The dominance component represents the

deviation of the heterozygote from the mid-parent or average of the homozygous parents. The dominance effects on the expression of a quantitative character in a population are generally small in comparison to additive effects. The interaction variance results from deviations caused by epistatic effects of non allelic genes. Examples of epistatic effects include additive x additive, additive x dominance, dominance x dominance, and additive x additive x additive interactions. The magnitudes of interaction or epistatic effects are difficult to evaluate, but they are generally believed to be small in comparison with additive and dominance effects and they are often ignored in calculating heritability estimates.

Heritability, the proportion of the phenotypic variance that is due to genetic causes, can be expressed as the following: $H = V_G/V_P = V_G/ (V_G + V_E + V_{GE})$. Heritability estimated from total genetic variance, without taking into consideration the components of genetic variance is referred in the broad sense (H^2), because it estimates heritability on the basis of all genetic effects. A more restrictive and often more useful estimate, is obtained if heritability is estimated solely from additive variance. The result is represented as heritability in the narrow sense (h^2). Narrow-sense heritability cannot exceed and is usually less than the broad-sense heritability (Poehlman and Sleper, 1995). Because narrow-sense heritability estimates the portion of genetic variation that is selectable, these are considered the more useful estimates. However, in many crops, obtaining accurate estimates of partitioned variation is difficult. In these situations, broad-sense heritability estimates provide a starting point for the development of

breeding approaches for a certain trait. In addition to the estimate, the type of crop, the genetics, and the modes of pollination and reproduction must be considered.

Response to Selection

The response to selection, which is symbolized by *R*; it is the difference of mean phenotypic value between the offspring of the selected parents and the whole of the parental generation before selection. The measure of the selection applied is the average superiority of the selected parents, which is called the *selection differential*, and is symbolized by *S*. It is the mean phenotypic value of the individuals selected as parents expressed as a deviation from the population mean, which is from the mean phenotypic value of all the individuals in the parental generation before selection was made. The ratio of response to selection differential is equal to heritability, and the response is given by:

$R = h^2 S$

The response to selection can be used as a means of estimating the heritability in the base population, because the expected value of the ratio *R/S* is the heritability. The heritability estimated this way is called the realized heritability because it is primarily a description of the response (Falconer and Mackay, 1996).

Heritability in Switchgrass

The heritability of several important traits in switchgrass has been determined. Talbert et al. (1983) obtained heritabilities at a single location of 0.91 to 1.49 for

maturity and concluded there was sufficient variation in flowering date in native lowland switchgrass populations to hasten or delay flowering time. In calculating individual heritability, the assumption must be made that plants are not correlated. For those traits in which plants within plots are correlated, within plot variance is underestimated, and heritability is biased upward. This appears to be the case for maturity. Van Esbroeck et al. (1998) reported realized heritabilities of 1.00 and 0.92 for early and late panicle emergence, respectively in Alamo.

Newell and Eberhart (1961) determined the heritability among clonal selections within selected endemic strains of switchgrass. Approximately 300 clones were selected from more than 100 endemic strains of switchgrass. Clones of 3 different types were evaluated in isolated crossing blocks in a 2-year period, and progenies were grown from groups of clones selected for superiority of several plant characters in each type. Average realized heritability for three types of plants for forage index, seed yield, and rust index was 0.56, 0.20, and 0.62, respectively.

Boe (2003) estimated narrow-sense heritability for seed weight to be 0.88 in the cultivar ‘Sunburst’ and 0.58 in the cultivar ‘Summer’. Alexander et al. (2003) calculated the realized heritability for seedling tiller number in big bluestem and switchgrass to be 0.26 and 0.23, respectively. Das et al. (2004) concluded that selection for number of tillers per plant would be the most effective means of indirectly increasing biomass yield in switchgrass.

Besides the above discussed factors influencing stand establishment of grasses, there are others influencing stand establishment in crops. Planting depth, adventitious roots, soil fertility, soil texture, insect pressure, moisture content, and weeds are among the factors affecting stand establishment.

The objectives of this study were: (1) to conduct two cycles of half-sib family selection for seedling vigor and measure the progress; (2) to calculate an estimate of heritability for seedling vigor, which would be helpful in making an assessment on how much of the genetic variance is available for selection and improvement. This will be helpful in assessing how much improvement in seedling vigor can be expected in switchgrass. This knowledge will be useful for those wanting to develop varieties with greater seedling vigor, which should improve establishment.

Materials and Methods

Seedling Vigor – Intrapopulation Improvement Methods (C_0 Half-sibs)

A half-sib population improvement program was initiated to determine if seedling vigor can be improved in switchgrass. The base population for this program was created at Oklahoma State University (C.M. Taliaferro, 2000 personal communication) as part of a seed and forage yield component study. The base population consisted of breeding derivatives of the cultivar Kanlow and the first generation seed from intercrossing Alamo and PMT 279 and commercial certified class Alamo. Plants from these sources were grown in a spaced planted nursery at Chickasha and Perkins, OK where they were allowed to randomly intermate. Each nursery was created with seedlings from the same parentage. The rainfall at Perkins averages approximately 813 mm per year, while only 712 mm are normally received at Chickasha. However, the soils at Chickasha are somewhat more fertile than those at Perkins. Seed was harvested from individual plants at each location during the fall of 1998. For each plant, the mass per 100 seed was measured to identify plants with extremes for seed mass. Seed from 400 half-sibs were available for evaluation from each location. However, seed from 180 plants were selected from the 800 seed lots that were available, independent of nursery location. The 800 seed lots were ranked by seed mass and 60 seed lots from each of three seed-mass classes were selected. The range of seed mass for the heaviest 60 half-sib families was 0.1029 to 0.1247 g 100 seed^{-1}. The range of the medium 60 half-sibs was 0.0885 to 0.0915 g 100 seed^{-1}. The range of the lightest 60 half-sibs was 0.0619 to 0.0802 g 100 seed^{-1}.

Seedling mass was determined in a growth chamber on three different occasions between February and June 2000. In each half-sib, a set of 50 seedlings was measured to determine the relationship between seed mass and seedling mass. The seedlings were grown in Scotts Ready Earth® potting mix. Temperature was maintained at 30ºC and the light intensity was not less than 1000 μmol m^{-2} s^{-1} using a combination of incandescent and fluorescent lamps. The photoperiod was 12-hr day: 12-hr night. The seedlings were grown in Conetainers (2.5 cm diameter and 15 cm length). Three seed were placed on the potting mix in each Conetainer and then covered with a small amount of potting mix. The potting mix was watered with distilled water that was applied with a watering can. The Conetainers were placed in racks with 200 Conetainers per rack, and 16 racks were placed into a growth chamber located in the Heep Center at Texas A&M University. Three runs were required to screen all 180 half-sib lines. Each run had 20 half-sib lines from each of the above mentioned weight class.

Seedlings emerged 3 days after planting and they were thinned to one seedling per Conetainer 3 days after emergence (6th day after planting). Peters® soluble fertilizer 20-9-17 (N-P-K) was applied at a rate of 6 gm L^{-1} to enhance seedling growth 7 days after emergence with a watering can. Seedlings were grown in the Conetainers until 14 days after emergence. They were harvested with a razor blade, cutting them at the soil's level. After harvest, the seedlings were placed in coin envelopes size 5.5 (7.8 cm by 13.8 cm), and dried in an oven at 65° C for at least 24 hours. Dry weights were determined by weighing individual seedlings.

Seedling mass was compared with seed mass using SAS (2001) statistical software and there was a poor relationship between the two traits. Therefore, the original selections were from plants that produced the heaviest seedlings and were from all the seed-mass classes. A total of 18 plants were selected. (Table 1).

Table 1. C_0 parental clones selected based on half-sib seedling weight from half-sib seed provided by Dr. C.M. Taliaferro of Oklahoma State University.

