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UNDERSTANDING SKINNING RESISTANCE INHERITANCE AND DIFFERENTIAL GENE EXPRESSION IN SWEETPOTATO [*IPOMEA BATATAS* (L.) Lam.]

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by
Reeve D. Legendre
B.S., Louisiana State University, 2011
May 2015

ACKNOWLEDGEMENTS

I would like to acknowledge my professors, my family, my friends, and my wife, without whom this thesis would not be possible.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF TABLES iv

LIST OF FIGURES v

ABSTRACT vi

CHAPTER 1: ESTIMATING THE HERITABILITY OF SKINNING RESISTANCE IN SWEETPOTATOES 1
- 1.1 The State of Sweetpotato 1
  - 1.1.1 Skinning in Sweetpotato 1
  - 1.1.2 Heritability in Sweetpotato 3
- 1.2 Materials and Methods 3
  - 1.2.1 Plant Material and Experimental Design 3
  - 1.2.2 Skinning Resistance Evaluation 4
  - 1.2.3 Heritability Estimates 5
- 1.3 RESULTS AND DISCUSSION 6

CHAPTER 2: DIFFERENTIALLY EXPRESSING GENES IN CURING AND AMBIENT CONDITIONS INVOLVED IN SKINNING OF SWEETPOTATO 10
- 2.1 Skinning and curing 10
  - 2.1.1 Wound healing process 10
  - 2.1.2 Genes involved with wound healing in Sweetpotato 11
- 2.2 MATERIALS AND METHODS 12
  - 2.2.1 Plant Materials and Skinning Treatments 12
  - 2.2.2 RNA Extraction, cDNA synthesis, and qRT- PCR 12
  - 2.2.3 Quantitative Reverse Transcription-PCR (qRT-PCR) 13
- 2.3 RESULTS AND DISCUSSION 14

REFERENCES 20

APPENDIX 23

VITA… 27

LIST OF TABLES

Table 1. Sweetpotato skinning resistance estimates as determined by the torque wrench in newton-centimeters 7

Table 2. Additive genetic variances, phenotypic variances, and heritability estimates for individual years as well as both years combined for sweetpotato using half-sib family analysis and parent-offspring regression.. 7

Table 3. Differentially expressed genes (DEGs) with skinning response in sweetpotato that were analyzed using qRT-PCR and their primer sequences. 13

LIST OF FIGURES

Figure 1. Expression of cinnamyl alcohol dehydrogenase (*IbCAD*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. 14

Figure 2. . Expression of early light-inducible protein (*IbELIP3*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. 15

Figure 3. Expression of cytochrome p450 (*IbCytP450*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. 16

Figure 4. Expression of extensin (*IbExt*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. 17

Figure 5. Expression of phenylalanine ammonia lyase (*IbPAL*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. 18

ABSTRACT

Burgeoning demand for sweetpotato [*Ipomoea batatas* (L.) Lam] is being driven by strong consumer consumption of fries and robust fresh market sales. Acreage has rebounded to meet needs, but increased supply has weighed negatively on fresh market prices, and processing sweetpotato is generally at a low price point. Growers are challenged with increasing production costs and labor is the most expensive input; reducing labor costs through mechanized harvest is critical. Many popular varieties of sweetpotato are highly susceptible to skinning damage and the problems that are associated with exposed root flesh such as rotting, weight loss, and sunken areas. The present study compared methods to assess skinning damage and determine if it is possible to breed for a more skinning resistant variety to aid in further mechanization. A torque wrench was found best at quantifying the amount of force required to cause skinning damage. A two year study of a parent and offspring population using the torque wrench found a heritability estimate of 0.10 on an individual plant basis and a 0.63 heritability on a family basis. It is assumed that an estimate above 0.50 is sufficient to improve a trait and data showed utility using families in breeding scheme, albeit this brings complications. The research also examined genes differentially expressed at skinning injury sites in 2 different environments. Over expression of genes involved in healing may decrease damage that does occur after skinning damage and complement a more durable skin. Consequences of skinning are lessened by curing roots (32° C; 85% relative humidity for 5 days); wound sites are rapidly healed with nominal desiccation. Cyt P450 and Ext, genes associated with periderm formation and wound healing, were found up-regulated in a curing environment compared to skinned sites at ambient outside conditions (conditions varied from approximately 26-30°C, with 50-70% RH) over multiple time points (2 h, 4 h, and 24 h after skinning). Multiple genes associated with stress were found up-regulated in the ambient conditions. It is hypothesized that selection of genotypes with a more durable skin and enhanced repair mechanisms may further the quest towards mechanized harvest.

CHAPTER 1: ESTIMATING THE HERITABILITY OF SKINNING RESISTANCE IN SWEETPOTATOES

1.1 The State of Sweetpotato

Sweetpotato has surged in the marketplace as per capita consumption has increased from 1.7 kg in 2002 to 2.9 kg in 2012. From 2002 to 2012, demand has been met as total production of sweetpotato has more than doubled in the U.S., rising from 580,562.64 metric tons to 1,201,223.52 metric tons. In the same time frame, yield per acre is up more than 25% from 19,613 kg/ha to 26,276 kg/ha (The United States Sweet Potato Council, 2013). The industry has grown rapidly through high yielding varieties and a narrowed production window to optimize yield under ideal conditions. The industry is, however, constrained by a high production cost of $8440 per hectare. Labor costs account for $3643 or 43% of the total (Stoddard, et al., 2006). Mechanization is moving quickly to reduce labor inputs and increase the speed of harvest while conditions are favorable. Meeting a narrowed harvest window necessitates further mechanization.

Traditionally, sweetpotatoes are harvested and sorted by hand to minimize the amount of damage that is incurred during harvest. The higher yield per acre necessitates the need to switch from the traditional method of harvesting sweetpotatoes. A study conducted by O'Brien and Scheuerman (1969) concluded that complete and economical mechanization of sweet potato harvesting, with the proper adjustments to machinery, is possible. Unfortunately, sweetpotatoes are highly susceptible to skinning damage which can lead to desiccation, weight loss, and rot (Rees et al., 2003). The combination of sweetpotato susceptibility to skinning and the inherent damage caused by a mechanized harvest can be extremely detrimental to a grower's crop.

