Characterization and application of Arkansas male sterile lines for hybrid rice production

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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CHAPTER 1: LITERATURE REVIEW

A Short History of Hybrid Rice Production

The development of hybrid rice began in China in 1964. Research by Dr. Yuan Longping led to the finding of a wild male sterile plant from a field that was later determined to be cytoplasmic male sterile. This cytoplasmic male sterile was then incorporated into the three-line method of hybrid rice production. In the three-line method there are three rice lines (as the name suggests) involved in the production of hybrid seed. One line required as already mentioned, is a cytoplasmic male sterile (CMS) line. It is referred to as the A-line and is required because the pollen is sterile due to an interaction between genetic factor(s) present in cytoplasm and the nucleus. A maintainer line referred to as a B-line is isogenic to the corresponding A-line and is required because its pollen is fertile and must be crossed with the A-line to produce seed of the A-line. The third line is a restorer line referred to as a R-line, and is required because it possesses dominant fertility-restoring genes and must be crossed with an A-line to produce the hybrid seed (Virmani, 1997).

Soon the three-line method became successful and hybrid rice was introduced into China rice acreage which resulted in a 10% - 20% yield advantage compared to the top conventional varieties (Cheng et al., 2004). The added yield advantage was sought after due to the population growth from 800 million in 1970 to 1.38 billion in 2017 and decrease of land for rice production from 36.5 million ha in 1975 to 30.5 million ha in 2000 (Worldometers, 2017; FAO, 2005). The commercialization of hybrid rice in China led to an increase of rice production by approximately 400 million tons from 330 million hectares of hybrid rice during the period of 1976 – 2002 (Cheng et al., 2004). In 2009, China's rice yields averaged at 6.6 tons per hectare, which was

much greater than the world average of 4.2 tons per hectare in the same year (IRRI – Hybrid Rice, 2018).

There is not as much hybrid rice history in the U.S. as compared to Asia as it is relatively new in U.S. rice production. It was not until 2000 that the first U.S. hybrid rice was developed. "XL6" was released by RiceTec Inc. and has since been commercially grown in the Mid-South of the United States (Nalley et al., 2015). Hardke et al., (2016) reported that hybrid rice production has grown rapidly in Arkansas and about 50% of Arkansas' rice acreage are now devoted to hybrid rice production. With the increase of hybrid rice acreage and demand for greater yields in the U.S., more companies and universities are working to develop more and better-performing hybrids.

Hybrid rice is first generation (F_1) seed which differs compared to conventional rice cultivars that are F_8 or later generation. Hybrid rice is the direct progeny of two genetically diverse parents which results in better performance due to the phenomenon known as heterosis. Because hybrid seed is F_1, it must continuously be produced by crossing its parents to produce enough seed for the next growing season. Due to the continuous replication of crossing the hybrid's parents there is a great amount of seed production required, however, it is feasible due to the characteristics of its female parent. The female parent is developed expressing either environmental genetic male sterility (EGMS): the sources of sterility are several genes and their expression is regulated by specific environmental conditions; or cytoplasmic male sterility (CMS). EGMS parents are used in the two-line method (two parents required for hybrid production), whereas CMS lines are used in the three-line method (as previously discussed) (Virmani et al., 2003). This study focuses on the two-line method.

Application of the Two-Line Method

The two-line method requires an EGMS line and a pollen donor (male) parent to produce hybrid seed. The male parent can be any variety or line, however, for better chances of higher-performing hybrids elite varieties are commonly used. For the two-line method to be possible there must be a stable EGMS line in which sterility can be induced for hybrid seed production and fertility can be maintained for self-pollination. Pollen sterility is induced by environmental conditions such as photoperiod (PGMS), temperature (TGMS), or both (PTGMS) at certain thresholds which affects the expression of sterility genes in the EGMS lines. Pollen fertility can be maintained, however, when environmental conditions meet certain thresholds so that the EGMS lines can self-pollinate for seed increase. It can be convenient for hybrid seed production when environmental conditions are right, but it also can result in impure hybrid seed if environmental conditions are not right and result in some self-pollinated EGMS seed. EGMS lines must be developed considering the environment in which the line will be used in (Virmani et al., 2003).

The large-scale application of the two-line method in hybrid seed production began with the discovery of the PGMS line, Nongken 58s, at Hubei Province, China in 1973 (Wang et al., 2011). Nongken 58s has been a donor parent to many male sterile lines used for hybrid seed production in the two-line method. The two-line method had begun early, but it was not until 1994 before the first commercial two-line hybrid was released in China (IRRI – By-products, 2018). By 2002, nine two-line hybrids were available for commercial production and accounted for 18% of total hybrid rice acreage (Cheng et al., 2004).

In the U.S., the application of the two-line method started later and most, if not all, EGMS lines come from Asia. The initial problem with getting the two-line method started in the

U.S. was lack of access to EGMS lines. The first documentation of U.S. environmentally sensitive male sterile rice plants was by Rutger and Schaeffer (1989) who described a mutant conditioned by a single recessive gene found from anther culture-derived plants. Oard et al. (1991) described a separate mutant conditioned by two genes with epistatic effect found in the M2 generation of ethyl methane sulfonate-treated material from the rice cultivar M-201. Pollen sterility was induced by photoperiod for both mutants.

With the evidence of EGMS lines being developed via induced mutagenesis, scientists at Dale Bumper National Rice Research Center, USDA-ARS at Stuttgart, AR conducted a study to develop new sources of male sterility conferred by a dominant sterility gene by using gamma-irradiation in 1993 and 1994 (Zhu and Rutger, 1999). They believed such mutant genes could produce a stable male sterile line preferable for variety development. Four male sterile mutant lines were developed from the following cultivars: "Kaybonnet" (GSOR 1), "Orion" (GSOR 2), "Cypress" (GSOR 3), and "LaGrue" (GSOR 4). The results from the study determined that GSOR 1, 2 and 4 possessed dominant male sterility genes and GSOR 3 possessed recessive male sterility genes. Pollen sterility was induced by photoperiod in all four lines (Zhu and Rutger, 1999). No publication describes an attempt of using U.S. developed EGMS lines for hybrid seed production. It is difficult to describe the application of the two-line method in the U.S. further since all hybrid rice is produced by private companies, thus there is no shared information or studies of the EGMS lines used for U.S. hybrid rice.

Genetic Basis of EGMS Traits

Several early studies indicated that TGMS traits are controlled by a single recessive gene. It was also discovered that there are different alleles associated with different TGMS lines (Virmani et al., 2003). *Tms$_1$* on chromosome 8 (Wang et al., 1995), *tms$_2$* on chromosome 7 (Yamaguchi, 1997), *tms$_3$* on chromosome 6 (Subudhi et al., 1997), *tsm$_4$* on chromosome 9 (Reddy et al., 2000), *tsm$_5$* on chromosome 2 (Yang et al., 2007), *tsm$_6$* on chromosome 5 (Lee et al., 2005), *tsm$_{6(t)}$* on chromosome 10 (Liu et al., 2010), *tms$_8$* on chromosome 11 (Hussain et al., 2012), and *tms$_9$* on chromosome 11 (Sheng et al., 2013) are the current genes associated with thermally-induced pollen sterility.

It is also evident that PGMS traits are controlled by a single recessive gene, although there may be some minor genes also responsible for sterility (Virman, 2003). There have not been many genes associated with photoperiod pollen sterility; *pms$_1$* on chromosome 7, *pms$_2$* on chromosome 3, and *pms$_3$* on chromosome 12 are the only three found so far (Chen et al., 2010).

PTGMS traits are more complex as induction of pollen sterility is influenced by both temperature and photoperiod. The most accepted concept is that the genetic control of PTGMS is governed by multiple genes having major and minor effects. It is expected that many genes are responsible for PTGMS as mapping studies have assigned PTGMS genes to six of the 12 rice chromosome: 3, 5, 6, 7, 11, and 12 (Virmani, 2003).

University of Arkansas EGMS Line Development via Spontaneous Mutations

In 2010, the University of Arkansas established a hybrid rice breeding program to produce new, improved hybrid cultivars in response to the success and popularity of commercial hybrids under the direction of Drs. Zongbu B. Yan and Chris W. Deren. Since EGMS lines were not accessible for this program, efforts were made to develop EGMS lines via spontaneous mutations through screening germplasm materials introduced into a different environment and by making wide crosses among genetically dissimilar parents (Virmani et al., 2003). A small core collection made up of 203 rice accessions from 30 countries were selected from the U.S. Small Grains Collection in Aberdeen, Idaho based on their genetic and geographic diversity as well as their agronomic characteristics. F_3 populations resulting from approximately 1,278 crosses were grown in field condition and each progeny plant was evaluated based on seed set which is determined using the following formula:

$$Seed\ Set = \frac{\text{number of filled grains}}{\text{total number of florets in a panicle}} \times 100$$

The resulting plants identified as male sterile were selected as being EGMS lines adaptable to Arkansas' climatic conditions. The lines were sterile during the time of Arkansas rice production; thus, these lines would be capable of producing hybrid seed. To produce seeds from the plants with aborted pollen, the selected plants were transplanted into pots, ratooned (cutting below the plants' matured panicles to allow for the development of additional panicles) and placed in a greenhouse with conditions simulating an autumn season (low temperature and short daylight). It was not noted, however, what specific conditions (temperatures, day length, or a combination of both) expressed pollen fertility. The evaluation of these populations was

continued for several generations and as a result, several EGMS lines, 236s, 801s, 805s, and 811s were developed (Yan et al., 2010).

The Arkansas EGMS lines are unique because they are developed explicitly by the University of Arkansas Hybrid Rice Breeding Program. They are not available anywhere else, and any hybrids produced from these lines combined with Arkansas elite varieties will be solely owned by the University of Arkansas. For hybrid seed production, the lines will need to be further studied to determine the genetic basis of sterility (photoperiod, temperature, or both), the environmental threshold for inducing sterility, the optimum planting date for a sterile environment, and a recommended seeding rate for optimum sterile, panicle production.

Thesis Organization

This thesis is organized into three chapters. Chapter one (this chapter) is a review of the literature relevant to this thesis. Chapter two is a manuscript of research (following Crop Science journal publication style) to determine the genetic source of sterility (photoperiod, temperature, or both) of the Arkansas EGMS lines, the environmental threshold for inducing sterility, the optimum planting date for a sterile environment, and a recommended seeding rate for optimum sterile, panicle production. Chapter three is the conclusions drawn from the research. Dustin Glen North was the primary investigator and author for this work under the supervision of Drs. Shuizhang Fei, Ehsan Shakiba, Thomas Lubberstedt, and Mark Westgate. The authors would like to express their gratitude to Arkansas rice producers via monies administered by the Arkansas Rice Research and Promotion Board; and the University of Arkansas System, Division of Agriculture.

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CHAPTER 2: GENETIC SOURCES AND POLLEN STERILITY THRESHOLDS OF ARKANSAS MALE STERILE RICE LINES

Introduction

Hybrid Rice

Hybrid rice (*Oryza sativa*) production has expanded world-wide since it was first commercialized in 1976. The potential of hybrid cultivars yielding 15% to 20% higher than pure-line cultivars has led to an increase of rice production by approximately 400 million tons from 330 million hectares of hybrid rice during the period of 1976 – 2002 (Cheng et al., 2004). Hybrids differ from pure-lines. F_1 seeds derived from the crossing of two genetically diverse inbred parents result in greater performance due to heterosis (Kuyek et al., 2000). Hybrid seed cannot be saved and grown as pure-line seed because the F_2 population will segregate and lead to the loss of uniformity within the population. To maximize yield gain, continuous, commercial production of F_1 seed is thus required. Large scale production of hybrid seed is made possible via using male sterile lines developed to serve as the female parents. Two type of male sterility are utilized for F1 hybrid rice production with the first one known as environmental genetic male sterility (EGMS): the sources of sterility are several genes and their expression is regulated by specific environmental conditions. The second type is known as cytoplasmic male sterility (CMS) which results from specific nuclear and mitochondrial interactions. CMS is used in a three-line hybrid rice production system, whereas EGMS is used in a two-line hybrid production system (Virmani et al., 2003). This study focuses on male sterile lines used in the two-line production system.

In the two-line system, the pollen sterility is induced by environmental conditions such as photoperiod, temperature, or both, which affects the expression of sterility genes in EGMS lines.