Oklahoma Location	OSU plant Designation	(OSU) Rep	(OSU) Plant ID number	OSU Seed weight in g seedling^{-1}	Average weight in g seedling^{-1}
Chickasha	SL93	3	9	0.1247	0.024479
Chickasha	SL94150	4	6	0.1174	0.024200
Perkins	SL94150	1	8	0.0911	0.022432
Chickasha	SL93	4	7	0.1207	0.022233
Perkins	SL92150	4	8	0.1136	0.022173
Chickasha	SL93	4	10	0.1223	0.021879
Perkins	SL94	2	1	0.0900	0.021545
Perkins	SL94150	1	9	0.0898	0.021116
Chickasha	SL94	3	2	0.0906	0.020620
Perkins	ALAMO	1	8	0.1099	0.020605
Chickasha	SL94150	3	9	0.0909	0.020516
Chickasha	SL93	1	1	0.1141	0.020365
Chickasha	SL93	2	8	0.0905	0.020323
Chickasha	SL94150	2	4	0.0896	0.020300
Perkins	SL92150	4	1	0.0905	0.020109
Perkins	ALAMO	2	6	0.0905	0.019821
Chickasha	SL94150	3	3	0.0893	0.019777
Chickasha	SLCO	2	6	0.0708	0.019604

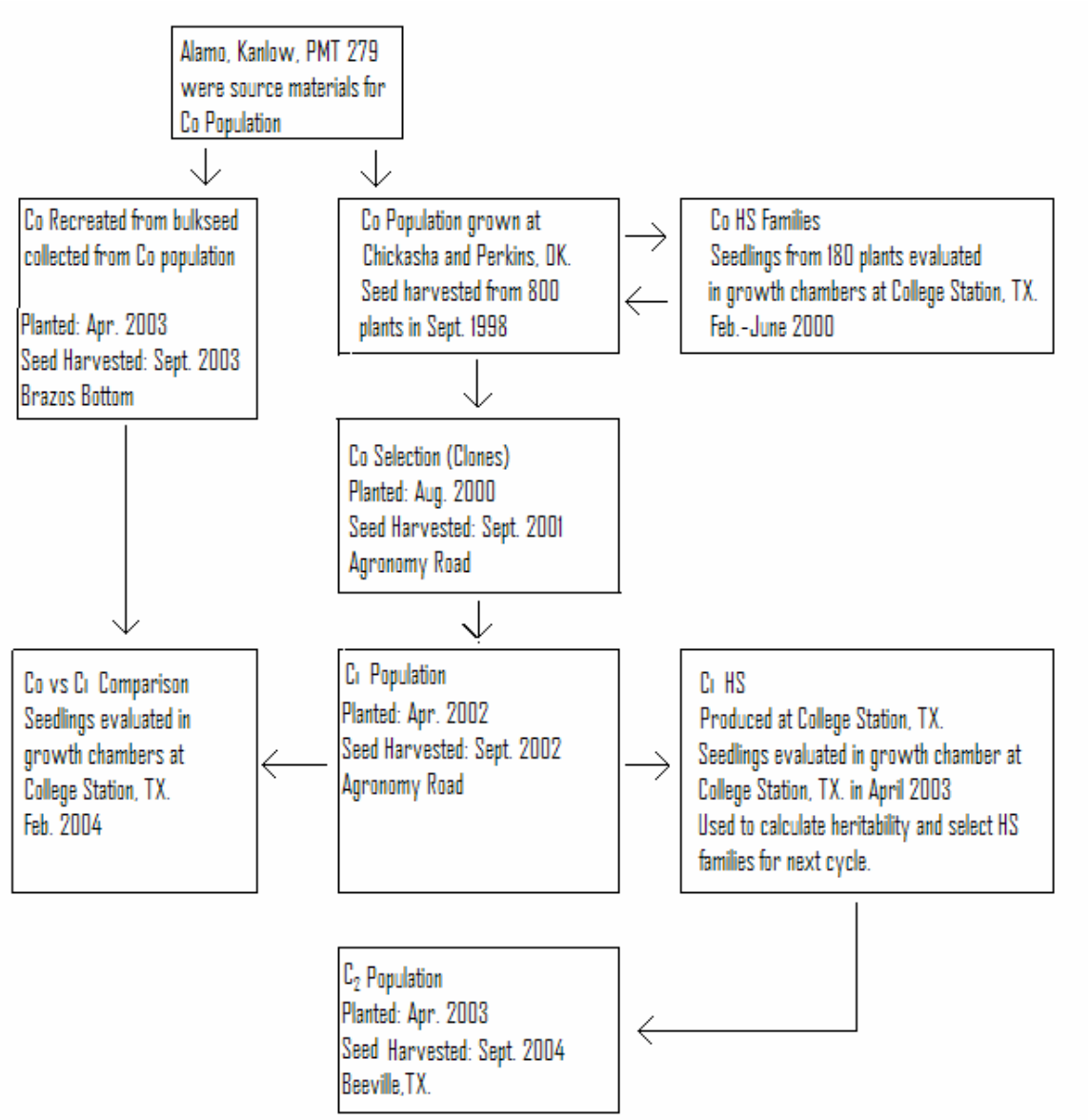

Figure 1. Flow chart of the half-sib evaluation methodology used in the current study, including the location of production or evaluation, and the populations used for specific scientific study.

C_0 Selections (Clones)

The parental plants that produced the heaviest half-sib progeny were identified in the Oklahoma nurseries, and the 18 parental plants were dug and transported to College Station, TX in July 2000. Each parent was cloned into seven ramets and these were used to create the C_0 selection crossing block (Table 1). The clones were kept in a greenhouse until August 2000 and then they were transplanted into a Booneville fine sandy loam soil (fine montmorillonitic, thermic Ruptic-Vertic Albaqualfs) at Texas A&M University located off of Agronomy Road in College Station, TX (30° 38'N, 96° 26'W) (Figure 1). The clones were space planted at a distance of 1.2 m between rows and plants. The crossing block was irrigated as needed by applying a minimum of 5 cm of water with sprinklers (approximately 2 hours of irrigation), and it was fertilized at a rate of 100, 45, and 0 kg ha^{-1} $year^{-1}$ of N-P-K.

During the summer 2001, the plants were allowed to intermate and seed were harvested from the nursery in September 2001. Weeds were kept in check by cultivation with a tractor at early stages, but when the switchgrass plants had grown too tall to use a tractor; the plot was manually hoed. Individual switchgrass plants were identified by designating the row and plant within the row. When seed started maturing, plants were checked several times to determine when the seed was ready to be harvested. This process was performed by holding the inflorescences and shaking them above a 20 L bucket. If seed were released by the shaking motion, it was mature and the seed were harvested (September-October 2001). Usually it took only one harvest to obtain sufficient seed. However, if not enough seed were collected, a second harvest was

performed about 2 weeks later. This seed was used to create the C_1 population. The seed were bulked to create the C_1 population. Equal quantities of seed were bulked from each plant and 150 seedlings were started in the greenhouse in February 2001 to create the C_1 population.

C_1 Population

In April 2002, the C_1 population was established with the 150 greenhouse grown plants in an isolated crossing block to create the next set of 120 HS families. This block was planted into a Booneville fine sandy loam soil (fine montmorillonitic, thermic Ruptic-Vertic Albaqualfs) at Texas A&M University in College Station, Texas (30° 38'N, 96° 26'W). The crossing block was space planted with a distance of 1.2 m between rows and plants. The nursery management and seed harvest was similar to that used for C_0 selection nursery. Seed were September and October 2002. Seed from these 120 HS families were screened for seedling weight (see C_1 Half-sib evaluation methodology below). The heaviest 15% (18 HS families) were selected based on dry weight of evaluated seedlings. Seedlings from the top 18 HS families were grown in a greenhouse and transplanted in the field at Beeville to create the C_2 population nursery.

C_1 Half-sib Evaluation Methodology

Seedling vigor of the half-sib progeny was evaluated as previously described in the growth chamber study. The only differences were that within a replication, only 10 seedlings (10 plastic Conetainers) were used for each half-sib family. Because the

number of entries to be evaluated was too large to fit in a single growth chamber, a sets within rep design with 6 sets and 4 replications was used in the screening. Each growth chamber was considered one replication, and the growth chamber used in this study was located in the Institute for Plant Genomics and Biotechnology at Texas A&M University. Two control checks were used in each replication. Both Alamo switchgrass and 'Frio' buffelgrass (*Pennisetum ciliare* L.) were included to measure variation from chamber to chamber (replication to replication). The dry weight for each HS family was based on the mean seedling weight averaged across replications.