1.1.1 Skinning in Sweetpotato

The damage incurred during harvest is a barrier preventing sweetpotatoes from being harvested mechanically. While there are other types of damage that can occur such as bruising, shattering, and breakage, skinning is the primary focus of this study. Skinning has been well studied in potatoes

(*Solanum tuberosum* L.) and involves removal of the periderm (Lulai and Orr, 1993; Lulai and Freeman, 2001). This occurs by one of two distinct mechanism: tensile fracture and shear fracture. Tensile fracture is the breaking of the lignified phellem layer, also commonly referred to as the skin, perpendicularly to the surface of the root. Shear fracture occurs when there is breakage across the phellogen, separating the phellem from the phelloderm (Hammerle, 1970; Lulai, 2002; Webster et al., 1973). This lack of the epidermal layer can lead to weight loss, root desiccation, and increased incidence of root rot due to higher susceptibility to pathogens (Rees, et al., 1998). Once skinning damage occurs, the underlying cells of a skinned area desiccate and die. Lignification begins to occur under the desiccated cell layers, followed by the formation of a new wound periderm. Wound periderm formation occurs best in warm temperatures (28-30° C) and with a relative humidity of more than 85%, such as the conditions found in a curing chamber (Kushman and Wright, 1969). Superficial skinning can be successfully treated under these conditions. Desiccated and sunken areas are unappealing to the consumer's eye and lead to a less desirable fresh market product. Rees, et al (2003) showed that the water loss from desiccation on stored sweetpotatoes increases the amount of stress put on the root, and thus increases the susceptibility to rot and other forms of deterioration. The same study also compared different varieties of sweetpotato grown in East Africa and demonstrated a wide range of shelf-life. Beauregard, a leading variety in the United States, was considered resistant compared to African varieties. In the United States, Beauregard is considered susceptible given the mechanized nature of crop production. The wide variance in shelf-life was attributed to the amount of skinning that occurred during harvest, which leads to water loss and desiccation and rot. Beauregard produced a continuous layer of wound tissue in contrast to African varieties which produced discontinuous wound tissue.

Research underlying attempts to increase skinning tolerance is scant. Preharvest applications of ethephon at 3-7 days before harvest were found to reduce skinning incidence in 2 of the 3 years. The treatment was also found to increase suberin and lignification of the skin; however, this increase in suberin / lignin was weakly correlated ($r = 0.51$) with the force required to skin the sweetpotato. These

results showed that there are other factors to consider besides suberin / lignin content that affect skinning resistance (Wang, 2013). A 1993 study (LaBonte and Wright, 1993) showed preharvest canopy removal can reduce the total surface area skinned. Sweetpotato plants that were defoliated 10 days prior to harvest reduced skinning damage by 62%, while other treatments of removing the canopy 8 days and 4 days prior to harvest reduced skinning damage by 53% and 26%, respectively.

1.1.2 Heritability in Sweetpotato

Our present interest is to estimate the heritability of skinning resistance in sweetpotato. Heritability is measured using various approaches, most common use variance and covariance data, as well as parent-regression (Jones, 1986; Courtney, et al., 2008; Kim, et al, 1996). The underlying concept is to estimate the proportion of phenotypic variance caused by genetic variance. Estimates range from 0 to 1. The closer the number is to 1, the more heritable the trait. Prior research on sweetpotato indicates a measurement of 0.3 for parent-offspring regression and 0.4 using a variance component approach is suitable for trait improvement in sweetpotato (Jones, 1986). Other studies involving heritability of traits in sweetpotato have been conducted. Estimates for the broad-sense heritability of micronutrient composition of sweetpotato roots showed that dry matter, iron and zinc concentrations were highly heritable (Courtney, et al., 2008). The narrow-sense heritability of reaction resistance to chlorotic leaf distortion caused by *Fusarium lateritium* was estimated to be less heritable (Kim, et al., 1996). The approach used in the current study is to measure shear fracture using a torque wrench developed for potato (Lulai and Orr, 1993). A parent and half-sib progeny population is used to determine narrow-sense heritability using variance estimates and parent-offspring regression. These results will aid in developing effective methods to improve skinning resistance in sweetpotato.

1.2 Materials and Methods

1.2.1 Plant Material and Experimental Design

Field research was done in each of 2 years in 2012 and 2013 at the LSU AgCenter's Burden Research Center located in Baton Rouge, LA. Fifteen half-sib families with corresponding female parents were

obtained from the LSU Sweet Potato Breeding program. The true seed progeny were harvested from a 4 row open pollinated nursery in the summer of 2011. The fifteen female parents were 10-21, 05-111, 10-9, Beauregard, Evangeline, 10-70, 05-29, 07-146, 10-3, 10-78, 09-82, 08-25, 10-67, 10-46, and 07-6R. True seeds from each of the 16 parents were planted in greenhouse benches and 30 half sib progeny were randomly selected for each of the parents. Three replicates (10 plants each) were arranged in a randomized complete block design with 3 replications of 10 plants of each parent. All plants were spaced 0.3 m apart in row and 1 m between rows. Plots were established on 1 June 2012 and 4 June 2013. A single marketable root [U.S. #1 grade (5.1 to 8.9 cm diameter and 7.6 to 22.9 cm long)] was harvested from each individual plant for both the parents and the progeny. Over the growing season in 2012, 716 mm of rainfall was recorded with an average high temperature of 31.4° C and an average low temperature of 22.1° C. The average maximum relative humidity was 93.3% and the average minimum relative humidity was 52.1%. Over the growing season in 2013, 429 mm of rainfall was recorded with an average high temperature of 32.2° C and an average low temperature of 22.3° C. The average maximum relative humidity was 94.9% and the average minimum relative humidity was 50.1%. The roots were hand-harvested in order to minimize damage incurred during the harvest. Each block was harvested separately over a 3 week period beginning 90 days after planting (30 August 2012 and 2 September 2013).

Plants for the second year were grown from the roots harvested from the first year's study as to have genetically identical samples. Loss occurred to some progeny genotypes in storage while others failed to produce plants.

1.2.2 Skinning Resistance Evaluation

Skinning resistance was evaluated the day after the roots were harvested using a Snap-On™ torque wrench (Kenosha, Wisconsin) using a protocol adapted by by Lulai and Orr (1993). A 2.5 cm by 2.5 cm square of 3M™ 100-grain medium sandpaper (Saint Paul, Minnesota) was secured to the end of the torque wrench with double-sided mounting tape. Each root was gently brushed free of dirt around the

middle of the root in order for a secure grip to be established between the sand paper and an unblemished section of root periderm. The torque wrench face from which the measurements are recorded is set to zero. Downward force was applied, and once a secure grip was established, the handle was rotated until skinning occurred. Once the technique is mastered, very uniform skinning damage scores can be achieved. The torque was measured in ounce-force-inches, and then converted into newton-centimeters. For each progeny, three measurements were made from each root sample and then averaged. From each parent, only one measurement was made from each root and then averaged with the other samples from the same parent in each replication.

1.2.3 Heritability Estimates

Data was analyzed using SAS (2014) and heritability estimates based on previous statistical approaches (Kim, 1996). Heritability estimates were calculated using the skinning resistances of both the parent and the progeny using two different mathematical approaches, a variance-covariance method as well as using a parent-offspring regression method.

The variance component based on half-sib family analyses were able to provide estimates of narrow-sense heritability on an individual plant basis (h^2) and on a half-sib family basis (h_f^2) for each year, as well as for both years combined.