EGMS lines are classified into five categories according to which sterility gene(s) they possess: thermo-sensitive genic male sterile (TGMS), reverse thermo-sensitive genic male sterile (rTGMS), photoperiod-sensitive genic male sterile (PGMS), reverse photoperiod-sensitive genic male sterile (rPGMS), and photo-thermosensitive genic male sterile (PTGMS). A number of genes have been mapped that control these male sterile lines: *tms1*, *tms2*, *tms3*, *tms4*, *tms5*, *tms6*, and *tms6(t)* associated with TGMS; *rtms1* associated with rTGMS; *pms1*, *pms2*, and *pms3* associated with PGMS; *rpms1* and *rpms2* associated with rPGMS; and *ptgms2-1* and *pms1(t)* associated with PGMS (Zhou et al., 2012). Each sterility gene in these lines has a threshold environment in which above or below that threshold influences pollen sterility (Table 1). For hybrid rice production, TGMS, PGMS, and PTGMS lines are predominantly used. The threshold for sterility in most TGMS lines is >30^{o}C for daytime (dt) and >24^{o}C for nighttime (nt). The sterility threshold for rTGMS is <24^{o}C (dt) and <16^{o}C (nt). Most PGMS lines have a threshold of day-length >13.75 h (Virmani et al., 2003). The sterility threshold for rPGMS is <13h (Zhang et al., 2013). PTGMS lines are more sensitive to temperature than photoperiod when daytime temperatures are <30^{o}C or nighttime temperatures are <24^{o}C, thus photoperiod has no effect. However, when the temperature is within the range of 24^{o}C - 30^{o}C then photoperiod affects the sterility of the PTGMS lines; inducing sterility when photoperiod is 14hr or longer in comparison with inducing fertility when photoperiod is 13h (Virmani et al., 2003).

The primary advantage of the two-line system is that there is no need for a maintainer line, which is required for F1 seed production in the three-line CMS system. This reduces the time and labor of hybrid seed production. Another important advantage is that any fertile line can be used as a male parent. This advantage greatly increases the flexibility of producing commercial hybrids. However, EGMS female lines are susceptible to sudden temperature change

during the R1 growth stage, when meiosis occurs, causes pollen to become partially or fully fertile (Moldenhauer et al., 2001). This poses a serious problem for hybrid seed production because seed produced from the male sterile line could be from self-pollination, affecting the purity of the hybrid seed. Therefore, identification of thresholds for male sterility is important in hybrid rice production. Based on our knowledge there is no previous study to evaluate the threshold of EGMS lines developed in the U.S. and all previous studies on this topic were done in Southeast Asia. Male sterile lines developed in other countries have been studied in the U.S. via DNA sequencing, but to our knowledge there are no published evaluations of the threshold environment affecting male sterility. There might be studies of male sterile lines by private companies looking at sterility thresholds, but they have not released such data. In this study, we aim to identify the nature (type of gene(s) possessed) and environmental thresholds of EGMS male sterility in Arkansas male sterile lines.

Hybrid Rice Production in Arkansas

Arkansas is the top rice producing state in the United States. In 2015, Arkansas harvested rice from 512,000 ha (1,286,000 acres) farmlands with an average yield of 8,227 kg/ha (7,340 lb/ac) which accounted for 49% of total United States rice production (Hardke, 2017). Hybrid rice production has grown rapidly during the last decade in Arkansas. Although the cost of premium hybrid seeds is greater than conventional cultivars and milling quality of hybrids is relatively less than conventional cultivars, farmers in Arkansas are interested in growing hybrid rice due to its greater yield performance. Nalley et al. (2015) reported the economic advantage of the hybrid rice cultivars in Arkansas to conventional cultivars in a 10-year period from 2003 to 2013. The first U.S. hybrid rice cultivar “XL6” was developed by RiceTec Inc. in 2000, and it

has since been grown commercially in the Mid-South of the United States. Hardke et al., (2017) reported that hybrid rice production has grown rapidly in Arkansas and about 40% of Arkansas rice acreage is devoted to hybrid rice.

Development of Arkansas Male Sterile Lines

In 2010, the University of Arkansas established a hybrid rice breeding program to produce new, improved hybrid cultivars in response to the success and popularity of commercial hybrids under the direction of Drs. Zongbu B. Yan and Chris W. Deren. Since EGMS lines were not available for this program, efforts were made to develop EGMS lines through spontaneous mutation by making wide crosses between genetically dissimilar parents. A small core collection made up of 203 rice accessions from 30 countries were selected from the U.S. Small Grains Collection in Aberdeen, Idaho based on their genetic and geographic diversity as well as their agronomic characteristics. F_3 populations resulting from approximately 1,278 crosses were grown in field condition and each progeny plant was evaluated based on seed set which is determined using the following formula:

$$Seed\ Set = \frac{\text{number of filled grains}}{\text{total number of florets in a panicle}} \times 100$$

The resulting plants identified as male sterile were selected as being EGMS lines adaptable to Arkansas' climate conditions. The lines were sterile during the time of Arkansas rice production, thus these lines would be capable of producing Arkansas hybrid seed (Yan et al., 2010).

To produce seeds from the plants with sterile pollen, the selected plants were transplanted into pots, ratooned (cutting below the plants' matured panicles to allow for the development of additional panicles) and placed in a greenhouse with conditions simulating autumn season (low temperature and short daylight). The evaluation of these populations continued for several

generations and several EGMS lines were developed - 236s, 801s, 805s, and 811s (Yan et al., 2010).

Prior to the University of Arkansas research initiative, scientists at Dale Bumper National Rice Research Center, USDA-ARS at Stuttgart, AR conducted a study to develop new sources of male sterility conferred by a dominant sterility gene by using gamma-irradiation in 1993 and 1994 (Zhu and Rutger, 1999). Four male sterile mutant lines were developed from the following cultivars: "Kaybonnet" (GSOR 1), "Orion" (GSOR 2), "Cypress" (GSOR 3), and "LaGrue" (GSOR 4). The results from the study determined that GSOR 1, 2 and 4 possess dominant male sterility genes and GSOR 3 possesses recessive male sterility genes (Zhu and Rutger, 1999). However, the authors did not report whether the source of sterility was based on CMS or EGMS genetic mechanisms. Moreover, there is no previous study on the application of GSOR lines in a hybrid production system.

Objectives of Research

The ultimate goal of this study is to determine the environmental threshold for inducing sterility of these newly developed EGMS lines so that they may be properly and successfully used in the production of Arkansas hybrid seed. Some of the important challenges surrounding the development of male sterile lines and hybrid production in Arkansas are as follows: 1) the genetic basis of sterility in male sterile lines developed at the University of Arkansas and USDA-ARS are not known; 2) variable Arkansas weather during hybrid rice seed production could cause the male sterile lines to become fertile; 3) late formed tillers (shoots that develop from the leaf axils at each un-elongated node of the main shoots and from other tillers) that encounter low temperatures at the R1 growth stage shifts from sterile to partially fertile (Hardke, 2018).

Our objectives in this study, therefore, were to: 1) identify the genetic basis of pollen sterility (TGMS, PGMS, or PTGMS) in Arkansas and GSOR male sterile lines; 2) determine the environmental threshold for pollen sterility relevant to sterility gene(s) [i.e. mechanism] the lines possess; 3) determine the optimum/planting dates to ensure an sterility-inducing environment during seed set; and 4) identify an optimum planting density to reduce late-formed tillers that might be induced to produce male-fertile panicles. The results of this study can be used by rice breeders, geneticists for further genetic analysis of sterile gene(s) and by agronomists and seed producers for producing hybrid seed in Arkansas rice lands at optimum conditions.

Materials and Methods

Seed Materials

The seeds of the 811s, 236s, 801s, and 805s inbred lines were received through the seed source of the University of Arkansas Rice Research and Extension Center (RREC) hybrid breeding program. To increase genetic uniformity of male sterile lines of 236s and 811s, about 200 plants from each line were grown under greenhouse conditions in December 2015. Tissue samples from each plant were collected and tested via molecular markers to select for homozygous plants, thus improving purity. Several phenotypic characteristics such as heading date (when 50% of panicles have partially exerted from plant sheath), plant height, and percentage of sterility were recorded. Homozygous plants were identified, ratooned and placed in lower temperature (21^{o}C) for seed production (Moldenhauer and Slaton, 2001). In the summer of 2016, the same procedure was done for 801s and 805s except that the plants were planted in the field at RREC. The selections based on the same phenotypic characteristics described for 236s and 811s were made, ratooned, placed in a controlled environment during the

fall season, and allowed to set seed to establish a pure seed source of the Arkansas male sterile lines. Seeds from GSOR 1, 2, 3 and 4 experimental lines were obtained from the germplasm collection at Dale Bumper's National Rice Research Center, USDA-ARS, Stuttgart, AR.

Growth Chamber Study

A growth chamber (GC) study was conducted to determine the threshold for sterility/fertility of each line under different environmental conditions. This study followed a method described by Lee et al. (2005) in which different daily maximum and minimum temperatures, and photoperiods were used to affect spikelet sterility/fertility. Four GC located in RREC were used for implementing 16 environmental treatments on the four Arkansas and four USDA EGMS lines. The photoperiod treatments were 12.5hr, 13hr, 13.5hr, and 14hr. Four daytime/nighttime temperatures were: 21.1°C/23.9°C, 23.9°C/26.7°C, 26.7°C/29.4°C, and 29.4°C/32.2°C. Thus, 16 combinations of temperature and photoperiod were implemented for testing (Table 2). These treatment combinations were based on possible climate conditions in Arkansas during plant growth.

For each environmental treatment, four male sterile plants from each sterile line were grown in a 3.8-liter pot. There were two replications in each treatment, thus there were eight possible male sterile [only if treatments were successful] plants from each sterile line. Each treatment was applied for 30 days (during the first reproductive stages). The pots were placed in a plastic tub with the dimension of 15.2 x 15.5 x 15.5cm in a greenhouse to hold water at a 5-10 cm depth (Fig. 1). Watering and fertilizer – 120 units of urea (46-0-0) – applications were implemented according to the standard rice growth recommendations in Arkansas. The plants were transferred later to the assigned GC to apply the treatment prior to the plants' reproductive

growth stage 1 (R1) which is approximately 30 days prior to the R2 growth stage (Fig. 2; Moldenhauer and Slaton, 2001). R1 is determined by panicle differentiation of the main tiller in which internode length (the panicle is forming inside the plant and is being pushed upwards as it grows) has reached ½ to ¾ inches (1.3cm to 1.9cm) (Fig. 3; Moldenhauer and Slaton, 2001). The most critical time for inducing sterility and fertility occurs in the reproductive stages: R1 + 15 days (Virmani, 2003). The plant growth stages were monitored daily.

At panicle heading (exertsion) (Fig. 4), three panicles from each plant were randomly selected and tested for pollen sterility/fertility. Results from 24 panicles were considered adequate to represent the response of each line to each treatment. Of each selected panicle, the anthers were harvested between 7:00 and 10:00 am from four spikelets for pollen stain testing (Table 4). The spikelets were sampled from separate panicles branches and prior to flowering so that all pollen may be available for testing. Any spikelets that had begun flowering near the top of the panicles were not sampled. The anthers from all four spikelets were sampled together to represent each plant. Sterility levels were assessed based on pollen appearance (Fig. 5) as classified by Virmani et al. (1997). Pollen appearance is also shown by photographs taken during this study (Fig. 6). In this classification, plants possessing >99% sterile pollen are categorized as completely sterile (CS), <99% – >91% sterile pollen is categorized as sterile (S), <90% – >71% sterile pollen is categorized as partial sterile (PS), <70% – >31% sterile pollen is categorized as partially fertile, <30% – >21% is categorized as fertile, and <20% – >0% is categorized at fully fertile (Table 4; Virmani et al., 1997). Sterile and fertile pollen grains were counted using a 10x microscope lens. The percentage of sterility was calculated based on the amount of sterile pollen grains observed of the total amount of pollen sample present at the center of the microscope slide. For this study plants that were partially sterile (<90%) are

categorized as fertile because anything less than the sterile category (<91%) could increase self-pollinated seed which will lead to impure hybrid seed production. Based on this criteria the three panicles sampled determined if the plant was sterile or fertile. Eight plants represented each line/treatment. All eight of the plants must be sterile in a treatment so that the environment can be considered sterile. Pollen staining was used to assess treatment effects on pollen sterility/fertility (Table 3). The procedure used for pollen staining closely follows Guzman and Oard (2011) in which a stock solution of iodine crystals, potassium iodide, and denionized water is applied (one droplet) to the anthers collected from the spikelets on a microscope slide. After gently pressing the anthers and stirring the solution the pollen will absorb the solution and after 5 minutes it can be observed with a microscope using a 10x objective lens.