C_2 Population

The C_2 population was created by bulking equal amounts of seed from the "selected clones" in the C_1 population nursery. This is different from the process that was used to produce the C_1 population, as pollen from all plants in the C_1 nursery contributed to these seed. The bulked seed was planted in multi-cell flats in the greenhouse at College Station, and the seedlings were allowed to germinate and were thinned to one seedling per cell. About 400 cells were planted. Once the seedlings were large enough to transport to Beeville, they were transplanted into a Clareville sandy clay loam (fine montmorillonitic, hyperthermic Pachic Argiustolls) in May 2003. Approximately 360 seedlings were transplanted into a crossing block similar to those mentioned above except the distance between rows and plants was 2 m. The nursery management practices were as described above. Seed produced from this crossing block were collected during the fall 2004 using the collection technique described above. This

seed will be screened using the half-sib methodology described above to identify the superior parents. These parents will be dug, cloned, and planted to create a C_2 selected nursery.

Comparison of C_0 vs. C_1

Comparisons between the C_0 and C_1 populations were based on the mean dry weight of seedlings from bulked seed from each cycle. The C_0 population seed was obtained from Oklahoma, but it was older and did not germinate well; therefore, it did not provide a valid comparison with the C_1 seed. Therefore, C_0 seed was recreated by germinating a random sample of remnant seed originally provided by Dr. C.M. Taliaferro and plants from these seed were planted in a crossing block. These seedlings were started during February 2003. The C_0 regenerated crossing block contained 220 plants and was planted in April 2003. The block was established at the Texas A&M University research farm on a Norwood silty clay loam [fine-silty, mixed (calcareous), thermic Typic Udifluvent] near College Station (30° 67'N). The C_0 recreated crossing block was space planted at a distance of 2.1 m between rows and plants. The management of this nursery and seed harvests were performed as described for all other crossing block and seed were harvested in September 2003. This seed was bulked and used to compare with the bulked seed from the C_1 population. Seed of the C_1 population was stored from November 2002 until November 2003 in a cold room at 5°C, and it germinated well in December 2003. Seedlings from the C_1 population stored seed were compared with seedlings from the seed produced from the C_0 recreated population. The

experimental design for this comparison was a randomized complete block design with 6 replications. Each experimental unit consisted of 50 seedlings, which were grown and evaluated in a growth chamber (located in the Institute for Plant Genomics and Biotechnology) using the procedures described in the half-sib family evaluation. In addition to the C_0 and C_1 populations, Alamo, and 'Frio' buffelgrass were included as check varieties. The buffelgrass check was apomictic and was included to help measure environmental variation in the growth chamber.

The Frio buffelgrass seed was harvested in 1999 from a seed increase nursery from the forage breeding program at Texas A&M University, and the Alamo switchgrass seed was purchased in 2000 and was produced in 1999.

The linear model used for comparisons of the two populations was:

$$Yij = \mu + Ti + Bj + Eij$$

where:

μ = overall mean

$Ti = \mu_{i.} - \mu$ = effect due to the i-th treatment

$Bj = \mu_{.j} - \mu$ = effect due to the j-th block

Eij = random component explaining all extraneous variation

Analysis of variance was completed using an all random model, and the statistical analysis was completed using SPSS® 11 (2001). Table 2 shows the expected mean squares and degrees of freedom for the analysis of variance for C_0 vs. C_1 comparison.

Table 2. Expected mean squares and degrees of freedom for the analysis of variance for C_0 vs. C_1 comparison under growth chamber conditions at College Station, TX, at 14 days after seedling emergence in February 2004.

Source	df	SS	MS	EMS
Blocks	r-1	SSR	MSR	
Treatment	t-1	SSC	MSC	$\Sigma^2_\varepsilon + r\sigma^2_t$
Error	(r-1)(t-1)	SSE	MSE	Σ^2_ε

Seedling Vigor – Heritability Estimates

A broad-sense heritability (H^2) estimate of seedling vigor was produced by analysis of the variation present among HS families of the C_1 population. The linear model to be used for the heritability estimate was:

$$Y = \mu + R + B_{(R)} + G_{(B)} + E$$

where:

μ= mean

R= the effect of replication and is a random effect

$B_{(R)}$= the effect of blocks nested within reps and is a fixed effect

$G_{(B)}$= the effect of genotypes nested within blocks and is a fixed effect

E= error

The genetic variance is estimated using the variance component from the $G_{(B)}$ term, as this term is a direct estimate of the total genotypic variation (σ^2_G) in the population. Heritability estimates are the ratio between the genotypic and the phenotypic variances of the population evaluated. Heritability (H^2) for seedling vigor was estimated using the following formula:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + (\sigma^2_E/r)}$$

where:

σ^2_G is the genotypic variance;

σ^2_E is the error variance,

r is the mean of replications.

Analysis of variance was completed using a mixed model, and the statistical analysis of the data was performed using the statistical analysis program SPSS® 11 (2001). Table 3 shows the expected mean squares and degrees of freedom for the analysis of variance for heritability on seedling vigor.

Table 3. Expected mean squares and degrees of freedom for the analysis of variance for heritability on seedling vigor under growth chamber conditions at College Station, TX, at 14 days after seedling emergence in April 2003.

Source	df	SS	MS	EMS
Rep	r-1	SSR	MSR	$\Sigma^2_\varepsilon + r\sigma^2_{G(B)} + G\sigma^2B(r) + BG\sigma^2r$
Block (Rep)	(b-1)r	SSB(R)	MSB(R)	$\Sigma^2_\varepsilon + r\sigma^2_{G(B)} + G\sigma^2B(r)$
Genotypes (B)	(g-1)b	SSG(B)	MSG(B)	$\Sigma^2_\varepsilon + r\sigma^2_{G(B)}$
Error	(r-1)(g-1)b	SSE	MSE	Σ^2_ε

Results and Discussion

Relationship between Seed and Seedling Weight

Regression analysis between seed weight and seedling weight of the first set of evaluations using seed grown in Oklahoma indicated that there was no relationship between these two traits. Even though there was significance at the 0.001 level, the variation explained by the model was so small, it is meaningless (r^2=0.05; P<0.001). Of the 18 parental plants selected, 7 came from the heaviest seed-mass group, 10 from the middle seed-mass group, and 1 from the lightest seed-mass group (Table 1). Based on this single set of evaluations, it appears that seed weight cannot be used to select for increased seedling weight in this population. Perhaps in future experiments, the lightest seedlots could be left out of the evaluations, as this group only yielded one selection. Smart and Moser (1999) reported that seed size appeared to produce only slight differences in the morphological development of shoot and root systems, leaf area, shoot weight, and adventitious root weight from seedling emergence until 6 weeks of growth. They concluded that seed size in switchgrass appeared to have minimal long-term effect on the growth and development of seedlings. McLean and Nowak, (1997) indicated that while seed size is often highly correlated with seedling vigor, selection only for seed size is not likely to improve vigor.

If the seedling weights from the 2000 growth chamber study that was used to select superior plants from the C_0 plants in Oklahoma are compared with those from the 2003 growth chamber study used to evaluated the half-sib lines out of the C_1 population, it would appear that good progress was made in selecting for seedling size. The mean of

all C_0 seedlings was 0.014 grams seedling^{-1} (range 0.006 to 0.024), while the mean from the 18 superior parents was 0.021 grams seedling^{-1}. Comparable numbers from the C_1 seedlings are 0.029 (range 0.012 to 0.042), and 0.037 grams seedling^{-1}. These numbers indicate that seedling mass nearly doubled with one cycle of selection. However, this could be due to environmental factors affecting seed development and seedling growth. These differences could be because the seed were produced in different environments that include different soil types, year, moisture contents, and temperatures. Clapham et al. (2000) conducted a study to determine whether the maternal thermal environment of developing seeds influenced subsequent plant development in white lupine (*Lupinus albus* L.). They grew plants and produced seed at two different temperatures (13 and 28°C) inside a growth chamber for three generations. They concluded that thermal environment during seed development can affect subsequent crop performance, which can impact cultivar production and consistency among years. This suggests that in future studies, populations should be grown at a same environment and they must be treated the same way, because any variation could affect the performance of the crop.

Gain from Selection from C_0 to C_1

When seed from the C_0 recreated population was compared with seed from the C_1 population in the same growth chamber, variation due to cycle of selection was significant ($P<0.02$), indicating that differences in the performance of the cycle were present (Table 4). The blocking term was significant ($P<0.05$), which indicates that differences in the performance of blocks were present. Frio buffelgrass was included to

measure non-genetic variation. Since it is an apomictic, any variation among seedlings in the growth chamber is presumably due to environmental factors, experimental error, or effects of nutrition. Variation inside a growth chamber can have a significant effect on seedling growth, as expressed in the buffelgrass (0.041-0.068 g); indicating that variation does occur in these chambers (Table 5). However, nutrition could have caused the variation that occurred in buffelgrass. When fertilizing the seedlings, it was done with a watering can and unequal amounts of fertilizer could have been applied to each seedling. This could have been avoided by giving the seedlings the same amount of fertilizer using a measuring device. Interestingly, considerably less variation was observed in Alamo and the C_0 and C_1 seedlings (Table 5).