Equations for the heritability estimates are as follows (Nyquist, 1991):

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = \frac{4\sigma_f^2}{\left(\sigma_f^2+\sigma_\varepsilon^2+\sigma_W^2\right)}$$

$$h_f^2 = = \frac{\sigma_f^2}{\sigma_P^2} = \frac{\sigma_f^2}{\left(\sigma_f^2+\sigma_\varepsilon^2+\sigma_W^2/rp\right)}$$

The additive genetic variance (σ_A^2) is calculated using the variance of the mean genotypic values of the families in the population (σ_f^2). The phenotypic variance (σ_P^2) is calculated using the variance of the mean genotypic values of the families in the population (σ_f^2), as well as the variance of the total plot effect within replications (σ_ε^2), and the variance of the values of the plants within the plots (σ_W^2). The

phenotypic variance of the plot (σ_w^2) includes the variance of the genotypic values of half-sib individual genotypes and the environment, the environmental effects variance, and the error variance. Subscripts "r" and "p" stand for replications and the harmonic mean of the number of plants per plot. The narrow-sense heritability for half-sibs family (h_f^2) is calculated by the division of the genetic family variance component (σ_f^2) by the phenotypic variance among family (σ_P^2) (Nyquist, 1991).

For both years combined, the narrow-sense heritability was also calculated using the following formulas:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = \frac{4\sigma_f^2}{\left(\sigma_f^2+\sigma_\epsilon^2+\sigma_w^2\right)}$$

$$h_f^2 = = \frac{\sigma_f^2}{\sigma_P^2} = \frac{\sigma_f^2}{\left(\sigma_f^2+\sigma_{fy}^2/y+\sigma_\varepsilon^2/yr+\sigma_w^2/yrp\right)}$$

The VARCOMP =REML procedure in SAS 9.3 (2014) was used to calculate the variance components for all models.

The parent-offspring regression was calculated using the formula $h^2 = 2b$, where b is the regression coefficient of the parents versus the progeny. Regression coefficients were determined using the parent and progeny data from each year individually, as well as both years combined, through use of the PROC GLM procedure in SAS.

1.3 RESULTS AND DISCUSSION

Mean values for the skinning resistances measurements for the 14 parents varied from 25.53 newton-centimeters to 29.74 newton-centimeters for both years combined, a difference of 14% (Table 1). The individual progeny means for skinning resistance varied from 25.61 newton-centimeters to 28.11 newton-centimeters for both years combined, a difference of 9%. The parental mean was slightly higher than the progeny mean over both years, 28.02 newton-centimeters versus 27.37 newton-centimeters, respectively. There was a significant year effect ($p < 0.05$) demonstrating skinning resistance varied for the two years and illustrates the impact of the environment on the genotype.

Table 1. Sweetpotato skinning resistance estimates as determined by the torque wrench in newton-centimeters.

	Skinning Resistance Index					
	2012		2013		2012 + 2013	
Parent	Parent	Progeny	Parent	Progeny	Parent	Progeny
05-111	30.65 ± 2.30	28.81 ± 2.49	27.51 ± 3.58	27.01 ± 2.70	29.09 ± 3.38	28.11 ± 2.70
05-29	32.86 ± 5.14	28.34 ± 1.95	28.44 ± 2.74	27.11 ± 2.93	29.74 ± 4.67	27.79 ± 2.48
07-146	29.64 ± 2.60	29.35 ± 2.56	28.62 ± 2.46	25.14 ± 2.89	29.13 ± 2.58	27.91 ± 3.33
07-6R	28.96 ± 3.91	27.29 ± 2.80	28.32 ± 1.94	26.70 ± 2.58	28.64 ± 3.08	27.07 ± 2.70
08-25	29.34 ± 2.94	28.74 ± 2.12	22.60 ± 2.68	25.80 ± 3.31	25.97 ± 4.39	27.61 ± 2.98
09-82	28.17 ± 3.28	28.70 ± 2.95	27.83 ± 4.00	25.03 ± 3.71	28.00 ± 3.63	27.75 ± 3.50
10-3	25.42 ± 3.06	28.70 ± 4.02	25.63 ± 3.02	24.45 ± 2.95	25.53 ± 3.02	27.13 ± 4.18
10-21	29.94 ± 2.20	26.60 ± 3.09	26.90 ± 2.29	25.90 ± 2.79	28.42 ± 2.70	26.29 ± 2.94
10-46	30.39 ± 1.88	28.54 ± 3.63	25.63 ± 2.46	24.47 ± 2.46	28.29 ± 2.61	26.99 ± 3.76
10-70	29.98 ± 3.09	27.83 ± 2.58	26.83 ± 2.46	25.25 ± 4.01	28.41 ± 3.19	26.82 ± 3.42
10-78	28.25 ± 3.76	27.47 ± 4.18	26.27 ± 2.46	21.66 ± 4.05	27.06 ± 3.16	25.61 ± 4.90
10-9	27.76 ± 2.82	27.36 ± 2.68	27.93 ± 3.03	25.09 ± 2.69	27.84 ± 2.90	26.66 ± 2.85
Beauregard	27.65 ± 2.32	28.78 ± 2.43	28.25 ± 3.24	25.94 ± 3.86	27.94 ± 2.81	27.58 ± 3.39
Evangeline	28.85 ± 3.04	28.55 ± 2.85	27.21 ± 2.82	25.17 ± 2.28	28.03 ± 3.02	27.19 ± 3.11

The narrow-sense family heritability (h_f^2) was calculated as 0.63 across both years (Table 2). However, when calculated on an individual plant basis it was only 0.10 for both years combined. Heritability was 0.17 on a family basis the first year and increased to 0.52 in the second year. On an individual plant basis, heritability was 0.04 in the first year and 0.26 in the second year. The parent-offspring regression estimates for the narrow-sense heritability were 0.19 over both years. The first year (0.09) was also lower than the second year (0.35) (Table 2). Both types of heritability estimates were lower in the first year and higher in the second year.

Table 2. Additive genetic variances, phenotypic variances, and heritability estimates for individual years as well as both years combined for sweetpotato using half-sib family analysis and parent-offspring regression.

Statistical Procedure	Year		
	2012	2013	2012 + 2013
Half-sib family analysis			
Individual plant basis			
Additive variance	0.98	5.43	2.04
Phenotypic variance	22.3	21.11	20.3
Narrow-sense heritability (h2)	0.04	0.26	0.1
Family mean basis			

Table 2 continued.

Additive variance	0.24	1.36	0.51
Phenotypic variance	1.37	2.61	0.81
Narrow sense heritability (h2)	0.17	0.52	0.63
Parent offspring regression			
Narrow-sense heritability (h2)	0.09 + 0.14	0.35 + 0.21	0.19 + 0.11

For sweetpotato, narrow-sense heritability estimates based on variance components are adequate for improving a trait at 0.4 or higher. For parent-offspring regression based heritabilites, 0.3 or higher is considered acceptable for breeding for a specific trait (Jones, 1986). The heritability estimates for both years combined do not meet the criteria set forth by Jones using the parent-offspring regression. They do meet the benchmarks based on the family mean basis at 0.63, but on an individual plant basis the estimates are low. These results suggest it would be best for a breeding program to select for skinning resistance on a family-wide selection scheme as opposed to selecting individual plants that display high amounts of skinning resistance. The present work underscores the need for various environments. Multiple years of data is needed to account for the environment. An individual plant can display a wide range of skinning resistance based on the environment that it is grown in.