2017 Field Study – 1 Location, 3 Planting Dates

In summer 2017, a field test was conducted to determine the optimum planting date for sterile lines at field conditions. The Arkansas male sterile lines, except 805s (due to limited amount of seed), and the USDA lines were tested in a randomized block design with three replications and three planting dates of April 25, May 02, and May 11 at RREC. The amount of seeds of 801s were limited, therefore, it was planted only on the third date (May 11).

The plots were composed of seven rows of 2.1 m long spaced 0.2 m apart. Approximately 200 seeds of each male sterile line were sown in each of plot. Irrigation, maintaining of water levels (5 – 10 cm depth), and fertilizer applications – 120 units of urea (46-0-0) – were implemented according to the standard rice growth recommendations in Arkansas.

Air temperatures were recorded every hour to observe any temperatures above or below the sterility thresholds of each EMGS line. Thresholds will be based on the results from the

growth chamber study. Temperatures are recorded by a weather station deployed by the University of Arkansas Cooperative Extension Service at RREC within 1 km distance from the plots (Weather stations, 2017).

Ten plants from each plot (Fig. 7) were randomly selected; panicles from the main stem, 1st tiller, and 2nd tiller were collected (Fig. 8.) and anthers were collected from 5 spikelets each from separate panicle branches for testing via pollen staining in the lab. Panicles sampled were placed inside of pollination bags to prevent adventitious pollination. Pollen staining was accomplished within 30 minutes of sampling the panicles. At the end of the season, 1,890 panicles were evaluated for sterility/fertility via pollen staining. Each plot was evaluated on the average number of sterile panicles using the same quantitative criteria as for pollen sterility (>90%) Observation of sterile pollen was accomplished by using the pollen staining procedure previously described (Table 3). The results were used to determine the optimum planting date for sterile conditions in Stuttgart which would help breeders/seed companies to decide when to plant for hybrid seed production.

2018 Field Study – 3 Planting Dates and 3 Locations

The objective for the 2018 field study was to determine an optimum planting date for a sterile environment at three locations in Arkansas. The planting dates varied depending on the location. Due to the results gathered from the 2017 field and from the growth chamber studies, the GSOR lines were excluded from this field study since these lines expressed mostly fertile pollen in all 16 environments tested.

The Arkansas male sterile lines 236s, 801s, 805s, and 811s were tested in a randomized complete block design with three replications, using three planting dates, at three locations:

RREC (34°27'52" N 91°25'11" W), Rohwer Research Station (RRS) (33°47'51" N 91°16'53" W), and Pine Tree Research Station (PTRS) (35°07'39" N 90°57'50" W). These locations were selected because they represent a large area of rice acreage in Arkansas (Fig. 9).

A HOBO MX2302 external temperature/RH sensor data logger was deployed in the plots at each location to provide accurate temperatures during growth (Onset Computer Corporation, Boume, MA). The HOBOs were used with a RS3-B solar radiation shield to reduce skewed temperature readings from sun light (Onset Computer Corporation, Boume, MA). Each HOBO was programmed to read every 5 minutes daily so that the low and high temperatures during growth could be recorded.

Because of the distance between locations, the lines were planted on different dates. The dates of planting were during the first week of May (5/01 at Rohwer, 5/02 at Pine Tree, and 5/03 at RREC), the second week of May (5/08 at Rohwer, 5/09 at Pine Tree, and 5/10 at RREC), and the third week of May (5/15 at Rohwer, 5/16 at Pine Tree, and 5/17 at RREC).

Due to limited amount of seed, 50 seed were hand planted for each plot (108 plots in total) with the dimension of 1.5m x 1.5m. There were 5 rows with spacing at 30.5cm and spacing between seeds at 15.2cm. Row spacing and distance between seeds were measured using tape measures. Irrigation, maintaining of water levels (5 – 10 cm depth), and fertilizer applications – 120 units of urea (46-0-0) – were implemented according to the standard rice growth recommendations in Arkansas.

Ten plants from each plot were randomly selected; three panicles from each plant including one from each of the main stem, 1st tiller, and 2nd tiller were collected and tested via pollen staining as done so for the previous study. Each plot was evaluated on the average number of sterile panicles observed using the pollen staining procedure previously described (Table 3).

At the end of the season, 3,240 panicles were evaluated for sterility/fertility via pollen staining and seed set. The results were used to determine an optimum planting date for sterile conditions at three locations in Arkansas which would help breeders/seed companies decide when to plant in the rice growing regions of Arkansas for hybrid seed production.

Planting Density Study

The reason of conducting this study is to determine the optimum seeding rate for environmental male sterile lines to reduce late tiller production. This is an issue in hybrid rice production because it results in an impure hybrid seed source due to self-fertilization. Another advantage this study is to determine the best seeding rate to maximize the amount of sterile panicles for hybrid seed production, while not using more seed than necessary. Because of the limited amount of male sterile seed usually available for hybrid seed production, an optimum seeding rate should be established for maximum hybrid seed production while diminishing the amount of male sterile seed wasted via over-seeding.

Due to the limited amount of seed, only one Arkansas EGMS line (811s) was tested. 811s was planted at RREC in a RCBD on July 20th with three replications using three different seeding rates: 1.8 (A), 3.6 (B), and 7.2 (C) seeds/m^2. The late planting date of July 20th was selected to increase the chance of having conditions to induce fertility of the late tillers, and to have isolation from nearby rice. Planting late also would result in isolation from nearby flowering rice to ensure that any seed production is due to self-fertilization.

Several samples of 811s seeds were weighed to determine the average of 39.6 seeds/g. This values was used to calculate the amount of seeds needed in each plot. Using a formula described by Runsick et al. (2009) for pounds of seed per acre, seeding rate A equals 47.2 lb/A

(52.9 kg/ha). Seeding rate B equals 92.1 lb/A (103.2 kg/ha). C seeding rate equals 189 lb/A (211.8 kg/ha). Plot dimensions were 1.52m long and 1.42m wide. There were 7 rows with 20.3cm row spacing. With the weight of seed configured the amount of seed per plot was determined. For the A seeding rate of 52.9 kg/ha, 11.6g of seeds were used for each plot, for the B seeding rate of 103.2 kg/ha, 22.7g of seed were used, and for the C seeding rate of 211.8 kg/ha, 46.6g of seeds were used. Irrigation, maintaining of water levels (5 – 10 cm depth), and fertilizer applications – 120 units of urea (46-0-0) – were implemented according to the standard rice growth recommendations in Arkansas.

Rows 2, 3, 5, and 6 of each plot were selected to record data (Fig. 10). On August 3rd and August 10th plants from the selected rows of each plot were counted to confirm a significant plant stand difference among the seeding rates. The amount of panicles was observed 7 times at random dates – Oct. 3rd, Oct. 8th, Oct. 12th, Oct. 18th, Oct. 29th, Nov. 2nd, and Nov. 9th – during heading (panicle exsertion) to compare number of panicles and compare panicle growth among the different seeding rates. The data were used to relate panicle production rate and seeding rates to establish an optimum seeding rate.

A final evaluation was conducted on Nov. 24th via tiller count after cutting rows 3 and 5 at plant maturation. Rows 3 and 5 of each plot (Fig. 11) were selected to conduct the tiller count evaluation because they were the centermost rows which would better mimic the similar performance of plants in a producer's field. Tillers from the selected rows were counted and separated into three categories: panicles with seed, panicles without seed, and no panicles. Panicles with seeds refers to tillers with exserted panicles containing developed seed resulting from self-pollination by fertile pollen. Panicles without seed refers to tillers with panicles exserted or non-exserted (panicles remaining within the flag leaf sheath) that contained no seed.

No panicles refer to tillers that did not form a panicle. The data from this evaluation were used to reveal which seeding rate is optimum for tiller production with exserted, sterile panicles. This will result in a better chance of available sterile panicles for hybrid seed production without using more male sterile seeds than necessary.

Results

Growth Chamber Test

Lines 801s, 805s, and 811s expressed similar results in all 16 treatments (Tables 5, 6, and 7). Pollen fertility was maintained at environments 1to 8, and pollen sterility was induced under environments 9 to 16. These results indicate 801s, 805s, and 811s are thermos-sensitive male sterile lines with a daytime temperature threshold >29.4°C and nighttime temperature threshold >26.7°C.

Line 236s expressed a higher temperature threshold for inducing pollen sterility (Table 8). Pollen fertility was maintained in environments 1 to 12; whereas pollen sterility was induced under environments 13 to 16. As for the previous lines, 236s was revealed to be thermo-sensitive male sterile, but with a higher daytime temperature threshold of >32.2°C and nighttime temperature >29.4°C.

All of the GSOR lines expressed pollen fertility in 12 of the photo-thermal treatments. Overall 89% of the GSOR plants were completely male fertile (Tables 9, 10, 11, and 12). There is a possibility that the gene associated with sterility was deleted from the genome or only fertile plants are present in the seed source.

2017 Field Test

EGMS line 236s expressed partial sterility among the 3 planting dates. For the 1st planting date, 87% of the 30 plants evaluated were sterile. For the 2nd planting date, only 53% of the 30 plants evaluated were sterile. For the 3rd planting date, 60% of the 30 plants evaluated were sterile. As shown in Figure 12, nearly all of the critical sterility-inducing days (R1 to heading date) for planting dates 2 and 3 were below the threshold for sterility, thus causing partial fertility among the plants. The evaluation suggests a critical growth stage for inducing pollen sterility is during the R1 growth stage, approximately 25 days prior to heading. According to Virmani et al. (1997), the critical timing for inducing sterility varies from 15 to 25 days prior to heading or 5 to 15 days after panicle initiation (R0), which corresponds with the results. This result confirms those of Moldenhauer and Slaton (2001) showing how the number of potential grains per panicle is greatly affected by the environment at the R1 stage. Taken together, these findings indicate that the most critical timing for inducing sterility is from the beginning of the R1 stage to 10 days after. These results suggest that 236s must be planted earlier than any of three planting dates due to late heading (95 days) in order to be sterile.

Line 801s expressed nearly complete sterility with 93% of the 30 plants testing as completely sterile. All of the critical sterility-inducing days (R1-10 days) were above the temperature threshold, while ten days before heading the temperatures were below the threshold (figure 13). Thus there is little to no effect on inducing sterility ten days before heading. The results suggest that the planting date used (May 10) is the latest date to avoid a fertile environment.

Line 811s expressed complete sterility at all three planting dates. As shown in figure 14, all of the critical sterility-inducing days were above the threshold. The evaluation suggests that

all of the daytime temperatures were above the daytime temperature threshold of 29.4°C, thus all three of the planting dates are acceptable to meet sterile conditions. The nighttime temperature threshold might be lower than what the growth chamber study suggested (26.7°C), or it has no effect on sterility.

The nighttime temperatures during the R1 stage were below the thresholds (as determined by the growth chamber study) of 236s, 801s, and 811s, but it seemed to not affect pollen sterility. There are two possibilities to explain these results: (1) the nighttime temperature thresholds are lower than the growth chamber study results suggested, or (2) nighttime temperature does not have a great enough affect on sterility as long as daytime temperatures are above the high temperature threshold. Further investigation is required to distinguish between these possibilities.

The GSOR lines were fertile under most of the conditions of the 2017 trial. Four of the 90 GSOR 2 plants tested were sterile. Twelve of the 90 GSOR 3 plants tested were sterile. Three of the 90 GSOR 4 plants tested were sterile. To test whether these sterile plants were PGMS or EGMS, they were ratooned (cut back) transferred into a growth chamber with a setting of photoperiod <13hr. These plants became fertile and set seed, thus the genetic source must be PGMS because the plants were sterile in the field when photoperiod was >13.5hr. Due to the low amount of seed and the high rate of fertile pollen results, the GSOR lines were removed from testing in 2018. The possible PGMS plants will be evaluated by another study.

2018 Field Test – 3 Locations

All four of the Arkansas EGMS lines, 236s, 801s, 805s, and 811s expressed sterility at the three locations for all three planting dates. The mean daytime high temperature during the

critical stages for inducing sterility for all three planting dates at the three locations was 37.1°C, which is above the sterility threshold of the four EGMS lines.

At PTRS, 96% of the days during critical stages were above both sterility thresholds for all of the EGMS lines (Fig. 15). PTRS had the lowest day time temperatures with a mean of 36.4 °C. Even though three days were below the thresholds it did not affect sterility. If PTRS had the lowest day time temperatures and all of the EGMS lines were sterile, then there is strong indication that all the lines should also be sterile at the other locations.

At RREC 94% of the days during critical stages were above both sterility thresholds and 100% were above the 801s, 805s, and 811s sterility thresholds (Fig. 16). RREC had the second lowest day temperatures with a mean of 37.1°C. There were four days below the sterility thresholds.