Comparing the mean of the C_0 recreated and C_1 populations indicates that progress in increasing seedling weight was not made from one cycle of HS selection (Table 5). The mean of the C_0 recreated seedlings (0.019 grams seedling^{-1}) was heavier than the C_1 population seedling mean (0.016 grams seedling^{-1}) (Table 5). The range of seedling mass for the seedlings from the three switchgrass populations used was 0.013 to 0.024 g seedling^{-1} (Table 5).

The findings of this section of the study indicate that selection for seedling weight using HS population improvement techniques is not effective after only one cycle of selection. There are several possible explanations for this. First, the evaluation of gain from selection from a single cycle of selection is often risky and does not always produce the desired effect. In many long term selection studies, gain from selection

Table 4. Analysis of variance for C_0 vs. C_1 comparison under growth chamber conditions at College Station, TX, at 14 days after seedling emergence in February 2004.

Source	df	SS	MS	F	Significance
Blocks	5	.000	2.35×10^5	7.255	0.024
Cycle	1	3.72×10^5	3.72×10^5	11.465	0.02
Error	5	1.62×10^5	3.24×10^5		

Table 5. Mean weight for six repetitions and overall mean weight for Frio buffelgrass, Alamo switchgrass, and the means of Cycle 0 and Cycle 1 under growth chamber conditions at College Station, TX, at 14 days after seedling emergence in February 2004.

Cycle of selection	Repetition in the same growth chamber	Mean dry weight of 50 seedlings in gm
Buffelgrass	1	0.068
	2	0.045
	3	0.042
	4	0.053
	5	0.041
	6	0.042
	Mean	0.049
Alamo	1	0.020
	2	0.017
	3	0.017
	4	0.017
	5	0.022
	6	0.016
	Mean	0.019
C_0	1	0.024
	2	0.021
	3	0.013
	4	0.021
	5	0.023
	6	0.015
	Mean	0.020
C_1	1	0.019
	2	0.017
	3	0.013
	4	0.013
	5	0.019
	6	0.014
	Mean	0.016

is clearly seen as a general trend. However, if the gain from one cycle to the next is randomly selected and used to measure progress, trends often are not clear (DeHaan et al., 2001). In many cases, the results mirror the findings observed in the current study. Therefore, it is critical to continue selection for at least two cycles to allow for the trends to be seen.

Another possible cause for these findings is the environmental variation for the locations where the seed were produced. The plants that produced the seed for the C_1 population were grown on a Booneville fine sandy loam soil, while seed of the C_0 population had to be recreated and these plants were grown on a Norwood silty clay loam. The Boonville fine sandy loam has a thin top soil with an impermeable clay pan; whereas, the Norwood silty clay loam is a deep fertile soil. Another difference is that the C_1 population was grown in a space planted nursery with 1.2 m in between plants; whereas, the C_0 recreated population was grown in a spaced planted nursery with 2.1 m between plants. This also could have affected seed development because the plants from C_0 had less competition and/or more nutrients. However, this should have been detected in weighing the seed and determining if there were differences in seed weight. However, this was not done in this study. If seed quality, not seed mass, affects seedling mass, it appears that a valid comparison may only be made when seed is grown in the same environment.

Another factor that could have had a large impact on this comparison is that the C_0 population had to be recreated from remnant seed. Given that this seed had deteriorated in storage and only a small percentage of the seed germinated, it would be

logical to conclude that only the highest quality seed (those that might produce superior seedling vigor) had survived several years of storage. If this is true, then this storage process may have indirectly selected for seed with the best seedling vigor.

Heritability Estimates

In this study, heritability estimates were based on the variation among HS families in the C_1 population (Table 4). In the HS progeny evaluation, significant variation was detected among HS families (Block) and among Blocks within Replications (Table 6). The variation among replications was not significant. The significant Blocks within Replication term is important because it indicates that spatial variation occurred within the replications. These results can have an effect on selection based on the mean (see previous selection). However, significant variation due to blocking does not affect variance estimates (Hallauer and Miranda, 1981).

Based on the analysis of variance, total genetic variance was estimated to be 1.034 and total error variation was estimated to be 6.801 resulting in a broad-sense heritability estimate of 0.60 with a standard error of 2.663. This heritability is moderate to high relative to other estimates of heritability for other traits in switchgrass. Eberhart and Newell (1959) calculated broad-sense heritabilities for switchgrass forage quality and seed quality (seed yield) to be 0.038 and 0.77, respectively. They also calculated broad-sense heritabilities for height of leaves, plant height, and plant yield as 0.92, 0.90, and 0.78, respectively. Vogel et al. (1981) calculated realized heritabilities for high and low IVDMD as 0.55 and 0.59, respectively.

Conclusions

Considering that there was no gain in selection from C_0 to C_1 but the H^2 was estimated at 0.60, these results seem to be contradictory. There are several possible explanations for these observations. First, there were differences in the selection environments which may have influenced selection and variance. The initial population was originally grown in Oklahoma and the C_0 selections were based on seed grown and collected in Oklahoma. Because the environment in which the seed were produced can have an effect on seed development, it is realistic to expect this to be a factor in this study. Additional cycles of selection should result in additional gain from selection. DeHaan et al. (2001) studied the potential of recurrent phenotypic selection under greenhouse conditions to increase total plant weight and reduce root/shoot ratio to increase seedling vigor in kura clover (*Trifolium ambiguum* M. Bieb.). The greatest increases in fresh shoot and total plant weight were obtained in the third cycle of selection.

A second possible explanation for the inconsistency between the heritability estimate and gain from selection results is specific to the heritability estimate. A broad-sense heritability estimate contains total genetic variance, of which only a portion is selectable. Included in this estimate are not only additive genetic variance but also dominance and epistatic variance. Of these sources of variance, only the additive and a small portion of the epistatic variance are responsive to selection. Thus, broad-sense heritability overestimates the amount of useful genetic variance present in a population.

Table 6. Analysis of variance for the heritability study on seedling vigor in a growth chamber for the C_1 cycle of selection at College Station, TX, at 14 days after seedling emergence in April 2003.

Source	df	SS	MS	F	Significance
Rep	3	.001	1	.898	.461
Blocks (Rep)	18	.006	1	13.409	.000
Genotypes (Block)	120	.008	6.8×10^{-5}	2.554	.000
Error	344	.009	2.66×10^{-5}		

In addition, several researchers have studied emergence and seedling vigor from agronomic and genetic viewpoints. They found that additive effects are more important than dominant effects for germination and seedling vigor under cold conditions in maize (*Zea mays* L.) (Haskell and Singleton, 1949; Ventura, 1961; Grogan, 1970; Eagles, 1982; Ajala and Fakorede, 1988).

Finally, directional selection may have increased linkage disequilibrium. Increased linkage disequilibrium underestimates additive genetic variance and overestimates the dominance variance (Falconer, 1989). While this is a possibility, there is no effective method of testing and evaluating its feasibility. Compared to the previous two possibilities, this is the least likely.

CHAPTER III

METHOD OF POLLINATION IN SWITCHGRASS

Literature Review

An understanding of the mode of pollination of a species is necessary for breeding and genetic improvement. Some species are predominantly self-pollinated and have evolved given the constraints and benefits of self-pollination. Other species have evolved as cross-pollinating species. However, some species have the capacity to produce seed under both self- and cross-pollinated conditions.

Many different mechanisms promote cross-pollination such as separation of the male and female plants, monecious plants with separate flowers on different parts of the same plant, non-synchronous anthesis in perfect flowers, and self-incompatibility mechanisms. Of these, the self-incompatibility mechanisms are among the most interesting and complex systems.

Self-incompatibility

Self-incompatibility (SI) prevents inbreeding in flowering plants by preventing the fertilization of an egg cell in the embryo sac in the ovule by a sperm nucleus from pollen from the same plant. There are many different forms of SI, but they can all be grouped as either gametophytic or sporophytic. Gametophytic incompatibility occurs when the phenotype of the pollen is determined by its own alleles, and sporophytic

incompatibility is present if the phenotype of the pollen is determined by the genotype of the plant producing pollen.