Evangeline and Beauregard parents, two prevalent commercial varieties of sweetpotato, measured very similar for skinning resistances at 28.03 newton-centimeters and 27.94 newton-centimeters, respectively. These two varieties were middle range among all of the parents tested and only demonstrated 6-7% less force needed to break the skin in comparison to the most tolerant genotype, 05-29 (Bonita). 05-29 ranked highest over both years, indicating that varieties may possess tangible differences in skinning tolerance. The impact of canopy removal combined with a more skinning resistant genotype is unknown. It is possible that skinning resistance could be accentuated.

Other techniques may enhance accuracy in measuring the skinning resistance of sweetpotato (Arancibia, 2014 personal communication). Methods like the force gauge and Halderson shear tester (Lulai and Orr, 1993) are currently being investigated as a means of measuring the tensile fracture resistance and shear fracture resistance separately, whereas the method that was used in the present study was only able to

measure the shear fracture. The Halderson shear tester measures tensile fracture resistance. This combined with shear fracture resistance may provide insight into sweetpotato skinning resistance mechanisms. However, the torque wrench is a quick, simple method of measuring skinning resistance that could be employed in the field with minimal setup and time investment.

Data presented demonstrated that gain in resistance can be achieved by selecting on a family mean basis. Selecting a number of superior progeny from each of several high families could be combined in an open pollinated nursery to generate a population with enhanced levels of skinning resistance.

CHAPTER 2: DIFFERENTIALLY EXPRESSING GENES IN CURING AND AMBIENT CONDITIONS INVOLVED IN SKINNING OF SWEETPOTATO

2.1 Skinning and curing

Skinning is a profound production issue facing sweetpotato producers. High production costs ($3,500 to $4,000) have encouraged more efficient harvest strategies using mechanization (T. Smith, 2014 personal communication). Unfortunately, this has accentuated the problem of skinning and its underlying negative effects on storage and marketability. Skinning damage incurred during harvest results in increased susceptibility to postharvest diseases (Rees et al., 2003). *Rhizopus* and *Fusarium* spp. are the most common of the postharvest diseases in North America and can result in significant crop loss in storage (Clark et al., 2013). In order to minimize the amount of postharvest disease, curing the harvested roots at 30°C and 90% relative humidity (RH) immediately following harvest for 5-7 days is a recommended practice (Clark et al., 2013). Literature is scant on the benefits of curing; however, it is well recognized that skinned areas in curing conditions do not become sunken due to desiccation and new wound tissue forms well in this environment and generates a protective barrier against pathogen entry. Storage roots with even slight desiccation injury have lower marketable value as well as roots with disease symptoms. In contrast, the postharvest physiology of storage roots undergoing curing is well documented (Picha, 1986); curing nearly doubles the amount of sucrose present in the storage root. Storage at 32°C and 90% RH for 10 days builds higher sucrose levels and after 6 weeks storage roots are considered cured and suitable for market and long-term storage. There is also an absence of literature regarding skinning on the effect of storability, whether cured or not; however, it is generally accepted that with an increase in skinning, the storability is decreased.

2.1.1 Wound healing process

The wound healing process of sweetpotato roots begins immediately after skinning occurs. The surface cell layers desiccate, which leads into underlying cell layers becoming lignified and suberized. As the sweetpotato loses water from desiccation, the crop also loses value as sweetpotato is a crop that is sold

by weight. Additionally, water loss results in reduced root weight which is correlated to a higher rate of root rot. Weight loss can vary significantly between cultivars, ranging from 8-30% in an east African trial (Rees et al., 2003). The amount of lignification, as well as the amount of desiccation, can vary with cultivar and RH. Transpiration rates (a rate that measures water loss) of some cultivars consistently outperformed other cultivars across every time point. These transpiration rates were significantly correlated to the amount of lignification (van Oirschot et al., 2006). The final step in the wound healing process is the establishment of a wound periderm (Artschwager and Starrett, 1931). Wound periderm formation has been found to occur best at 28 - 30° C in high relative humidity (Kushman and Wright, 1969), such as the conditions found in a curing chamber. Different cultivars may have differences in gene expression that contribute to a more efficient wound healing physiology. Beauregard, a leading U.S. cultivar, was found to have superior wound-healing characteristics in an east African trial (Rees et al, 2008); however, it is deemed skinning susceptible in U.S. production regions. Identifying cultivars that have overexpression of wound-healing related genes and integrating superior genotypes into breeding programs could lead to the development of new varieties with better wound healing.

2.1.2 Genes involved with wound healing in Sweetpotato

As the sweetpotato industry continues the trend towards mechanization of harvest to reduce costs, it is imperative to take a multipronged approach to minimize skinning on sweetpotato roots. As producers make an effort to reduce skinning through proper harvest and cultural practices, breeders can assess a series of genes that have been identified with a possible connection to wound healing. Cinnamyl alcohol dehydrogenase (CAD) is an enzyme which plays a key role in catalyzing the synthesis of monolignols, an important precursor to lignin biosynthesis. Early light inducible proteins (ELIP3) may play a role in desiccation response (Effendy, 2013). Phenylalanine ammonia-lysase (PAL) and cytochrome P450 76C4 (Cyt P450) are enzymes responsible in part for lignin and suberin synthesis. Extensin (Ext) is a structural glycoprotein that is essential to constructing and sustaining cell walls (Lamport, et al., 2011), and physical wounding can induce extensin biosynthesis (Chrispeels et al., 1974). Recognizing and

classifying up-regulated genes may allow breeders to identify genes that would allow for marker based screening for sweetpotato lines with superior wound healing responses to skinning injury, whether cured or not.

The present research examines expression of previously described genes found differentially expressed in underlying epidermal tissue in response to skinning injury and how gene expression differs for underlying epidermal tissue for storage roots undergoing curing in comparison to storage roots at ambient conditions.

2.2 MATERIALS AND METHODS

2.2.1 Plant Materials and Skinning Treatments

In order to identify differentially expressed genes, two treatments were established. Freshly harvested U.S. #1 size roots (5.1 to 8.9 cm diameter, 7.6 to 22.9 cm long) of sweetpotato cultivar LA 07-146, also known as Bayou Belle, were skinned in a 50 millimeter by 50 millimeter square using a potato peeler (Victorinox, Monroe, CT) on 13 August, 2013. In the first treatment, skinned roots were placed outside in ambient conditions in the sun to emulate sweetpotatoes left in the sun after harvest (conditions varied from approximately 26-30°C, with 50-70% RH). In the second treatment, skinned roots were placed in a curing chamber in high humidity (90% RH) and high temperature (30°C). Three samples were taken from the skinned area of three separate roots (as biological replicates) at 0 h (control), 2 h, 4 h, and 24 h after being skinned for each treatment. The samples were removed using a potato peeler (Victorinox, Monroe, CT); care was taken to detach only the upper 1.2 millimeters of the sweetpotato flesh. The samples were immediately frozen in liquid nitrogen and then stored in a -80° C freezer until RNA extraction.