At RRS (Fig. 17) 97% of the days during critical stages were above both sterility thresholds and 99% were above 801s, 805s, and 811s sterility thresholds (Fig. 17). RRS had the least low day temperatures with a mean of 37.9°C. There were only two days below the sterility thresholds.

The daytime high temperatures at the three locations was shown to be significant ($p<0.0028$). RRS was shown to be grouped differently than PTRS, indicating a significant difference (Fig. 18). RREC is in both groups, thus shares high temperature similarities and can be considered as the intermediate environment of the three locations. These three locations cover much of rice production acreage in Arkansas. These results indicate that maintaining male sterile inbred rice is possible across the Arkansas rice production area.

Planting Density Test

For total panicle production there were some significant differences among the different seeding rates. Seeding rates A and C were significantly different (p=0.01), as were rates A and B (p=0.05) (Fig. 21). Seeding rates B and C show no significant difference, thus there is no gain from the heavier seeding rate of C compared to seeding rate B for panicle production.

Do the seeding rates have an impact on fertile tiller production? Whole rows were harvested from each plot and separated into three categories by counting the tillers: tillers bearing panicles with seed (fertile panicles), tillers bearing panicles without seed (sterile panicles), and tillers without panicles. Total panicles is the sum of tillers bearing panicles with seed set and the tillers with sterile panicles. Total tillers is the sum of total panicles and the tillers without panicles.

The first category, panicles with seed, is an indication of fertile pollen in spikelets on panicles developing during temperatures below the sterility threshold. This test was planted on July 20th in an attempt to expose plants to temperatures below the sterility threshold during panicle initiation. Part of the test was to look at the potential of pollen fertility due to late tiller production during cooler temperature conditions. All tillers with panicles producing seed were grouped and counted. These panicles had very a low amount of seed (<1 gram), but were still categorized as panicles with seed because any self-fertilized panicles would increase the impurity of hybrid rice seed production. From R1 plus 10 days, there were 3 days with maximum temperatures below the threshold (Fig. 22). From R1 to 50% heading, 29% of the maximum daytime temperatures were below the threshold. This resulted in 13.7% of all panicles having some seed set. Fertile panicle production (panicles with seed set) among all three seeding rates

showed no significant difference (Fig. 23). This means that the opportunities for any late tillers that might produce self-fertilized seeds was not significant ($p>0.05$)for all three seeding rates.

There were significant differences among the amount of tillers with sterile panicles. Because there were significant differences of panicle production there must be some significant difference for the amount of sterile panicles. Seeding rates A and C, and seeding rates B and C were significantly different comparing the amount of tillers with sterile panicle production (Fig. 24). Seeding rate B appeared to be the best option.

There was a significant different of total tillers with panicles between seeding rates A and C ($p = 0.0031$) (Fig. 25). Seeding rate B shows no significance with either A or C when comparing total tillers with panicles produced. Again, seeding rate B is the best option when considering the amount of seed used and panicle production.

Tillers without panicles were counted to observe the amount of non-productive tillers (no panicle development). There is no significant difference among the three seeding rates (Fig. 26). While there were fewer tillers without panicles for seeding rate A compared to the other seeding rates it is not significant ($p>0.05$). Seeding rate B is the best option because of the amount of non-productive tillers is optimized with the amount of seed used.

For the final category – total tillers – there is no significant ($p>0.05$) difference among the three seeding rates (Fig. 27). Seeding rates B and C similarity of total tillers produced ($p = 0.8704$) indicating that increasing the seeding rate beyond 103.2 kg/ha does not result in more tillers production. As a final decision, seeding rate B is the optimum choice for an EGMS parent used in hybrid seed production because.

Discussion

We determined that the four Arkansas EGMS lines, 236s, 801s, 805s, and 811s are TGMS genotypes, in which male sterility is induced by temperatures above a certain threshold. Growth chamber studies revealed temperature conditions inducing sterility. Moreover, photoperiod did not influence sterility. Lines 801s, 805s, and 811s sterility threshold is >29.4°C. Line 236s sterility threshold is >32.2°C. The GSOR lines (1, 2, 3, and 4) were fertile in almost all of the treatments. This may be due to the loss of the sterility gene(s) over time from the GSOR seed source. The sources of sterility of the GSOR lines were not determined.

In the 2017 field test it was verified that lines 801s and 811s share a similar sterility threshold of 29.9°C, whereas 236s has a higher threshold of 32.2°C. This was evident when looking at the sterility response from the different planting dates and temperatures during the critical sterility inducing stages of the EGMS lines. The critical growth stage for inducing pollen sterility is at/during R1 which is approximately 25 days prior to heading. According to Virmani et al. (1997) the critical timing for inducing sterility varies from 15 to 25 days prior to heading or 5 to 15 days after panicle initiation (R0), which corresponds with our results. Based on our findings the most critical timing for inducing sterility is from the beginning of the R1 stage to 10 days after. This is important to know for both hybrid seed production and seed production of the EGMS lines. Knowing the thresholds and critical timing for inducing sterility enables the breeders/producers to successfully plant in the right conditions considering which seed production is desired. The GSOR lines expressed sterility and fertility in different conditions, thus they can at this time not be used in hybrid seed production.

Planting date is important for successful hybrid seed production because the EGMS lines must be grown in an environment that is above sterility thresholds during the R1 growth stage.

Proper planting date must be considered and an optimum planting date of April 25th in Arkansas was determined considering the results. In 2017, 236s expressed some fertility for all 3 planting dates; however, it was less fertile for the April 25th planting. In 2018, all four Arkansas EGMS expressed sterility for all three planting dates at all three of the locations used. Weather is unpredictable, but the Arkansas EGMS lines will have the greatest chance of being sterile when planted on that date. April 25th is also the average, optimum planting date for Arkansas rice production which suggests that the EGMS lines will grow more successfully (Hardke et al., 2018).

The final objective was to find an optimum planting density to reduce late tiller panicles that may become fertile if the environment changes by planting with different seeding rates. Ultimately the seeding rate does not significantly affect the amount of late tiller panicles that could become fertile. Among the three seeding rates, A (52.9 kg/ha), B (103.2 kg/ha), and C (211.8 kg/ha), there was no significant difference for the amount of fertile panicles produced. What was found to be the most important difference is that seeding rate B was shown to be the most efficient when considering the amount of panicles produced. An optimum seeding rate is required because the cost and risk of producing EGSM seed is high and time consuming, and the results from the plant density study suggests a maximum seeding rate of 103.2 kg/ha. The results concluded a maximum seeding rate recommendation, but further studies can be conducted to determine an optimum seeding rate looking at different seeding rates between seeding rates A (52.9 kg/ha) and B (103.2 kg/ha).

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Tables

Table 1 Environmental threshold of each EGMS line for inducing sterility

EGMS LINE	*DL	**DT	***NT
TGMS	-	<30^{o}C	<24^{o}C
RTGMS	-	>24^{o}C	>16^{o}C
PGMS	>13.75hr	-	-
RPGMS	<13hr	-	-
PTGMS	>13hr†	<30^{o}C	<24^{o}C

* Day length required for inducing sterility, ** Daytime temperature required for inducing sterility, *** Nighttime Temperature required for inducing sterility † Day-length for PTGMS lines only affects sterility when DT and NT are within the range of 24^{o}C – 30^{o}C

Table 2 Sixteen environments for growth chamber study

	12.5hr	**13hr**	**13.5hr**	**14hr**
TEMP 1	T1 23.9°C, 21.1°C†	T2 23.9°C, 21.1°C	T3 23.9°C, 21.1°C	T4 23.9°C, 21.1°C
TEMP 2	T5 26.7°C, 23.9°C	T6 26.7°C, 23.9°C	T7 26.7°C, 23.9°C	T8 26.7°C, 23.9°C
TEMP 3	T9 29.4°C, 26.7°C	T10 29.4°C, 26.7°C	T11 29.4°C, 26.7°C	T12 29.4°C, 26.7°C
TEMP 4	T13 32.2°C, 29.4°C	T14 32.2°C, 29.4°C	T15 32.2°C, 29.4°C	T16 32.2°C, 29.4°C

† Each cell represents a different environment in which the lines were grown in and evaluated for sterility (For example, the first cells shows that T1 at 12.5 hours of day-length with a daytime temperature of 23.9°C and a nighttime temperature of 21.1°C).

Table 3 Pollen stain protocol (Guzman et al., 2011)

STEP	PROCEDURE
1	Stock solution of a mixture of iodine crystals, potassium iodide, and deionized water is purchased through VWR and is made by the company BDH.
2	Dilution of stock solution – for every 20 ml of stock solution add 80 ml of distilled water
3	Select 4 florets/spikelets from a panicle from individual branches (between booting and flowering stages).
4	Manually separate the palea from the lemma. The exposed anthers should remain covered by the palea. This should be done within 15 minutes of collecting florets/spikelets.
5	Remove anthers with surgical blade placing them onto a glass slide.
6	Place a droplet of stock solution on the anthers, gently press anthers with surgical blade, and stir to mix solution with pollen.
7	Allow the pollen to absorb the solution for about 5 minutes, then view with a microscope using a 10x objective lens.
8	Pollen grains that are fertile will stain a dark purple color whereas sterile pollen grains will appear as a light, transparent color (Fig. 2).
9	Count pollen grains to determine the ratio of sterile: fertile grains.

Table 4 Category of percentage of pollen grain that are sterile (Virmani et al., 1997)

POLLEN STERILITY (%)	CATEGORY
100	Completely sterile (CS)
91 – 99	Sterile (S)
71 – 90	Partially sterile (PS)
31 – 70	Partially fertile (PF)
21 – 30	Fertile (F)
0 – 20	Fully fertile (FF)

Table 5 Growth chamber results for 801s EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)
T4	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)

Table 6 Growth chamber results for 805s EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)
T4	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)

Table 7 Growth chamber results for 811s EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Sterile)
T4	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)

Table 8 Growth chamber results for 236s EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)
T4	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Sterile)

Table 9 Growth chamber results for GSOR 1 EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)
T4	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Fertile)

Table 10 Growth chamber results for GSOR 2 EGMS

	12.5hr	13hr	13.5hr	14hr
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Sterile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Sterile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Sterile)
T4	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Sterile)

Table 11 Growth chamber results for GSOR 3 EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Sterile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)
T4	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Fertile)

Table 12 Growth chamber results for GSOR 4 EGMS

	12.5hr	**13hr**	**13.5hr**	**14hr**
T1	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)	23.9°C, 21.1°C (Fertile)
T2	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)	26.7°C, 23.9°C (Fertile)
T3	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)	29.4°C, 26.7°C (Fertile)
T4	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Sterile)	32.2°C, 29.4°C (Fertile)	32.2°C, 29.4°C (Fertile)

FIGURES

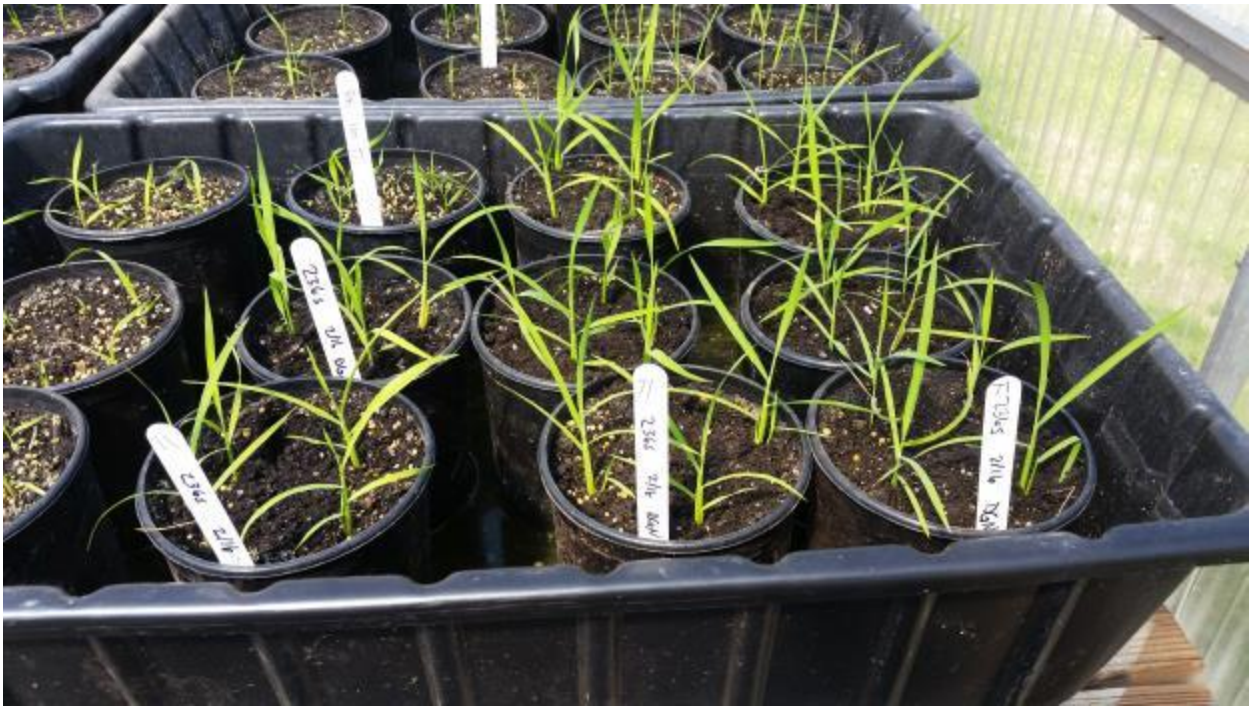


Figure 1. Plants grown in the greenhouse at the stage the plants were moved into the growth chambers. Photographed by author in 2017.