In each of these systems, different mechanisms control pollination. In most gametophytic SI systems, pollen that is incompatible germinates successfully on the stigma surface, penetrates the stigma, and grows into the style. At this point, the tube grows between the longitudinal files of cells of the central transmitting tract, and eventually, pollen tube growth through the transmitting tract toward the ovary is arrested (de Nettancourt, 1977). In the sporophytic SI system, pollen may germinate, but pollen tube growth is usually arrested on the surface of the sigma (Allard, 1964; Brewbaker, 1957; de Nettancourt, 1977; Poehlman and Sleper, 1995).

The incompatible reactions described above are genetically controlled. The genetics can be simple and qualitative or very complex involving several loci which are influenced by the environment. The simplest systems are single gene gametophytic SI. If the pollen parent's genotype is S_1S_2, this plant will produce S_1 and S_2 pollen. When the allele in a haploid pollen grain matches any allele in the diploid tissue of the style, pollen tube growth is arrested. For example, both S_1 pollen and S_2 pollen are inhibited in an S_1S_2 style; whereas, S_2 pollen will grow in a S_1S_3 style. When there is no match of the alleles (e.g., pollen grains from an S_1S_2 plant on an S_3S_4 pistil), the pollen tubes of both genotypes will grow through the style, ovary, and into the embryo sac (Newbigin et al., 1993).

A simple single-gene sporophytic system is similar but is governed by the parental phenotype of both the male and female gametes. If the genotype of the pollen

parent is S_1S_2, all pollen grains have this phenotype regardless of whether they possess an S_1 or S_2 allele in the nuclear genome. When an allele in the pollen parent matches that of the pistil (e.g., S_1S_2 or S_1S_3), pollen germination is arrested on the stigma surface. Where there is no match with the pistil (S_3S_4), the pollen will germinate and grow through the style, ovary, and into to the embryo sac (Newbigin et al., 1993).

The most common SI system in grasses is controlled by two independently segregating, polyallelic genes, S and Z (Lundqvist, 1956; Hayman, 1956; Hayman, 1992). When a pollen grain has the same alleles as the style, it is incompatible. The S-Z self-incompatibility system is not broken down by polyploidy. In polyploids, all the alleles of the pollen do not need to be matched in the style to produce an incompatible reaction. Any S-Z allelic combination present in the pollen grain, which is also present in the style, is sufficient to produce incompatibility (Lundqvist, 1957).

A pollen grain is specified gametophytically by the complementary interaction of its S and Z genes. A pollen grain is incompatible when both the S and Z genes it possesses are present in the stigma on which it comes in contact with. A pollen grain with only a S or Z gene in common with the stigma expresses no impairment in its compatibility and is fully functional (Hayman, 1992).

Pollen Tubes

The pollen tube wall consists of two main layers of polysaccharides. The inner callosic wall contains mostly 1,3-β-glucan (Stone and Clarke, 1992), and the outer wall contains predominantly arabinan consisting α-L-arabinofuranosyl residues in 1→5

linkage, with some branching through C(O)2 and/or C(O)3 (Rae et al., 1985). The inner glucan layer fluoresces when it comes in contact with aniline blue stain allowing the pollen tubes to be visible, when observed with ultraviolet light (wavelength of 365 nm).

Compatibility in plants can be estimated by the behavior of the pollen grains. Compatible and incompatible pollen grains germinate similarly, and the pollen tubes of both penetrate the stigma surface. Compatible pollen tubes grow into the style and down towards the ovary. Callose is deposited inside the pollen tube. The nuclei in the pollen grain enter the pollen tube. Incompatible pollen tubes may cease growth in: 1) the stigma, 2) the style; and 3) the ovary. If the pollen tube does not grow into the micropyle, then the pollen grain does not release its contents and fertilization does not occur (Mogensen, 1990; Hayman, 1956).

Heslop-Harrison and Heslop-Harrison (1982) provided a basis for interpreting some of the principal features of the self-incompatibility response. In the physiological features they included four essential characteristics. First, the S, Z allele combination does not significantly affect the initial hydration and germination of the pollen. Second, in an incompatible combination, although hydration and germination are not affected simply by contact between the exine and the stigma, the growth of the tube ceases when the tip touches the stigma surface, or very soon thereafter. Third, the arrest or retardation of growth in an incompatible tube is associated with the formation of nodules, seemingly composed of microfibrillar pectins, in the wall at the extreme apex. Finally, starch and lipid degradation and the continued deposition of callose, the general

metabolism of the male gametophyte are not blocked following arrest of tube growth in an incompatible pollination.

Burson and Young (1983) investigated the impediments to hybridization between different *Panicum* species. Accessions of *P. antidotale* Retz. (blue panicgrass), (kleingrass), and *P. deustum* Thunb. were self- and cross-pollinated. When self-pollinated, pollen germination was approximately 90% for each species and germination occurred shortly after pollination. Pollen tubes grew to the micropyle within 1 hour after pollination in kleingrass and 2 hours in blue panicgrass and *P. deustum*. One *P. deustum* accession (PI 364953) was self-incompatible. When the species were crossed with one another, the pollen germinated shortly after pollination, and overall germination ranged from 77 to 88% for the different crosses. In crosses between kleingrass and *P. deustum* essentially all of the tubes failed to grow beyond the stigmas. However, in crosses between blue panicgrass and *P. deustum*, the tubes grew into the styles but not into the ovary. In the kleingrass x blue panicgrass crosses, they grew into the ovary, but the tubes became disoriented and grew in a random manner, never entering the micropyle. They concluded that different cross-incompatibility systems are responsible for the failure to produce hybrids among these *Panicum* species.

Burson (1987) also investigated the reason for low crossability between *Paspalum* species. Pollen germination and tube growth were examined in self- and cross-pollinated pistils of *Paspalum intermedium* Munro. ex Morong, *P. jurgensii* Hackel and *P. dilatatum* Poir. When self-pollinated, pollen germination ranged from 57 to 80% for all species. However, most of the *P. intermedium* tubes did not grow beyond the stigma. Apparently stylar-incompatibility is the reason for the low seed set when this species is self-pollinated. When the three species were cross-pollinated, germination ranged from 57-88% and the pollen tubes grew to the micropyle indicating that cross-incompatibility is not the cause for low hybridization among the species. Examination of the post-fertilization events in the embryo sacs revealed that the gametes usually failed to unite and this is a primary reason for low crossability.

Martinez-Reyna and Vogel (2002) investigated incompatibility in switchgrass. Seed set and seed characteristics of reciprocal matings of tetraploid, octaploid, and tetraploid x octaploid were used as a measure of incompatibility. They concluded that there are pre- and post-fertilization incompatibility systems in this species; however, they also indicated that additional research is needed to fully resolve them.

Seed Set in Switchgrass

Talbert et al. (1983) tested the assumption that switchgrass is predominately a cross-pollinated species. They compared the seed yields of bagged (self-pollinated) inflorescences with that of open-pollinated inflorescences for 33 plants. The bagged inflorescences yielded on the average less than 1% of the seed as that of the open-pollinated inflorescences. Taliaferro and Hopkins (1996) also determined seed set for switchgrass when self- and open-pollinated, and they reported similar findings. Martinez-Reyna and Vogel (2002) reported the seed set for tetraploid and octaploid plants under self-pollinated conditions were 0.35 and 1.39%, respectively.

The objective of this study was to observe pollen germination and tube growth under self- and cross-pollinated conditions to determine the actual method of pollination of switchgrass.

Materials and Methods

Plant Material

Six different plants of Alamo switchgrass were used in this study. These plants were dug from the field in December 2002 and cloned vegetatively to ensure adequate plant material. Individual clones were planted into 3.7 L pots containing a commercial potting mix Ready Earth®. These clones were grown in a greenhouse at College Station, TX under a 14-hr photoperiod (6:00 am-8:00 pm) and a mean temperature of 30°C. Mercury-vapor lamps were used to extend the day length when it was less than 14 hours. The plants were watered daily or as needed. Fertilizer was applied using both a soluble and a granular form. The soluble form was a solution of 6 gm L^{-1} of water soluble fertilizer 20-9-17 (N-P-K) and it was applied every 30 d during the growing season. The growing season consisted of the time after clipping until the time of flowering. Osmocote® 13-6-11 (N-P-K) was the granular form of fertilizer, and 5 g were applied when the plants were clipped after flowering. After flowering was initiated, some of the inflorescences were used for pollinations (see next section). When flowering was completed, the plants were clipped at a height of 20 cm and re-fertilized. The same plants were kept in the greenhouse and were clipped several times (March, July, and September 2003).