2.2.2 RNA Extraction, cDNA synthesis, and qRT- PCR

RNA was extracted using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA) using the established protocol of the manufacturer, with minor modification. The RLT buffer was replaced with Trizol (Invitrogen, Carlsbad, CA), in order to more effectively break down the starch in the samples. RLC

buffer was also tried, but Trizol was found to be a better lysis agent. DNA was removed from the total RNA using RNase-free DNase (Qiagen, Valencia, CA). The quantity and quality of the total RNA was assessed using a ND-1000 spectrophotometer (Nanodrop Technologies, Wimington, DE). First strand cDNA was synthesized from total RNA using the iScript Select cDNA synthesis kit (Bio-Rad, Hercules, CA) as described by Effendy et al. (2013). A 20 µl reaction volume consisted of 2 µg of RNA, 4 µl of 5x iScript Select Reaction mix, 2 µl of oligo (dT) primer, 1 µl of iScript reverse transcriptase, and the remainder was nuclease-free water. The mixture was incubated at 25°C for 10 min, and then for 50 min at 42°C. The reaction was stopped by inactivating the reverse transcriptase at 85°C for 5 min.

2.2.3 Quantitative Reverse Transcription-PCR (qRT-PCR)

The 1st strand cDNA was diluted to 5 times and 2 µl was used for qRT-PCR to establish the relative expression of the differentially expressing genes. For 20 µl reaction volume for each sample, 10 µl of SYBR^{TM} green supermix, 7.6 µl of the DNAse-free water, 2 µl of the diluted cDNA, and 0.2 µl of both forward and reverse primers (50 ng/µl) were mixed well. The thermal profile for the PCR was: 40 cycles at 95°C for 10 sec followed by 65.0° for 30 sec followed by 71 cycles of 60°C for 30 sec. The gene specific primers that were used were for early light-inducible protein (ELIP3), phenylalanin ammonia lyase (PAL), extensin (Ext), cytochrome P450 (Cyt P450), and cinnamyl alcohol dehydrogenase (CAD) (Table 3). The sweetpotato elongation factor (*IbEF1α*) was used as the reference (Solis, 2012). The formula $2^{-\Delta\Delta Ct}$ was used in order to obtain the relative expression ratio (Ramanarao et al., 2011).

Table 3. Differentially expressed genes (DEGs) with skinning response in sweetpotato that were analyzed using qRT-PCR and their primer sequences.

DEG	Name	Organism	Forward Primer 5' x 3'	Reverse Primer	Product Length (BP)
lbCytP450	Cytochrome P450 76C4 (Cyt P450)	*V. vinefera*	CGCTGGCTATAAGGATGGTG	ACTGCTCCTCCATGTCCAAC	141
lbELIP3	Early light-inducible protein (ELIP3)	*Populous trichocarpa*	GTCGAAGTCCAAAGGGTTGA	ACAAGAGCAGTGGGGTATGG	131
lbExt	Extensin (Ext)	*I. batatas*	ACTTGCCCTAGCCCTAAACC	GCCCTTCAATGAGAGAGCAG	178

Table 3 continued

lbPAL	Phenylalanine ammonia lyase (PAL)	*I. batatas*	GAAAGACTTGCTC CGAGTGG	GCAGCCTCAAC TICTTTTGG	222
lbCAD	Cinnamyl alcohol dehydrogenase (CAD)	*I. batatas*	GTCGCCAGGAACT AGCGTCT	GCTGCTTGGCTT ATGGGTGT	240
lbEFla	Elongation factor (EF)	*I. batatas*	CCAAGATTGATAG ACGGTCTGG	CAGTTGGGTCCT TCTTGTCAAC	100

2.3 RESULTS AND DISCUSSION

Expression of differentially expressed genes varied between the 2 treatments for all 5 genes that underwent qRT-PCR. Cinnamyl alcohol dehydrogenase (*IbCAD*) showed a spike in up-regulation at 4 h after skinning injury in the treatment under the ambient conditions. For the curing chamber treatment (high RH and high temperature), low *IbCAD* expression gradually increased over the 24 h period (Figure 1). CAD is responsible for catalyzing monolignols, which are essential lignin precursors. CAD transcripts have been found to be induced due to biotic and abiotic stresses (Kim, et al. 2010).

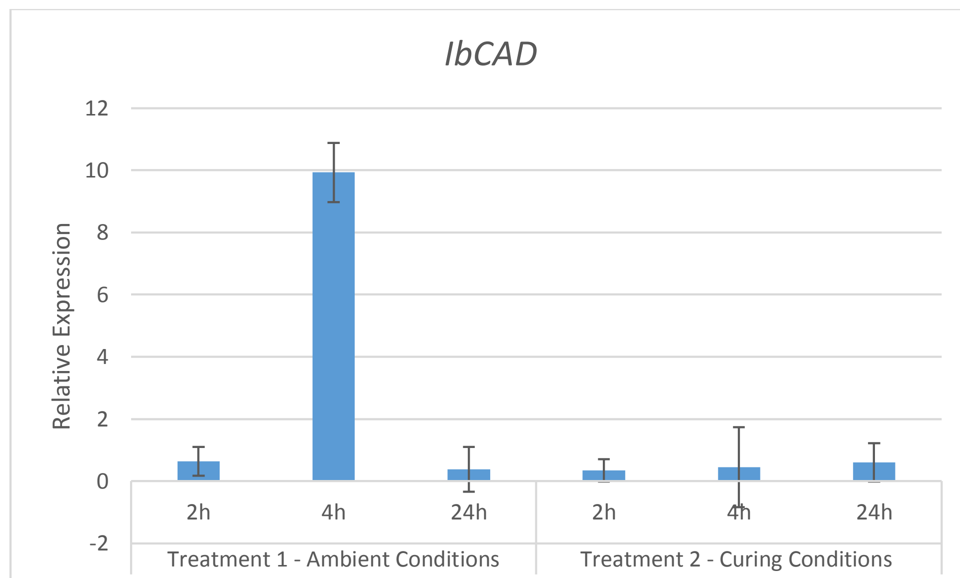

Figure 1. Expression of cinnamyl alcohol dehydrogenase (*IbCAD)* at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. The error bars indicate SE.