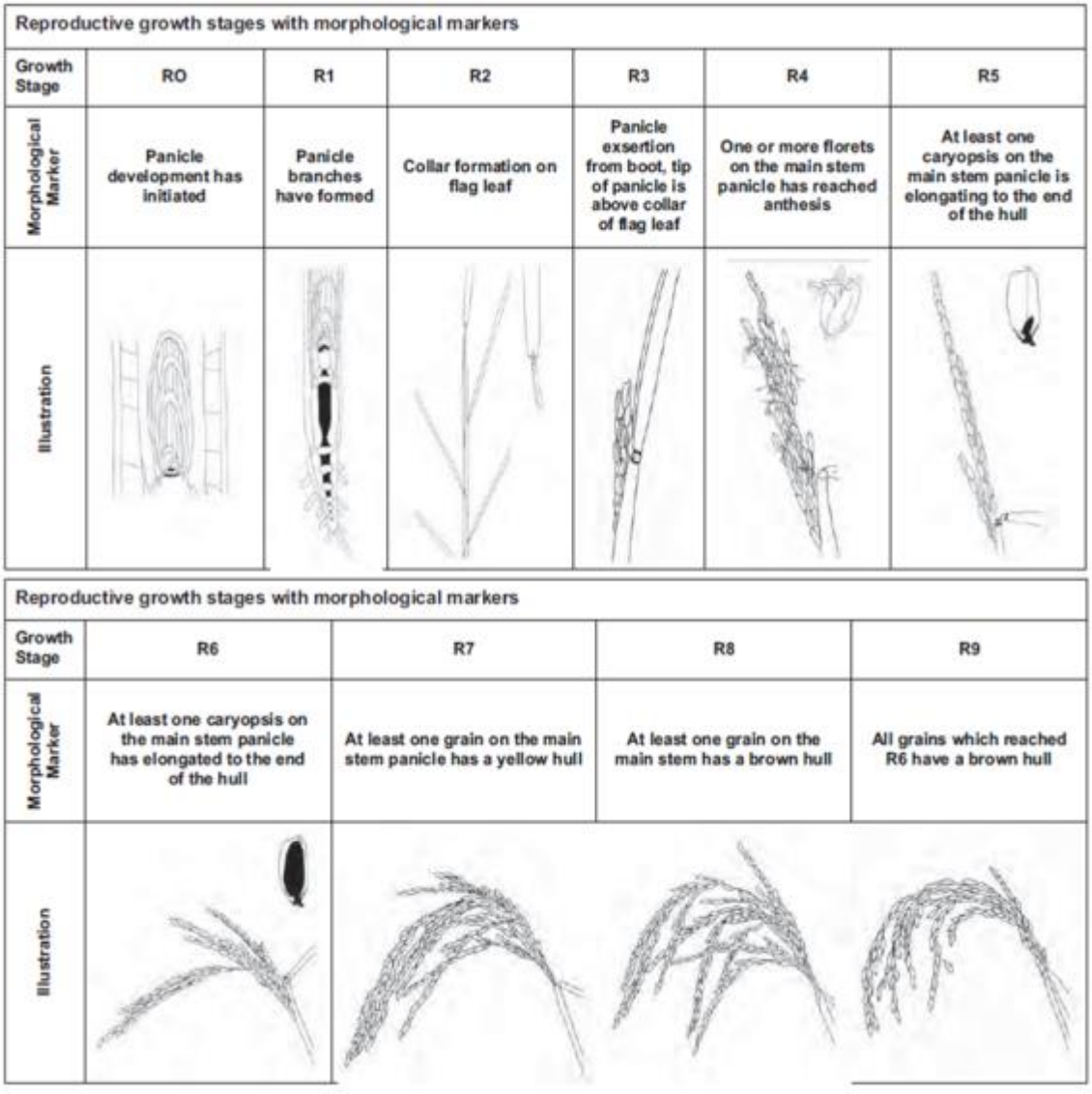


Figure 2. Reproductive growth stages of rice (Moldenhauer and Slaton 2001). Received from https://www.uaex.edu/publications/pdf/mp192/mp192.pdf

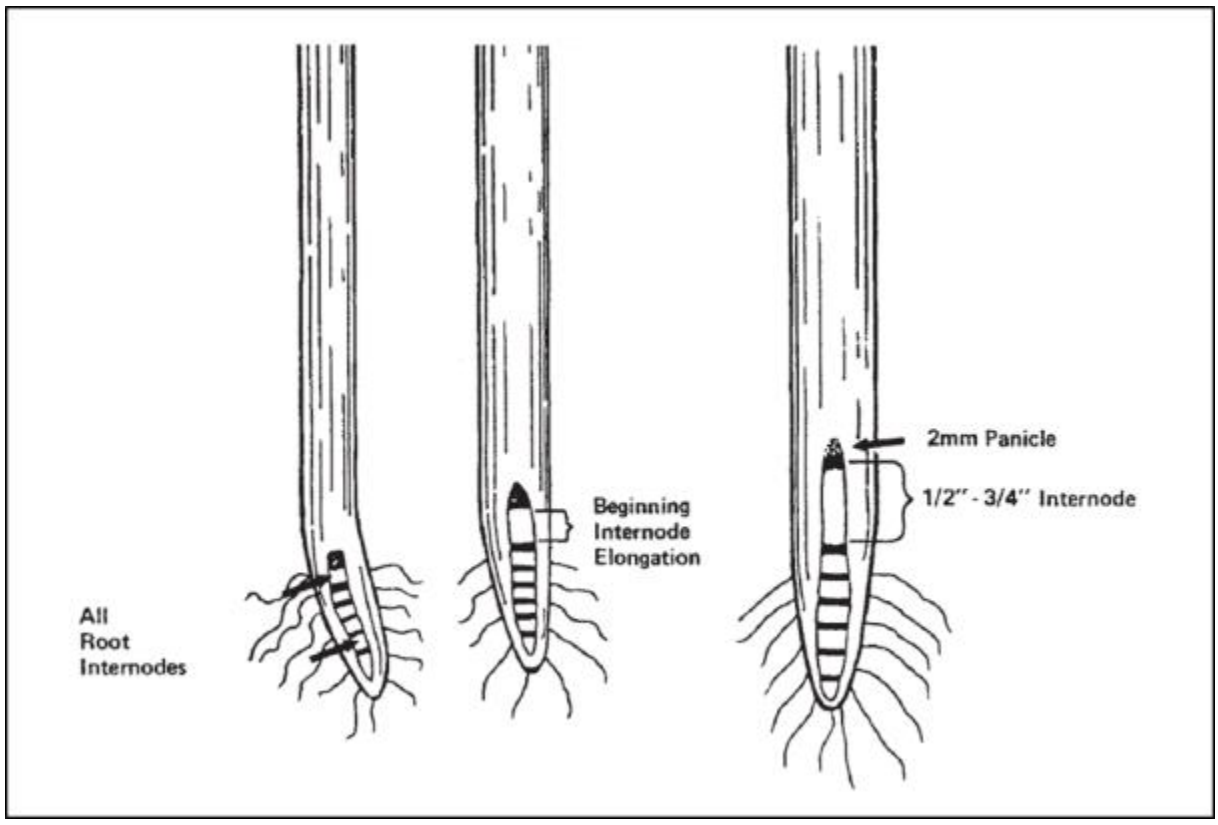
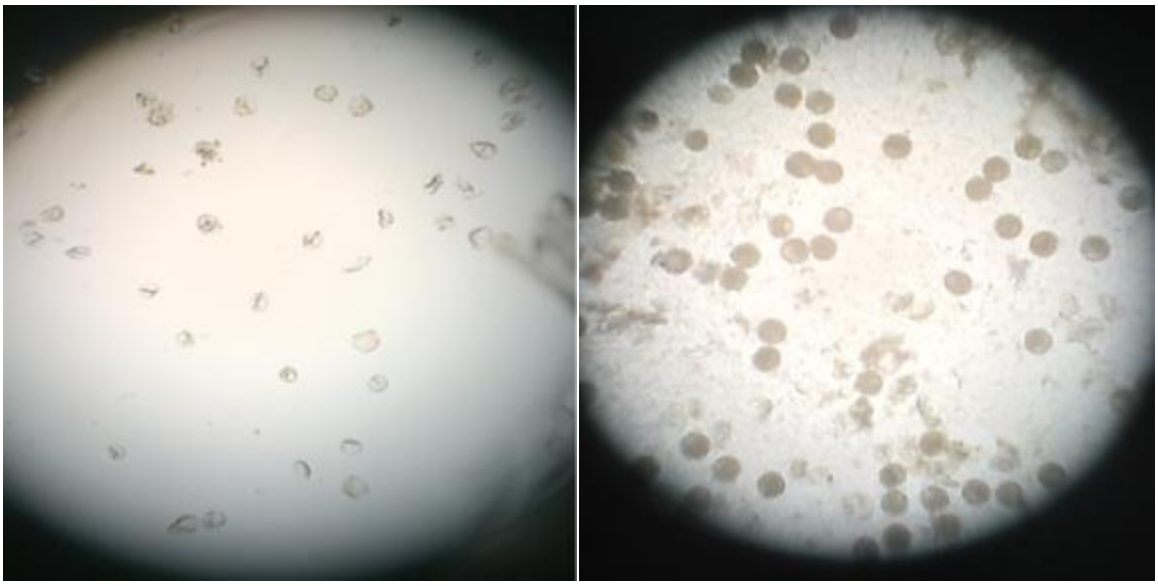


Figure 3. R1 stage (far right) as determined when Internode Elongation has reached ½ to ¾ inches (1.3cm - 1.9cm) (Moldenhauer and Slaton, 2001). Received from https://www.uaex.edu/publications/pdf/mp192/mp192.pdf

Figure 4. Panicles ready to pollen stain in a growth chamber. Photographed by author in 2017.