Emasculation

The plants used as the female parents in the crosses were emasculated shortly before anther exsertion. This was usually done between 8:00 and 10:00 AM, prior to pollen shed in the greenhouse. The plants used as the female parents were moved to a different area in the greenhouse to prevent them from being pollinated with pollen from other plants. Branches on the same panicle with florets at a similar stage of development were selected for emasculation. Prior to anther exsertion from the floret, the lemma and palea were gently pulled apart using forceps and the anthers were removed without damaging the stigmas.

Spikelets with florets that had already extruded anthers and those that opened shortly after pollination were removed from the panicle to avoid contamination. Martinez-Reyna and Vogel (1998) had to emasculate and/or remove the lower floret to avoid self-pollination. However, this was not a concern in this study because only the upper florets were used and they were already removed before the lower florets opened. If a floret opened after pollination, it was removed to avoid self-pollination. This frequently occurred which reduced the number of florets that could be analyzed.

Pollination

The pollination technique used is described by Martinez-Reyna and Vogel (1998). In switchgrass pollen shedding occurs typically between 10:00 and 11:00 AM. Mature anthers were collected from the male parent, placed into a petri dish, and shaken to promote pollen shed. This pollen was used to pollinate the emasculated florets.

Pollination was accomplished by touching the stigmas of emasculated florets to the pollen in a petri dish.

Switchgrass plants were expected to flower primarily in the morning as reported by Jones and Newell (1946); however, when grown in a greenhouse at College Station, the plants flowered throughout the day which made it very difficult to control pollinations. It was necessary to closely monitor the inflorescences in which controlled pollinations had been made. Those florets that initiated flowering after the pollinations had been made were removed from the inflorescences and discarded because they could contaminate the pollinated florets.

Pollen Tube Detection

At one-hour intervals after pollination, 10 spikelets from each self- and cross-pollination were removed from the inflorescences. These spikelets were fixed in FAA (18:1:1, 70% ethanol; glacial acetic acid; formaldehyde) for at least 30 minutes and stored in 70% ethanol. Pollen germination and tube growth were determined by using a modified version of the technique reported by Kho & Baer (1968). Pistils were dissected from the florets inside the spikelet, placed in 1N NaOH for 30 minutes, and transferred into a 0.1% aniline blue solution for 30 minutes. They were placed on a microscope slide, cover slipped, and examined with a Zeiss universal microscope® equipped with a F1 Epi-fluorescence condenser with an Osram® HBO 50W high pressure Hg lamp. The excitation wavelength for this light was 365 nm and an emission wavelength of 420 nm.

Pollen tube germination was determined by counting the actual number of germinated and non-germinated pollen grains on the stigmas and the percent germination was calculated. Figure 2 shows how the distance of tube growth into the pistil was recorded based on the number of pollen tubes that had grown into the (1) stigma, (2) style, (3) ovary, and (4) micropyle.

Bagged Inflorescences

Seed set under self-pollinated conditions was also used to determine the mode of pollination of the six switchgrass plants. Panicles from each plant were self-pollinated by enclosing an inflorescence into a pollination bag prior to anthesis. Each bagged panicle was supported with a bamboo stake to prevent the culm from breaking. During anthesis, the bags were shaken daily to facilitate pollen dispersal inside the bag to enhance pollination. After the seed had matured, self-fertility was estimated by counting the number of florets with developed seed and the total number of florets. Self-fertility was measured as the percentage of seed set in the panicle. The results were then used for comparison to the information obtained from the pollen tube study.

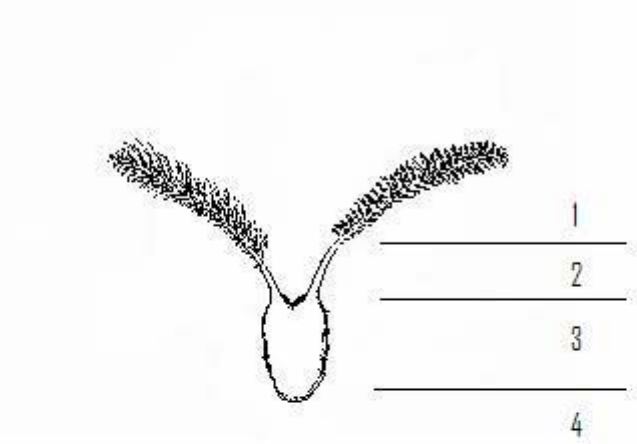

Figure 2. Switchgrass pistil showing pollen tubes recorded to: (1) stigma, (2) style, (3) ovary, and (4) micropyle.

Results and Discussion

Self-pollination

Pollen tubes were observed within one hour after pollination in all plants. Mean pollen germination under self-pollinated conditions was 73, 69, 70, 73, 40, and 78% for clones 2, 3, 5, 6, 7, and 8, respectively. The grand mean for total pollination across all entries was 67% (Table 7). Pollen germination was not associated with time after pollination because the germination percentage was similar at 1, 2, and 3 hr after pollination, meaning the germination percentage did not increase with time after pollination.

Following pollen germination, the tubes immediately elongated and penetrated the stigma papillae where they continued growing through the stigma branches and into the central axis of the stigma. However, none of the pollen tubes reached the style with the exception of plant 3. This plant had 6 tubes in the style and 2 tubes in the ovary at 2 hr after pollination, but none of the tubes had reached the micropyle (Table 7). This means that plant 3 is either slightly self-compatible or there was some contamination with pollen from a different plant. Because no seed were produced on the bagged inflorescences of plant 3 (see next section), this must be due to contamination. The remaining switchgrass entries had a few pollen tubes growing into the style at 3 hours after pollination but none had tubes in the ovary (Table 7).

Findings reported by Shivanna et al (1982) are similar to these findings. Working with *Gaudina fragilis* (L.) Beauv. and *Secale cereale* L., they found that incompatible pollen grains germinated normally, but the tubes were arrested at the stigma surface. However, pollen tubes of *Alopecurus pratensis* L. grew into the transmitting tracts of the stylodia before they were inhibited. They concluded that species vary in the strength of their self-incompatibility response.

Findings from microscopic analysis of pollen tube growth in self-pollinated panicles of switchgrass demonstrate that pollen tubes do not grow to the micropyle, indicating that self-fertilization must be low if not absent altogether. Talbert et al. (1983) and Taliaferro and Hopkins (1996) reported similar results; however, they only bagged inflorescences and compared the percentage of seed set of self-pollinated inflorescences (1%) with those that were open-pollinated.

Table 7. Pollen germination and tube growth of self-pollination switchgrass plants under greenhouse conditions at College Station, TX, during February, June, and October 2003.

Accession	Time after pollination	Pistils observed	Pollen Grains			Pollen Germination	Tubes to:		
			Germinated	Not Germinated	Total		Style	Ovary	Micropyle
	Hours	----------No.----------				%	----------No.----------		
Self 2	1	8	238	92	330	72	0	0	0
	2	9	570	272	842	68	0	0	0
	3	10	483	126	609	79	5	0	0
Self 3	1	10	190	90	280	68	0	0	0
	2	10	157	105	262	60	6	2	0
	3	9	111	32	143	78	1	0	0
Self 5	1	9	253	136	389	65	0	0	0
	2	10	330	135	465	71	0	0	0
	3	10	327	109	436	75	1	0	0
Self 6	1	10	327	118	445	73	0	0	0
	2	10	431	156	587	73	0	0	0
	3	10	563	198	761	74	1	0	0
Self 7	1	10	102	260	362	28	0	0	0
	2	10	40	100	140	29	0	0	0
	3	9	136	82	218	62	0	0	0
Self 8	1	10	345	124	469	74	0	0	0
	2	10	281	75	356	79	0	0	0
	3	10	332	73	405	82	0	0	0

Bagged Inflorescences

Individual inflorescences of the six switchgrass plants were enclosed in glassine bags prior to anthesis to determine if seed would be produced when self-pollinated. Approximately 4 weeks after anthesis, the bags were removed and the inflorescences were hand thrashed to determine seed set. A number of mature fertile florets with large, indurate lemmas and paleas tightly clasped together along their outer edges appeared to be seed. However, upon opening these florets, it was determined that they did not contain an embryo or any endosperm. Thus, caryopses were not produced under self-pollinated conditions for any of the six plants indicating that these plants are self-sterile (Table 8). This was expected because the pollen tubes of these same plants failed to grow into the ovary and fertilization of the gametes did not occur when self-pollinated (Table 7).