Early light inducible protein (*IbELIP3*) was found at higher levels in ambient outside conditions than the curing chamber across all 3 time points. In the ambient conditions, expression was higher at 2 h and 24 h

with a dip in expression at 4 h. Expression for this gene was minimal throughout the time points in the curing chamber (Figure 2). While the function of *IbELIP3* is not definitively known, a 2001 study (Alamillo and Bartels, 2001) found that an ELIP-like protein, dsp 22 (desiccation stress protein), amassed in thylakoid membranes in PSII as a reaction to photoinhibition damage caused by desiccation in order to prevent further damage. Other ELIPs have been found to accumulate due to other stresses such as heavy metal build up, exposure to light, temperature extremes, drought, high salinity levels, and the stress hormone ABA (Tao, et al. 2011). High levels of *IbELIP3* after 12 hours were found in skinned sweetpotato as a result of desiccation stress (Effendy et al. 2013). The high levels of relative humidity in the curing chamber could have contributed to the lower *IbELIP3* levels inferring a less stressful environment for skinned areas undergoing healing.

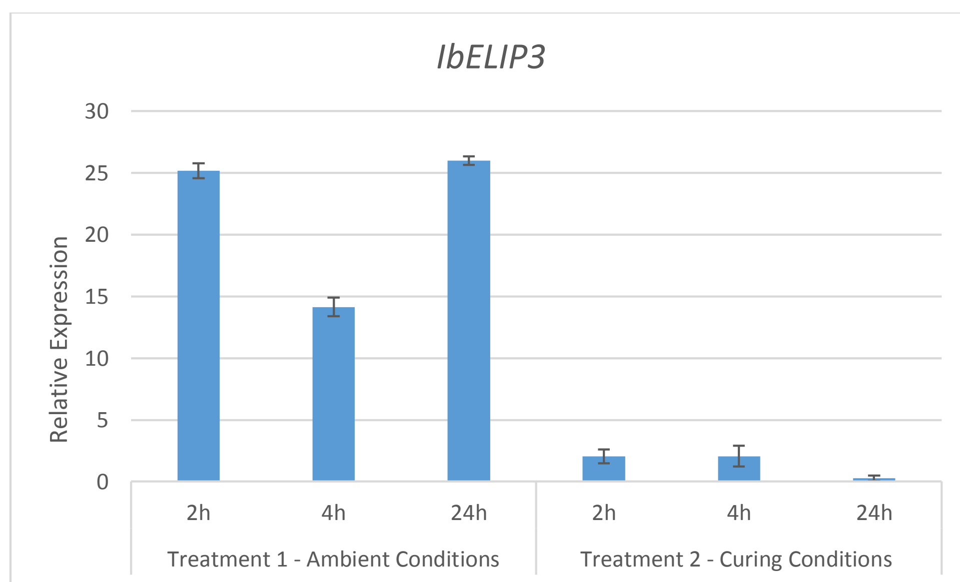

Figure 2. Expression of early light-inducible protein (*IbELIP3*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. The error bars indicate SE.

Cytochrome P450 (*IbCytP450*) showed moderate expression under ambient conditions, increasing from 2 h to 4 h and then declining at 24 h. Under curing conditions, the majority of the expression occurred at 24 h, with low levels of expression at 2 h and 4 h (Figure 3). Cyt P450 is a significant protein in biosynthetic pathways of phenylpropanoid compounds that includes products used in lignin synthesis (Dixon, et al. 1995; Schuler, 1996). This particular gene, *IbCytP450*, is a significant component of

suberin biosynthesis. The curing chamber conditions seems beneficial for *IbCytP450* expression after 24 h which could allow for more lignin and suberin production as the root stays in storage.

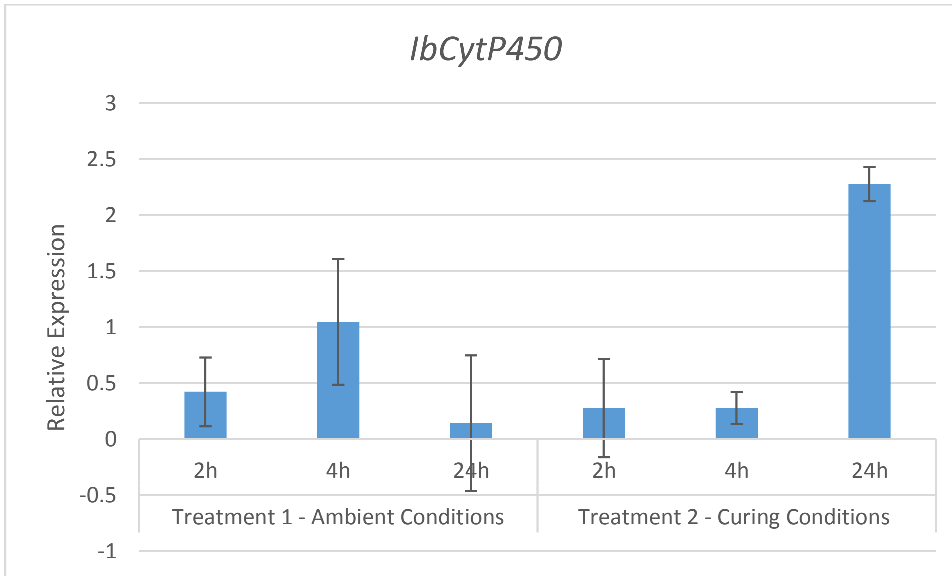

Figure 3. Expression of cytochrome p450 (*IbCytP450*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. The error bars indicate SE.

Extensin (*IbExt*) showed a slight increase in expression in the ambient treatment from 2 h to 4 h before falling off at 24 h. The curing chamber treatment showed more than 2 times higher levels of *IbExt* accumulation at 2 h. These levels decreased sequentially in the 4 h and 24 h time points (Figure 4). Extensin is a structural glycoprotein that has vital roles in constructing and sustaining plant cell walls (Lamport et al. 2011). Mechanical injury has also been linked to extensin biosynthesis in carrot storage tissue (Chrispeels et al. 1976). The enhanced extensin expression is evident in the curing chamber which corroborates the evidence that curing chamber conditions can enhance wound healing. The ambient conditions may inhibit the production of extensin which may hinder wound healing.

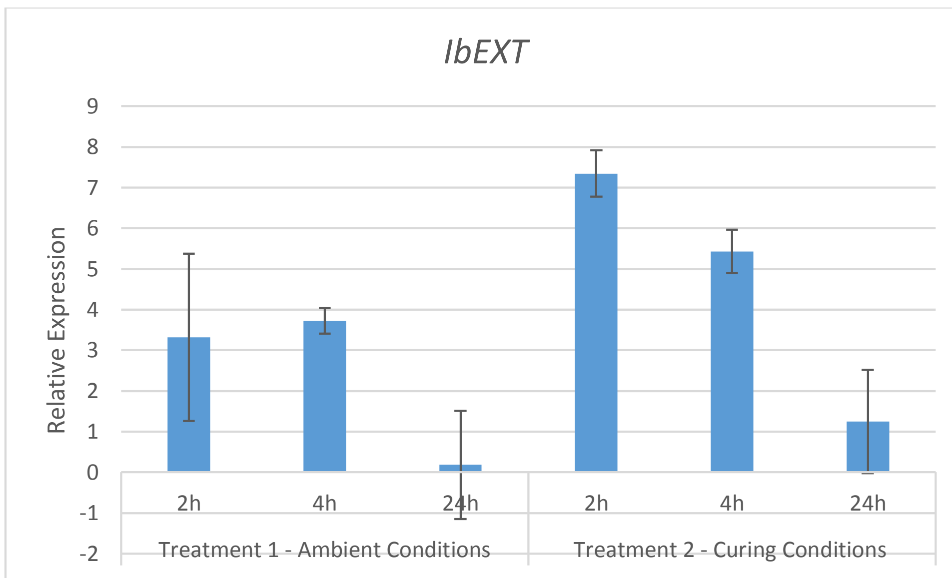

Figure 4. Expression of extensin (*IbExt)* at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. The error bars indicate SE.