Category	Appearance	Classification
Unstained withered sterile (UWS)		Sterile
Unstained spherical sterile (USS)		Sterile
Stained round (light) sterile (SRS)		Sterile
Stained round fertile (SRF)		Fertile

Figure 5. Pollen grain appearance after staining (Virmani et al., 1997). Received from http://books.irri.org/9712201031_content.pdf

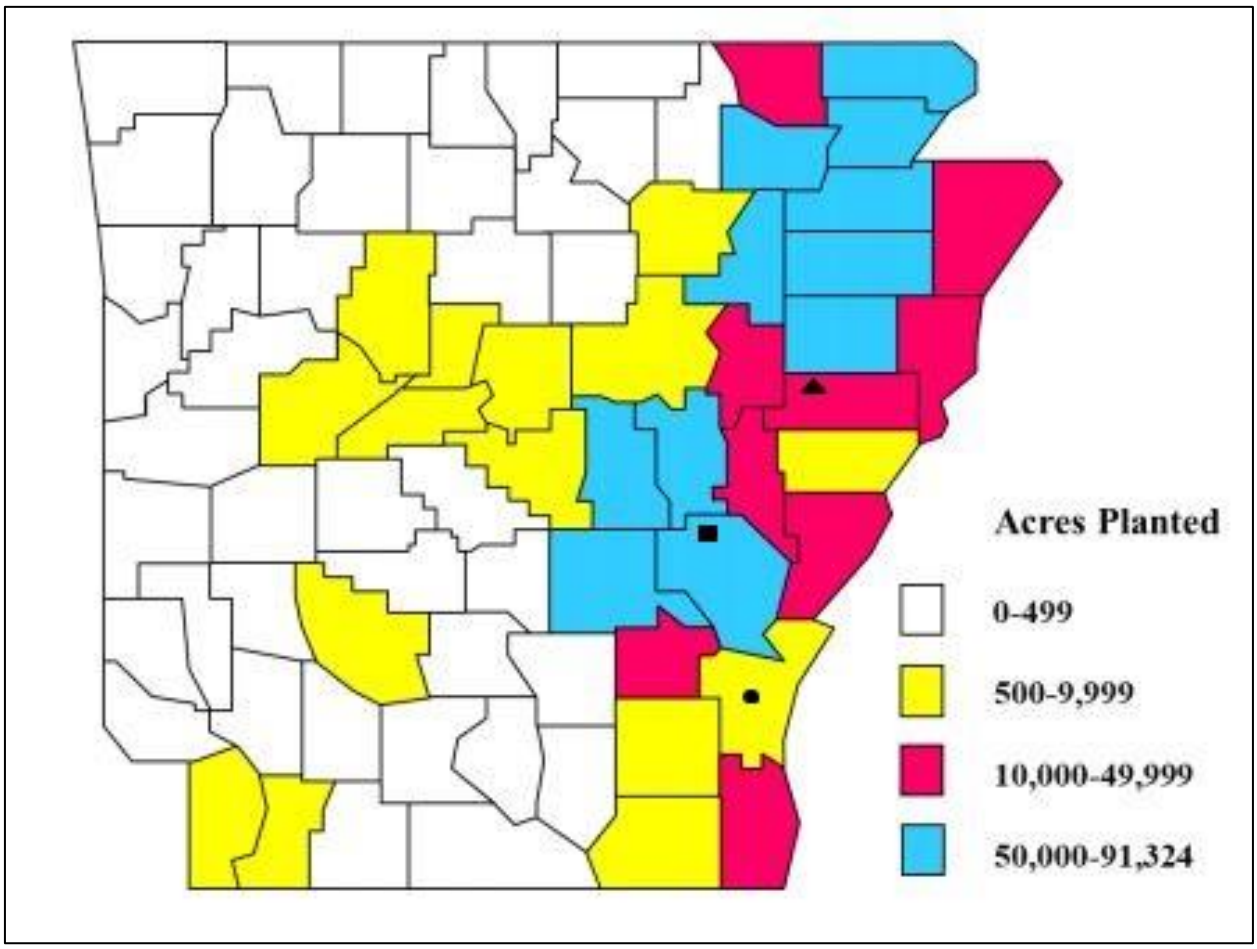


A. Sterile Pollen Grains B. Fertile Pollen Grains

Figure 6. Pollen grain appearance under a microscope (10x magnification) after staining. Photographed by author in 2017.

Figure 7. Plot ready to collect anthers for testing pollen viability. Photographed by author in 2017.

Figure 8. Panicle ready to pollen stain. Photographed by author in 2017.



▲ - Pine Tree Research Station (PTRS)
● - Rohwer Research Station (RRS)
■ - Rice Research and Extension Center (RREC)

Figure 9. Locations of 2018 field test in Arkansas in correspondence to 2017 rice acreage (University of Arkansas, 2018). Received from https://www.uaex.edu/farm-ranch/economics-marketing/farm-planning/2017%20Crop%20Acreage%20Distribution-Preliminary.pdf,

Figure 10. Plant density test plot (7 rows). Photographed by author in 2018.

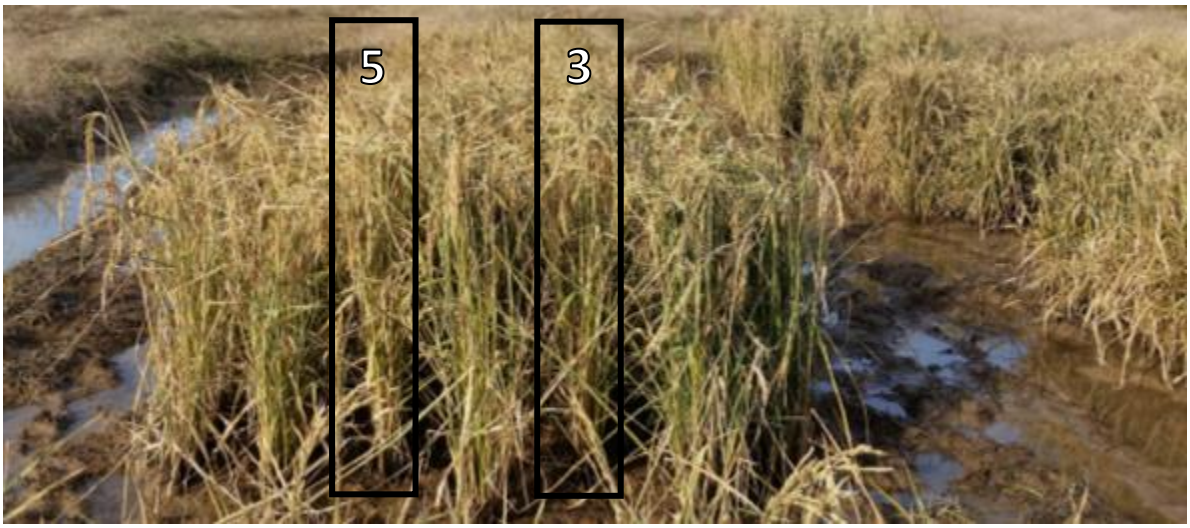


Figure 11. Rows 3 and 5 were hand harvested and tillers were seperated and counted. Photographed by author in 2018.

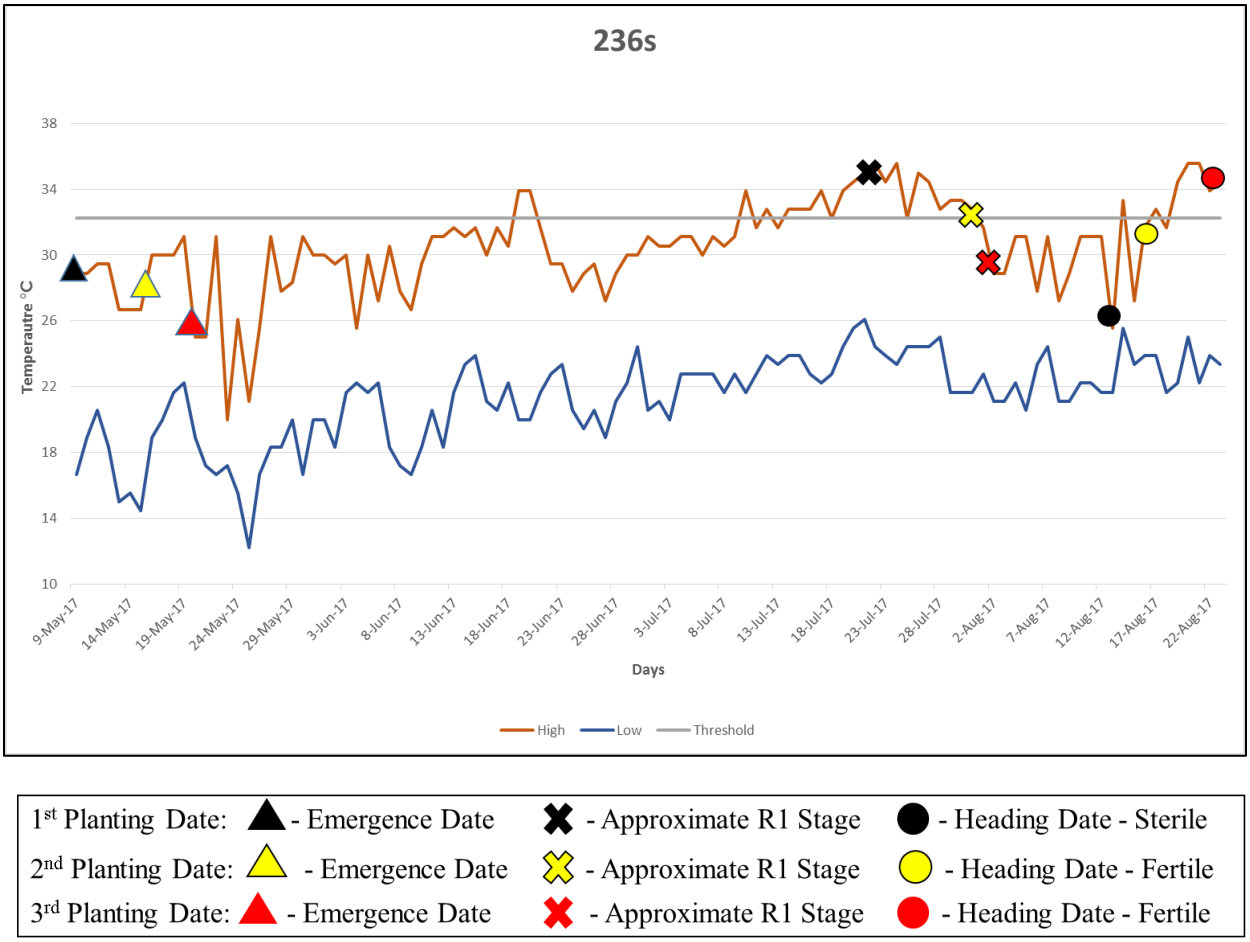


Figure 12. High and low temperatures during 236s EGMS inbred rice line growth.

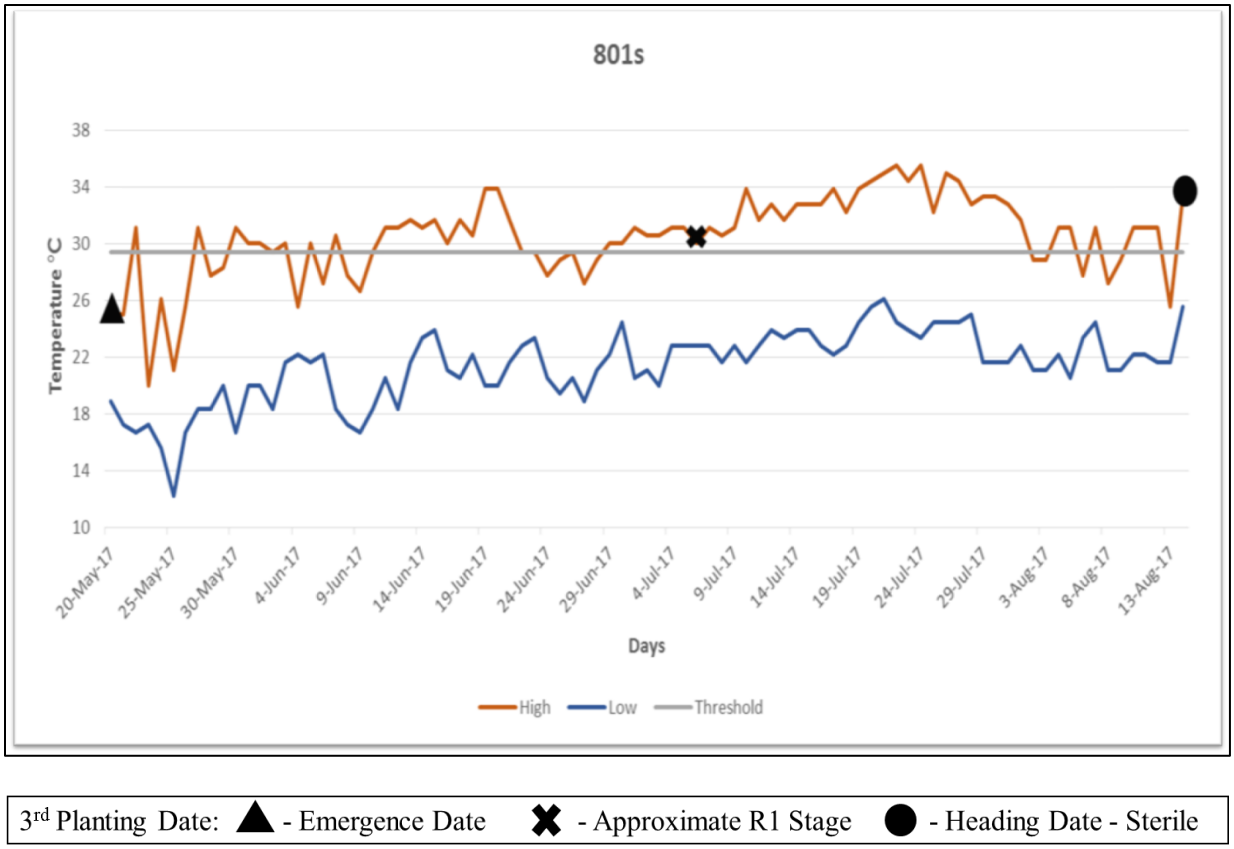


Figure 13. High and low temperatures during 801s EGMS inbred rice line growth.