Table 8. Seed set under self-pollinated conditions of switchgrass plants under greenhouse conditions at College Station, TX, February 2004.

Plant	Number of fertile florets pollinated	Number of Caryopses
2	452	0
3	541	0
5	632	0
6	589	0
7	378	0
8	471	0
Mean	510.5	0

Cross-pollination

Because of the irregular flowering of the switchgrass plants used, it was not possible to use all of them. Only plants 2, 3, 5, and 8 were used for cross-pollination and the crosses made were 2 x 3, 3 x 2, 5 x 8, 8 x 5, 2 x 8, and 8 x 2.

Pollen tubes were observed within one hour after pollination in all cross-pollinations. Pollen germination ranged from 14 to 78% with a mean of 65% (Table 9). Pollen germination does not appear to be associated with time after pollination as the germination percentage was similar at 1, 2, and 3 hours after pollination. This same behavior as was observed in the self-pollinated pistils and the mean percent pollen germination under self- and cross-pollinated conditions were similar, 68% and 65% respectively.

After the pollen germinated, the tubes immediately elongated and penetrated the stigma papillae. They continued growing through the stigma branches and into the central axis of the stigma. In most cases, the tubes grew through the stigmas into the styles more rapidly when cross-pollinated than when self-pollinated. This was true for all crosses except for the 3 x 2 cross, where only one pollen tube had grown into the style at 1 hour after pollination. Interestingly, pollen germination in this cross was greatly reduced (Table 9). None of the pollen tubes in this cross grew into the ovary. The lack of tube growth in the 3 x 2 cross may be due to the low number of pollen grains on the pistils. However, a more likely possibility is that an incompatibility mechanism exists between plants 2 and 3. Interestingly, pollen tube growth in the reciprocal cross (2 x 3) differed greatly from that in the 3 x 2 cross (Table 9). Over time, the number of

pollen tubes growing into the ovary and micropyle increased. These findings are similar to those reported by Shafer et al. (2000) regarding stigma receptivity and seed set in buffelgrass. They also examined tube growth at one hour intervals after pollination and the number of tubes reaching the style, ovary, and micropyle increased with time.

For all switchgrass crosses (except 3 x 2), pollen tubes grew into the micropyle within 2 or 3 hours following pollination and in some cases within 1 or 2 hours (Table 9). These results are similar to those reported by Burson (1987), where recorded the same amount of time was required for the tubes to grow to the micropyle in interspecific crosses among three *Paspalum* species; however, in two of the crosses, tubes reached the micropyle within 30 to 45 min.

In the 5 x 8 cross, the number of tubes growing into the ovary and micropyle increased as time after pollination increased. However, there is not a big difference between 2 and 3 hours after pollination. This could be due to the number of pistils examined and the number of pollen grains on each pistil. At 2 hours after pollination, the number of pistils examined was nine; whereas, at 3 hours the number of pistils examined was eight. This undoubtedly affects the number of pollen tubes present because the more pistils examined, there should be a greater number of pollen tubes present.

These findings regarding pollen germination and tube growth support what is reported in the literature regarding the mode of pollination of switchgrass. The reason why very little if any seed are produced when self-pollinated is because the pollen tubes

from the same plant do not grow beyond the style. When cross-pollinated, the tubes of most genotypes readily grew into the ovary and micropyle.

Table 9. Pollen germination and tube growth of reciprocal cross-pollinations between switchgrass plants under greenhouse conditions at College Station, TX, during February, June, and October 2003.

Accession	Time after pollination	Pistils observed	Pollen Grains			Pollen Germination	Tubes to:		
			Germinated	Not Germinated	Total		Style	Ovary	Micropyle
	Hours		----------No.----------			%	----------No.----------		
3X2	1	9	17	64	89	19	1	0	0
	2	9	7	35	42	17	0	0	0
	3	7	5	62	67	7	0	0	0
2X3	1	8	368	109	477	77	11	2	2
	2	7	136	95	231	59	17	14	8
	3	10	318	70	388	82	45	36	24
5X8	1	10	391	91	482	81	1	0	0
	2	9	434	124	558	78	53	34	23
	3	8	329	106	435	76	45	34	23
8X5	1	7	488	177	665	73	22	3	0
	2	7	716	86	802	89	73	53	3
	3	10	433	129	562	77	85	71	32
2X8	1	10	310	167	477	65	12	0	0
	2	10	338	113	451	75	31	23	10
	3	10	290	40	330	88	44	21	12
8X2	1	10	61	63	124	49	7	3	2
	2	10	182	87	269	68	6	3	1
	3	10	482	71	553	87	26	23	8

CHAPTER IV

CONCLUSIONS

One cycle of selection was not successful in improving seedling vigor in switchgrass. However, progress for the second cycle of selection remains to be evaluated and should reveal if selection for improving seedling vigor is possible using this procedure. Based on the variability observed in seedling mass of all half-sib families, from the one completed cycle of selection, it appears as though a positive response to seedling mass should be possible with another cycle of selection using a half-sib methodology.

The broad-sense heritability estimate was moderate to high. The calculated broad-sense heritability was $H^2 = 0.60$. However, this is somewhat difficult to measure because the initial population used was produced in a different environment which could affect the heritability estimate. Also, the broad-sense heritability has all the genetic parameters including those that are not heritable.

Several activities could have affected the success of selection. Among the factors that may have influenced the outcome of this research are the selection pressure used, location of experiment, and the environment that the various cycles were grown. The selection of the half-sibs from Oklahoma was done without the use of blocks inside the growth chamber. This could affect the selection process because spatial variation was present; therefore, selection of the half-sibs may have been due to the environment instead of genetics.

If a more strict selection pressure had been applied (selecting the top 5% instead of 15%), greater advances could have been made, but perhaps more half-sib families would have had to been evaluated so as to not restrict the population sizes. Perhaps if all populations would have been compared in the same environment, more of the differences would have been genetic factors. Other selection procedures (other than half-sib) might have resulted in more progress. Also controlling crosses in the field, although this is more time consuming, may have provided better estimates for heritability. Even though seed mass and seedling mass were not highly correlated, there may have been merit in not including the lightest 1/3 of the seed-mass lot, as only one plant was selected from this group. This would have allowed for a higher number of the heavier seedlots to have been screened, and perhaps may have made more progress in selection.

The manner in which fertilizer was applied to the seedlings in the growth chamber could have influenced the results. By applying the fertilizer with a watering can the amount of water (with soluble fertilizer) applied was not the same for all seedlings. This would have an effect on seedling growth. A better approach would be to use a measuring device, which would ensure that the same amount of nutrients was applied to each seedling.

Seedlings were grown in Scotts Ready Earth® potting mix in the growth chamber for all experiments. This was done to minimize the differences in growing media among plants. However, if the seedling were grown in field soil, they may have responded differently.

These results confirm what has been suggested by others and that is switchgrass is self-incompatible. When self-pollinated in the greenhouse, the pollen tubes did not grow into the ovary. However, when the same plants were cross-pollinated in a greenhouse, the pollen tubes grew into the micropyle. It appears that in switchgrass a self-incompatibility mechanism prevents the pollen tubes from growing beyond the style in the pistil and this is why self-pollinated seed are not produced.

In a cross-pollinated species, dominant effects are larger than additives effects; therefore, selection is more difficult. This could be a possibility why we did not make large gains in selecting for seedling vigor; however, future cycles of selection could show a positive response.

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APPENDIX

Table A-1. Seedling weight at 14 days after emergence of C_0 population. Seed was provided by C.M. Taliaferro, Oklahoma State University.