The cDNA encoding for phenylalanine ammonia lyase (*IbPAL*) for the ambient treatment showed expression at 2 h and 24 h, but there was no detectable levels of expression at 4 h. Lower levels of *IbPAL* were found in the curing chamber treatment where expression peaked at 4 h (Figure 5). PAL is a vital enzyme of phenylpropanoid metabolism which is part of the pathway that synthesizes protective compounds such as flavonoids and cell-wall compounds. PAL has been shown to be upregulated by wounding, pathogens, and UV light treatment (Lois, 1989). The higher levels of *IbPAL* expression in the ambient conditions at 24 h is likely due to the cells dying faster in the more stressful ambient conditions. It could also be a response to the higher exposure to UV light outside and pathogen activity that could be a result of the stress of less than ideal wound healing circumstances.

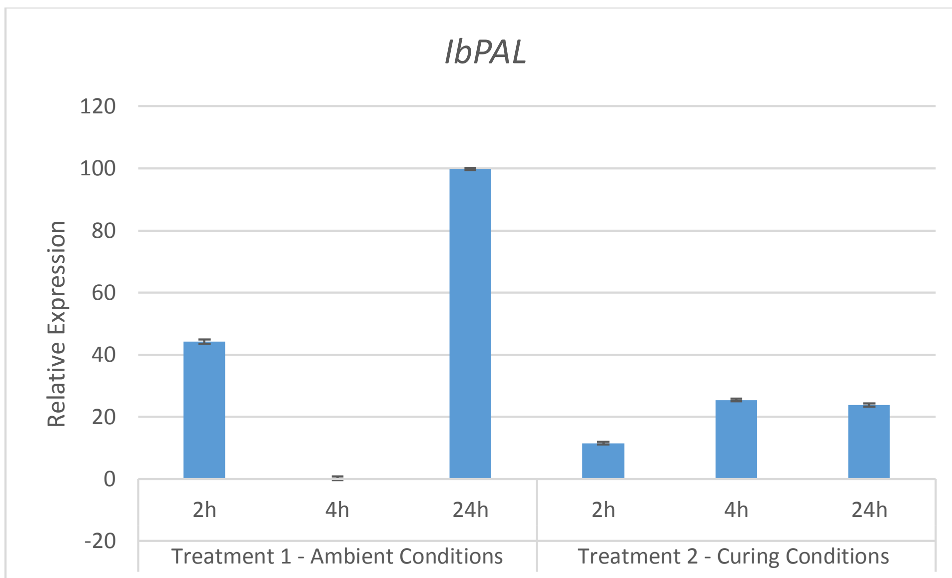

Figure 5. Expression of phenylalanine ammonia lyase (*IbPAL*) at 2 h, 4 h, and 24 h after skinning injury in sweetpotato for both ambient conditions and curing conditions. The error bars indicate SE.

The curing chamber treatment resulted in upregulation of *IbCytP450* and *IbExt* genes that play important roles for wound healing and periderm formation. In the ambient conditions, *IbPAL*, *IbCAD*, and *IbELIP3* all showed greater accumulation than the curing chamber conditions. In previous studies, these genes have been found to be expressed under biotic and abiotic stresses. This suggests that the curing chamber conditions are more conducive to wound-healing and present less stress to an injured sweetpotato root than the ambient conditions. Overexpression of *IbCytP450* and *IbExt* may circumvent detrimental outcomes of roots skinned and held even briefly under ambient conditions. Consistent overexpression of *IbPAL* over time suggested the process of lignification is initiated as early as 2 h of curing. Similarly an early stage upregulation of extensin under both conditions signaled the formation of suberin to act as a structural barrier in cell wall against desiccation and/or pathogen injury. Thus, if sweetpotato genotypes that have greater expression of genes that aid wound healing can be identified, breeders can integrate these genotypes into their breeding programs in efforts to develop varieties that exhibit better wound healing response. The sweetpotato industry could benefit greatly from such varieties.

Further research could be beneficial to the development of varieties that show higher skinning resistance as well as better response to skinning when it does occur. A study comparing skinning resistances in a variety of environments could identify superior phenotypes for specific environments, as well as establishing trends for the effect of agro climatic data on sweetpotato skin. Comparing skinning resistances at different harvest dates could possibly help develop better harvest practices for farmers. Measuring sweetpotato size and its effect on skinning resistance is another prospect. A variety trial analyzing the gene differentiation in response to skinning could isolate genotypes that have better wound-healing response to be integrated into breeding programs. It could also be possible to identify gene differentiation at time points prior to skinning. Possible factors to analyze like weed pressure, climate, and pre-harvest practices could establish some upregulated genes effecting skin set and skinning resistance.

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APPENDIX

Appendix for Heritability Calculations

Part 1: SAS Statement for variance components heritability estimates:

A. The SAS statement for the variance component heritability calculations was as follows:

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'Herit year two';
ods rtf file = 'Herityear2.rtf';
ods html file = 'Herit1year2.html';
data herit;
        input line $ year parent rep skin num ;
        harmean = harmean(skin);
        datalines;
10-46  2    0    1    30.8    1
10-46  2    0    1    35.6    2
10-46  2    0    1    41.06666667   3
10-46  2    0    1    32.13333333   4
10-46  2    1    1    36      1

run;
if parent=0 then output;
proc varcomp
method=REML;
        class rep line num;
        model skin = line rep line*rep num(line rep);
run;
Proc print data=herit(obs=1) noobs;
var harmean;
   title2 "Moment estimator of Harmonic Mean";
Run;
quit;
```

B. Data input

Data was input into SAS using the following setup:

Line – name of the line used
Year – year that the sample was taken from. 1 or 2.
Parent – determines if the sample was a parent or progeny. 0 for progeny, 1 for parent.
Rep – Replication that the sample was taken from. 1, 2, or 3.
Num – number of sample from each rep. 1-10.

Table 1. Sample of how data was formatted for input into SAS.

line	year	parent	rep	skin	num
05-111	2	0	1	39.06667	1
05-111	2	0	1	40.66667	2
05-111	2	0	1	40.66667	3
05-111	2	0	1	42.13333	4
05-111	2	0	1	37.33867	5

Part 2: SAS Statements for parent offspring regression heritability estimates:

A. The SAS statements for parent offspring heritability calculations are as follows:

```
title="Heritability Estimate";
data herit;
        input year line $ rep PAR PRO ;
        datalines;
1       05-111 1       42.24   38.4
1       05-111 1       42.24   37.33333333
1       05-111 1       42.24   38.93333333
1       05-111 1       42.24   46.93333333
1       05-111 1       42.24   46.4

proc glm;
class rep;
model pro= rep par / solution;
by year;
run;

proc glm;
class rep year;
model pro= year rep(year) par / solution;
run;
```

B. Data input

The data was input into SAS using the following setup:

Year – Year that the sample was grown

Line – name of the line

Rep – rep that the sample was grown

PAR – Mean skinning resistance of the parents

PRO – Skinning resistance from the sample

Table 2. Sample of how data was formatted for input into SAS.

year	line	rep	PAR	PRO
1	05-111	1	42.24	38.4
1	05-111	1	42.24	37.333
1	05-111	1	42.24	38.933
1	05-111	1	42.24	46.933
1	05-111	1	42.24	46.4

Part 3: Narrow-sense heritability using variance components

To determine the heritability on a plant basis using variance components, use the SAS output (see table 2) from corresponding year(s) from the table REML iterations (see Table 3):

$\hat{\sigma}_A^2$ = 4 * iteration var(line) estimate

$\hat{\sigma}_P^2$ = iteration 4 of the var(line) estimate + iteration 1 of the var(rep*line) + iteration 4 of the var(error)

$h^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_P^2}$

Table 3. REML Iterations table output from SAS from year 1 of the skinning resistance data.

REML Iterations						
Iteration	**Objective**	**Var(line)**	**Var(rep)**	**Var(rep*line)**	**Var(num(rep*line))**	**Var(Error)**
0	2449.00469	0.2617214627	0.6952664115	2.0053979522	0	20.0752890889
1	2448.98150	0.2420892514	0.6352129439	2.1242710791	0	20.0468674698
2	2448.98147	0.2442371732	0.6383781063	2.1206424864	0	20.0472130249
3	2448.98147	0.2440771090	0.6382230281	2.1208244748	0	20.0472090769
4	2448.98147	0.2440771090	0.6382230281	2.1208244748	0	20.0472090769

<u>Year 1 plant basis</u>

$\hat{\sigma}_A^2$ = 4 * 0.2440771090

$\hat{\sigma}_A^2$ = 0.976308436

$\hat{\sigma}_P^2$ =0.2440771090 + 2.0053979522 + 20.0472090769

$\hat{\sigma}_P^2$ = 22.2966341381

$h^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_P^2} = \frac{0.97630841381}{22.2966841381} = 0.0437871582$

<u>Year 2 plant basis</u>

$\hat{\sigma}_A^2$ = 4 * 1.359822549

$\hat{\sigma}_A^2$ = 5.439290196

$\hat{\sigma}_P^2$ = 1.359822549 + 2.0147026942 + 17.733359871

$\hat{\sigma}_P^2$ = 21.1078851142

$h^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_P^2} = \frac{5.439290196}{21.1078851142} = 0.2576899659$

<u>Both years combined on a plant basis</u>

$\hat{\sigma}_A^2$ = 4 * 0.5106504665

$\hat{\sigma}_A^2$ = 2.042601866

$\hat{\sigma}_P^2$ = 0.5106504665 + 0.2954317895 + 0.4773492171 + 19.0149693409

$\hat{\sigma}_P^2$ = 20.298400814

$h^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_P^2} = \frac{2.042601866}{20.298400814} = 0.1006287089$

Part 4: Narrow-sense heritability using variance components on a family basis:
To determine the heritability on a family basis, using variance components, use the SAS output from corresponding year(s) from the table REML iterations (see Table 3). The harmonic mean will also be required:

$$\hat{\sigma}^2_G = \textit{iteration 4 of var(line)}$$

$$\hat{\sigma}^2_P = \textit{iteration 4 of var(line)} + \frac{\textit{iteration 1 of var(rep*line)}}{\textit{\# of reps}} + \frac{\textit{iteration 4 of var(error)}}{\textit{harmonic mean}}$$

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_P}$$

<u>Year 1 family basis</u>

$$\hat{\sigma}^2_G = 0.2440771090$$

$$\hat{\sigma}^2_P = 0.2440771090 + \frac{2.005397955}{3} + \frac{20.0472090769}{43.7333}$$

$$\hat{\sigma}^2_P = 1.3709399915$$

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_P} = \frac{0.2440771090}{1.3709399915} = 0.1780363185$$

<u>Year 2 family basis</u>

$$\hat{\sigma}^2_G = 1.359822549$$

$$\hat{\sigma}^2_P = 1.359822549 + \frac{2.0147026942}{3} + \frac{17.733598971}{30.8}$$

$$\hat{\sigma}^2_P = 2.6071563141$$

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_P} = \frac{1.359822549}{2.6071563141} = 0.5215730801$$

<u>Both years combined on a family basis</u>

$$\hat{\sigma}^2_G = 0.5106504665$$

$$\hat{\sigma}^2_P = 0.5106504665 + \frac{0.2954317895}{2} + \frac{0.4773492171}{6} + \frac{19.0149693409}{43.7333}$$

$$\hat{\sigma}^2_P = 0.8103902037$$

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_P} = \frac{0.5106504665}{0.8103902037} = 0.6301291207$$

Part 5: Narrow-sense heritability calculations using parent offspring regression:
To determine the narrow-sense heritability using parent offspring regression, use the SAS output to locate the PAR intercept estimate for *b*. The standard error of estimate will be the Standard Error for *b*.

<u>Year 1</u>
$h^2 = 2b$
$h^2 = (2 * 0.0479310) \pm (2 * 0.07226478)$
$h^2 = 0.095862 \pm 0.14452956$
<u>Year 2</u>
$h^2 = 2b$
$h^2 = (2 * 0.17600462) \pm (2 * 0.1027741)$
$h^2 = 0.35200924 \pm 0.2055482$
<u>Both years</u>
$h^2 = 2b$
$h^2 = 2 * 0.09639782 \pm (2 * 0.05940453)$
$h^2 = 0.19279564 \pm 0.11880906$

VITA

Reeve Daniel Legendre was born to parents Mark and Mary Legendre as the youngest child of two. Raised in Baton Rouge, Louisiana, he graduated from Catholic High School in 2004 and enrolled at Louisiana State University that fall. Graduating with a Bachelor of Science in Horticulture in 2011, he worked in the LSU Sweet Potato Breeding Program under Dr. Don LaBonte who became his major professor. Reeve is a candidate for a Master of Science in the School of Plant, Environmental, and Soil Sciences with an emphasis on plant breeding and genetics.