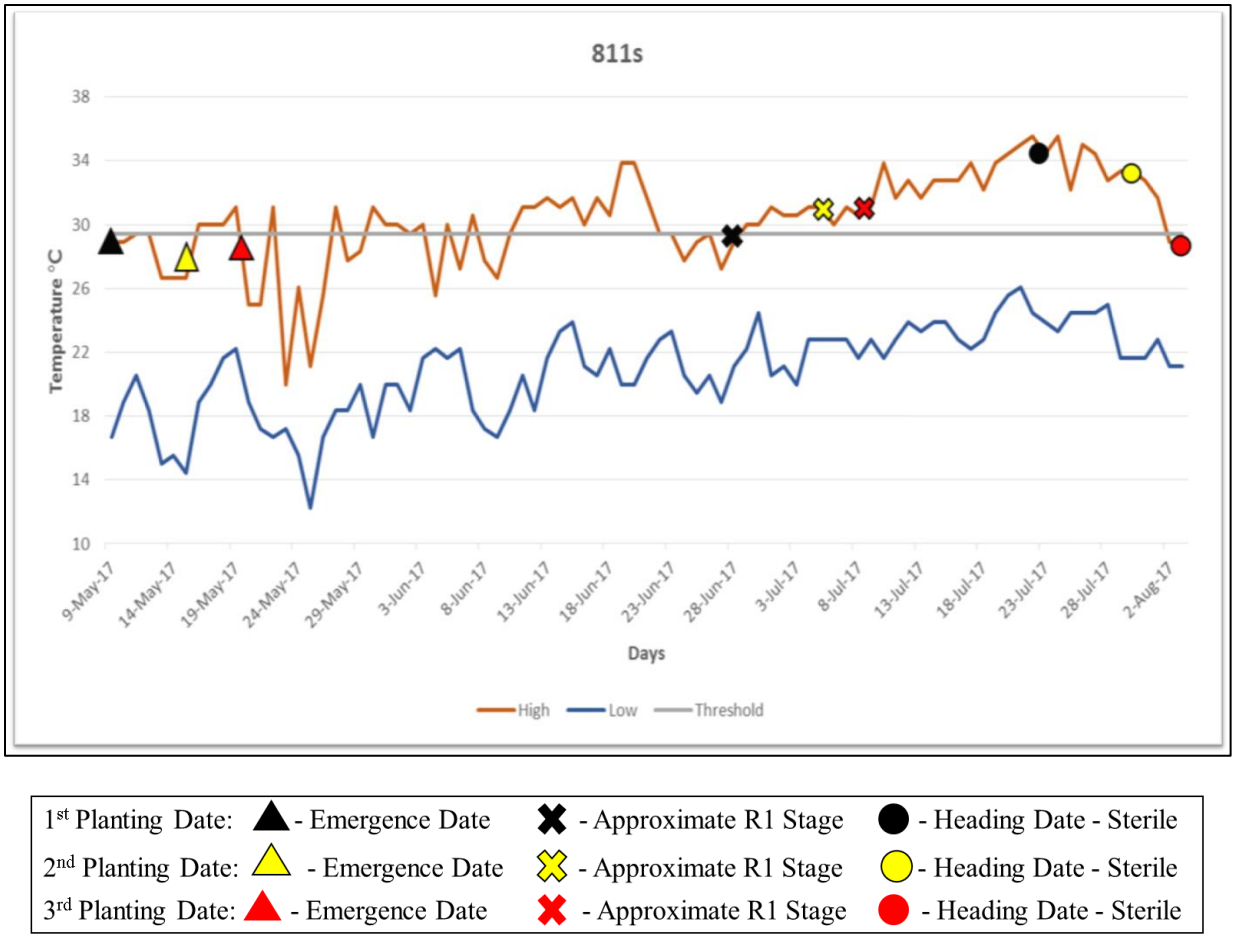


Figure 14. High and low temperatures during 811s EGMS inbred rice line growth.

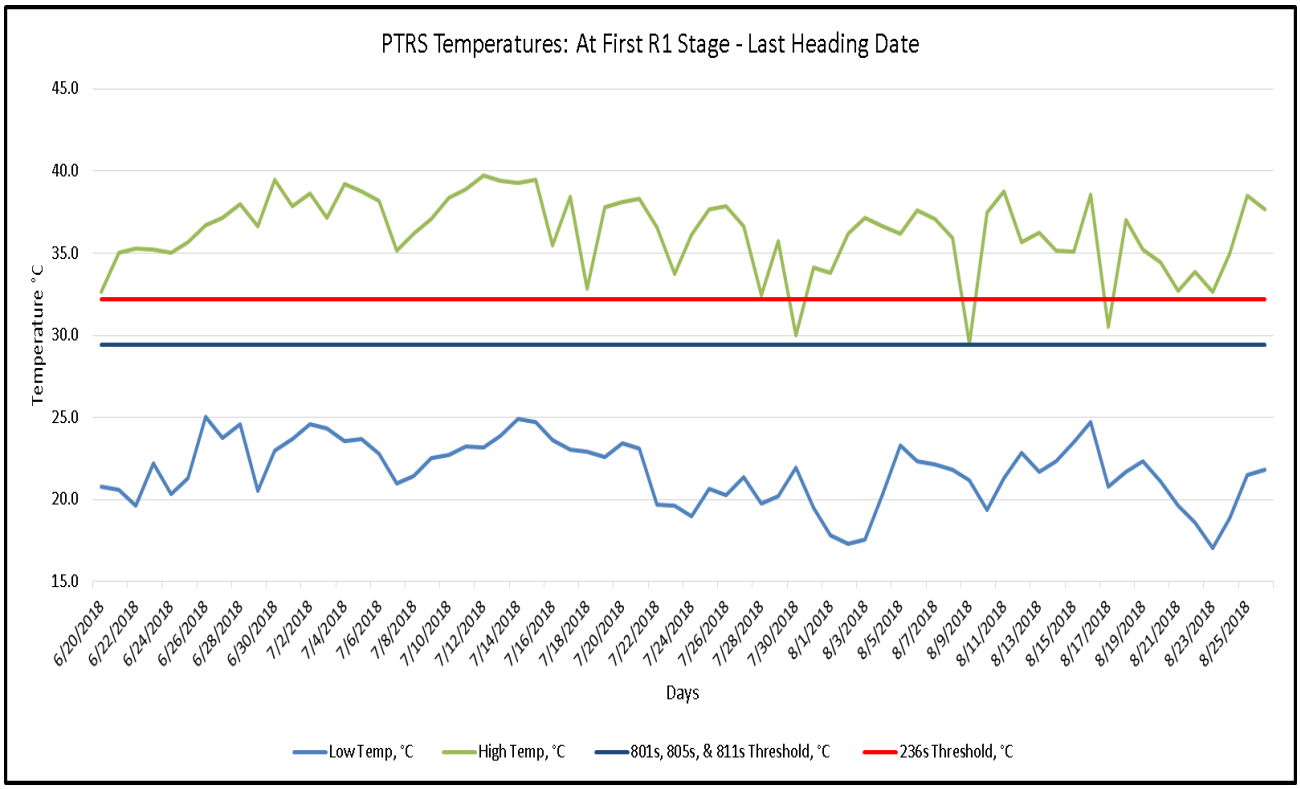


Figure 15. Pine Tree research station (PTRS) temperatures.

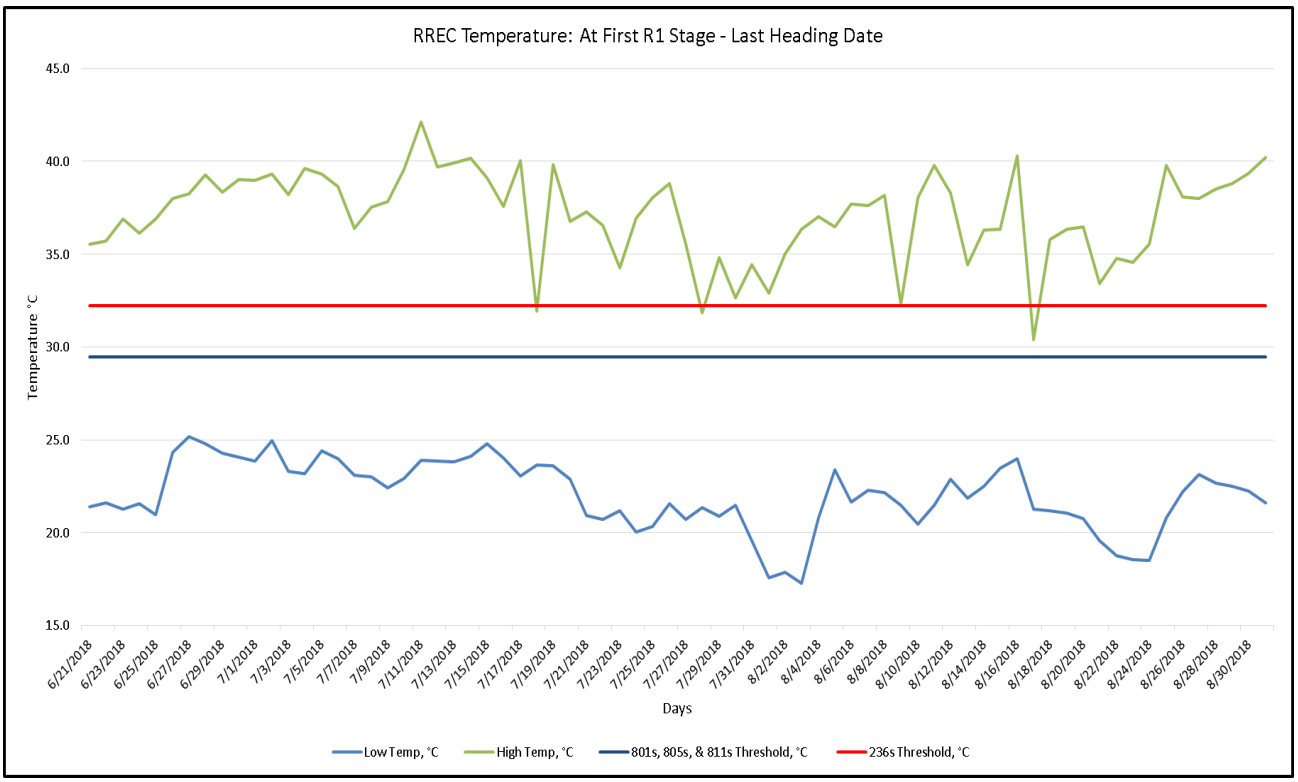


Figure 16. Rice research and extension center (RREC) temperatures.