OSU designation	Rep	Plant	Average weight in grams seedling^{-1}
SL93	3	9	0.024479
SL94150	4	6	0.024200
SL94150	1	8	0.022432
SL93	4	7	0.022233
SL92150	4	8	0.022173
SL93	4	10	0.021879
SL94	2	1	0.021545
SL94150	1	9	0.021116
SL94	3	2	0.020620
ALAMO	1	8	0.020605
SL94150	3	9	0.020516
SL93	1	1	0.020365
SL93	2	8	0.020323
SL94150	2	4	0.020300
SL92150	4	1	0.020109
ALAMO	2	6	0.019821
SL94150	3	3	0.019777
SLCO	2	6	0.019604
ALAMO	4	6	0.019593
ALAMO	1	9	0.019453
SL92150	4	2	0.019156
SL92150	1	2	0.019116
SLCO	3	7	0.019108
SL92150	4	1	0.019091
ALAMO	4	6	0.019028
ALAMO	1	5	0.018911
SLCO	3	3	0.018726
SL92150	3	10	0.018580
SLCO	4	3	0.018524
ALAMO	4	4	0.018263
SL93	1	7	0.018258
SLCO	4	6	0.018169
SL94150	4	10	0.018039
SLCO	3	6	0.017928
ALAMO	3	1	0.017851
SLCO	4	8	0.017847
SLCO	3	5	0.017667
ALAMO	3	1	0.017611
SL92150	4	5	0.017610
SL93	3	1	0.017600

SL93	2	4	0.017600
SL94	4	2	0.017512
ALAMO	4	9	0.017471
SL94150	3	10	0.017165
SL94	1	3	0.017070
SL93	1	3	0.017060
SL92150	3	6	0.017045
ALAMO	2	7	0.016945
ALAMO	2	3	0.016909
ALAMO	4	8	0.016889
SL92150	1	4	0.016865
SL94150	1	4	0.016784
SL94	2	10	0.016671
SL93	4	2	0.016548
SLCO	3	9	0.016413
SL92150	4	3	0.016242
ALAMO	3	6	0.016231
SL92150	3	5	0.016206
SL93	3	8	0.016168
SL92150	4	10	0.016148
SLCO	2	3	0.016143
SL92150	2	4	0.016136
SL94	2	4	0.016125
ALAMO Check			0.015910
SL94150	1	1	0.015886
ALAMO	4	1	0.015733
ALAMO	3	8	0.015726
SLCO	1	6	0.015623
SLCO	1	7	0.015524
SL94	4	7	0.015413
SL94	3	6	0.015196
ALAMO	2	9	0.015135
ALAMO	4	5	0.015134
SL92150	3	4	0.015133
SL92150	2	1	0.014970
ALAMO	4	1	0.014945
SL94150	3	5	0.014923
SL94150	3	3	0.014915
SL93	4	7	0.014689
SL94	2	5	0.014682
ALAMO	1	9	0.014587
SL94	3	4	0.014477
SLCO	4	9	0.014237
SLCO	2	9	0.014214
SL93	1	5	0.014084
SLCO	4	1	0.013987
SL94150	4	1	0.013970
SL92150	3	9	0.013955

SL92150	2	8	0.013900
SL94	1	10	0.013897
SL93	4	3	0.013896
SLCO	3	10	0.013879
SLCO	1	3	0.013857
SLCO	4	10	0.013823
SL93	4	1	0.013805
SL93	1	10	0.013754
SL94	2	7	0.013650
SL94	3	7	0.013608
SL94	2	9	0.013603
SL92150	4	3	0.013462
ALAMO	2	10	0.013223
SLCO	3	9	0.013209
SL94150	4	1	0.013079
ALAMO	1	7	0.013072
SLCO	1	5	0.013055
SL93	4	2	0.013051
SLCO	2	4	0.013006
SL92150	4	2	0.012920
SL93	2	6	0.012488
SL94	4	5	0.012435
ALAMO	2	6	0.012300
ALAMO	1	10	0.012117
SL94150	1	8	0.012070
SLCO	1	9	0.012056
SL94150	1	2	0.012029
ALAMO	1	1	0.011983
ALAMO	4	3	0.011929
SL92150	2	5	0.011863
SL92150	4	7	0.011750
SL93	3	6	0.011722
SL92150	3	5	0.011653
SLCO	1	7	0.011559
SLCO	3	1	0.011433
SLCO	3	8	0.011382
SL94	4	9	0.011367
SL94150	4	9	0.011300
SL93	4	9	0.011266
SL94150	3	7	0.011229
SL94150	1	6	0.011212
SL92150	3	8	0.011176
SL92150	2	3	0.011126
SL93	3	8	0.011043
ALAMO	2	1	0.010967
SL93	4	8	0.010940
SL93	4	1	0.010865
SL93	1	8	0.010709

SL93	2	1	0.010695
ALAMO	4	4	0.010621
ALAMO	3	9	0.010579
SL94150	2	7	0.010529
SL94	2	3	0.010450
SLCO	2	8	0.010290
SL94150	2	3	0.010288
SLCO	4	2	0.010242
SL94	4	6	0.010120
ALAMO	3	3	0.010112
SL94150	4	6	0.010052
SLCO	2	10	0.010020
SLCO	1	8	0.010011
ALAMO	4	10	0.009960
SL94	4	3	0.009947
SL94	2	3	0.009912
SL92150	2	1	0.009912
SL92150	2	9	0.009841
ALAMO	3	6	0.009706
ALAMO	2	7	0.009688
SL94150	1	7	0.009667
ASLCO	3	8	0.009634
SL94	4	5	0.009600
ALAMO	1	3	0.009481
SL94	1	2	0.009450
SL93	4	6	0.009329
SL94150	3	8	0.009133
SL94	2	4	0.008967
SL94	1	5	0.008957
SL94150	4	5	0.008951
SL92150	3	7	0.008700
SL93	4	8	0.008689
SL92150	3	3	0.008672
SL94	4	6	0.008537
SL94150	4	3	0.008512
SL92150	2	2	0.008467
SL94150	4	2	0.008388
ALAMO	4	9	0.008295
ALAMO	1	7	0.008245
SLCO	4	4	0.008030
SL93	1	5	0.007829
SL92150	3	2	0.007706
SLCO	3	2	0.007571
SL92150	2	10	0.007433
SL92150	1	9	0.005836
Mean seedling weight			0.014124

Table A-2. Average seedling weight at 14 days after emergence of C_1 population. Seed produced in spaced planted nursery off of Agronomy Road in College Station, TX in September 2002. Seed were evaluated for heritability study in February 2003.

Row	Plant	Average weight in grams seedling^{-1}
12	16	0.042
11	11	0.039
12	11	0.039
7	1	0.039
4	14	0.038
6	10	0.038
11	10	0.037
3	17	0.037
3	18	0.037
3	2	0.037
1	6	0.036
16	3	0.036
12	19	0.035
16	12	0.035
4	3	0.035
5	1	0.035
7	3	0.035
11	17	0.034
11	9	0.034
15	4	0.034
2	16	0.034
3	14	0.034
8	8	0.034
1	2	0.033
10	5	0.033
10	9	0.033
11	8	0.033
16	18	0.033
2	10	0.033
2	11	0.033
6	14	0.033
6	17	0.033
8	12	0.033
8	14	0.033
8	16	0.033
10	19	0.032
10	2	0.032
12	8	0.032

13	10	0.032
14	9	0.032
17	6	0.032
2	19	0.032
3	16	0.032
4	20	0.032
6	1	0.032
8	1	0.032
9	16	0.032
9	19	0.032
10	3	0.031
11	5	0.031
15	1	0.031
17	20	0.031
17	5	0.031
18	1	0.031
8	19	0.031
10	1	0.030
10	11	0.030
15	15	0.030
17	13	0.030
17	14	0.030
17	15	0.030
2	5	0.030
5	3	0.030
6	13	0.030
8	17	0.030
8	18	0.030
8	5	0.030
12	7	0.029
2	1	0.029
4	15	0.029
4	19	0.029
5	14	0.029
8	15	0.029
9	11	0.029
11	13	0.028
11	20	0.028
12	5	0.028
12	6	0.028
14	15	0.028
17	1	0.028
17	2	0.028
2	8	0.028
3	4	0.028
3	7	0.028
4	9	0.028
5	19	0.028

7	11	0.028
1	5	0.027
12	2	0.027
15	2	0.027
15	8	0.027
6	7	0.027
7	15	0.027
16	13	0.026
16	16	0.026
16	9	0.026
2	13	0.026
3	6	0.026
5	8	0.026
6	2	0.026
7	6	0.026
8	13	0.026
1	18	0.025
13	16	0.025
14	7	0.025
3	10	0.025
4	2	0.025
8	11	0.025
9	10	0.025
12	18	0.024
13	6	0.024
17	17	0.024
5	13	0.024
7	4	0.024
12	13	0.023
15	10	0.023
15	17	0.022
2	12	0.022
2	17	0.022
6	19	0.022
8	3	0.022
10	17	0.020
17	18	0.020
16	17	0.012
Mean seedling weight		0.029

VITA

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