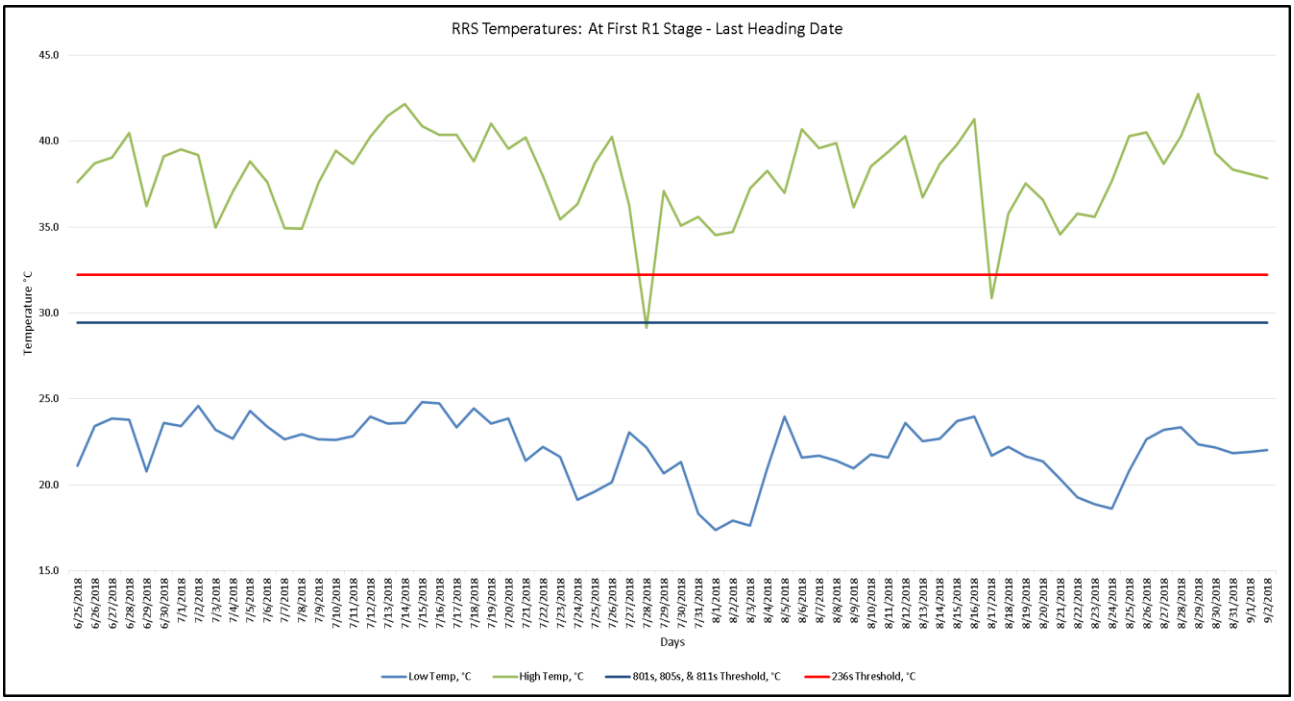
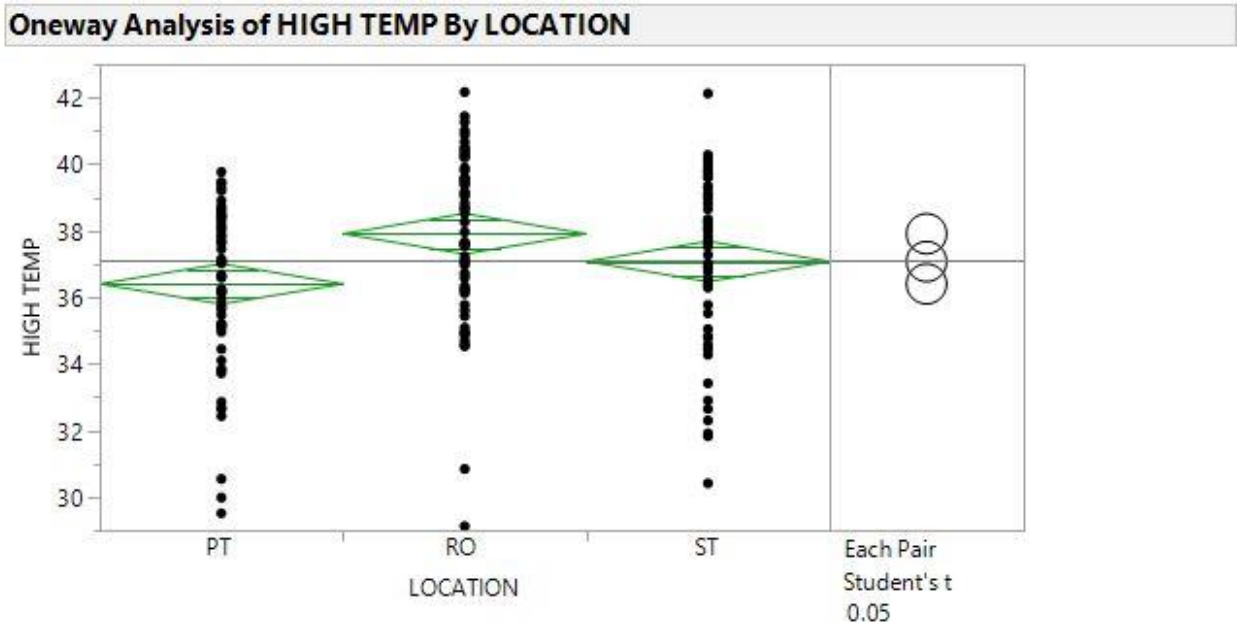


Figure 17. Rohwer research station (RRS) temperatures.



Oneway Anova

Summary of Fit

Rsquare	0.061168
Adj Rsquare	0.051074
Root Mean Square Error	2.435275
Mean of Response	37.12967
Observations (or Sum Wgts)	189

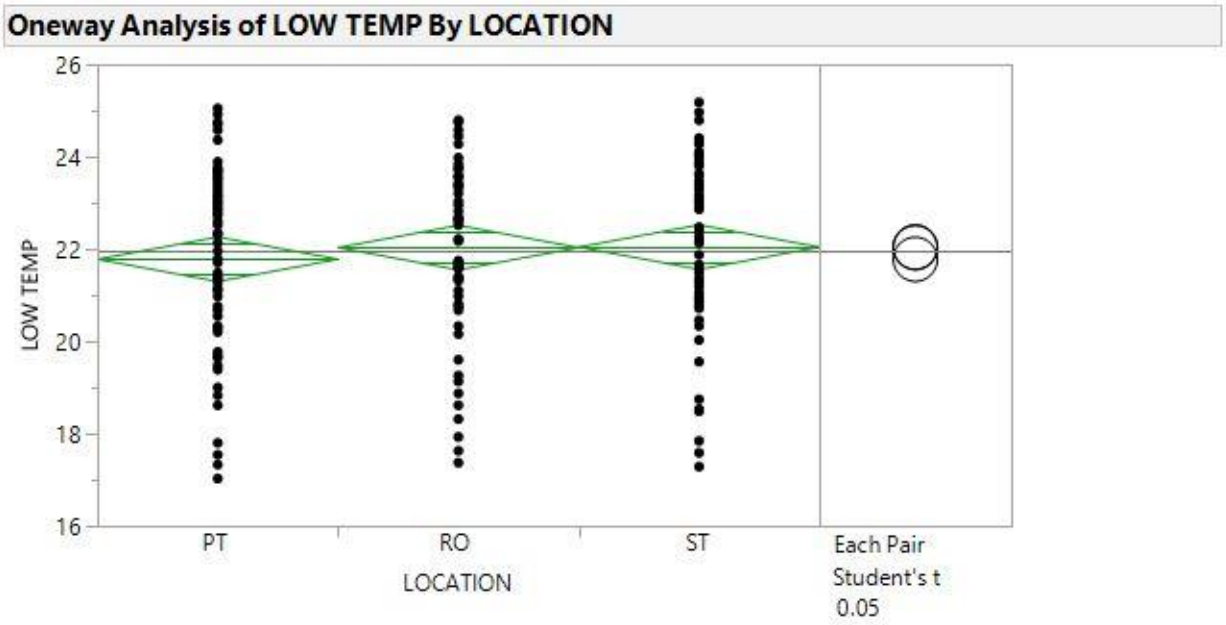
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
LOCATION	2	71.8702	35.9351	6.0593	0.0028*
Error	186	1103.0851	5.9306		
C. Total	188	1174.9553			

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
RO	PT	1.507642	0.4339031	0.651638	2.363646	0.0006*
RO	ST	0.834174	0.4339031	-0.021830	1.690178	0.0561
ST	PT	0.673468	0.4339031	-0.182536	1.529472	0.1223

Figure 18. Analysis of high temperatures at Pine Tree (PT), Rohwer (RO), and Stuttgart (ST). Ordered differences report shows significant differences of high day time temperatures between locations Pine Tree and Rohwer.



Oneway Anova

Summary of Fit

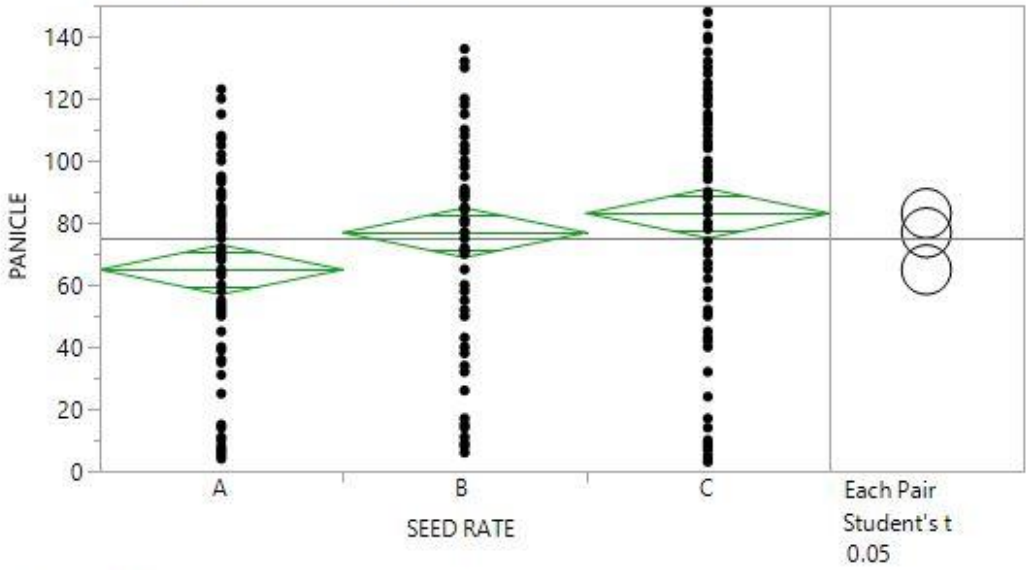
Rsquare	0.003992
Adj Rsquare	-0.00672
Root Mean Square Error	1.938163
Mean of Response	21.95179
Observations (or Sum Wgts)	189

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
LOCATION	2	2.80036	1.40018	0.3727	0.6894
Error	186	698.70457	3.75648		
C. Total	188	701.50493			

Figure 19. Analysis of low temperatures at Pine Tree (PT), Rohwer (RO), and Stuttgart (ST).

Oneway Analysis of PANICLE By SEED RATE



Oneway Anova

Summary of Fit

Rsquare	0.039963
Adj Rsquare	0.032252
Root Mean Square Error	37.07776
Mean of Response	74.90873
Observations (or Sum Wgts)	252

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	14249.56	7124.78	5.1826	0.0062*
Error	249	342315.35	1374.76		
C. Total	251	356564.90			

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
C	A	18.11905	5.721223	6.85089	29.38721	0.0017*
B	A	11.92857	5.721223	0.66041	23.19673	0.0381*
C	B	6.19048	5.721223	-5.07768	17.45864	0.2803

Figure 20. Panicle production analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha. Ordered differences report shows significant differences between seed rates A and C, and between seed rates A and B.

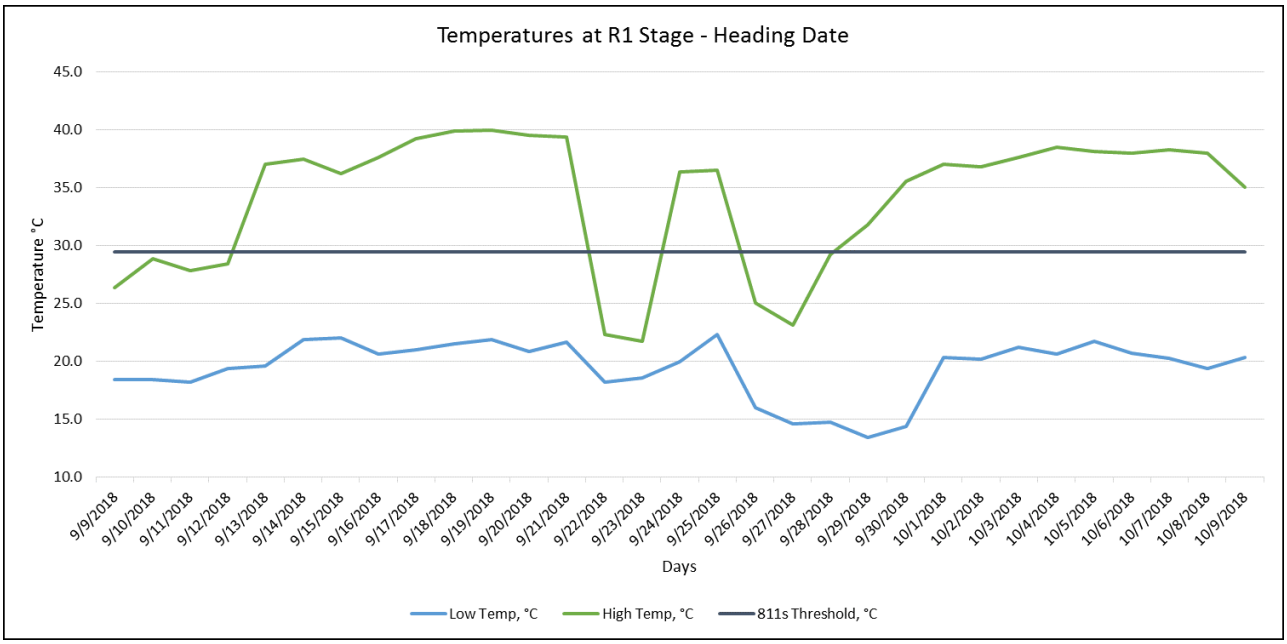
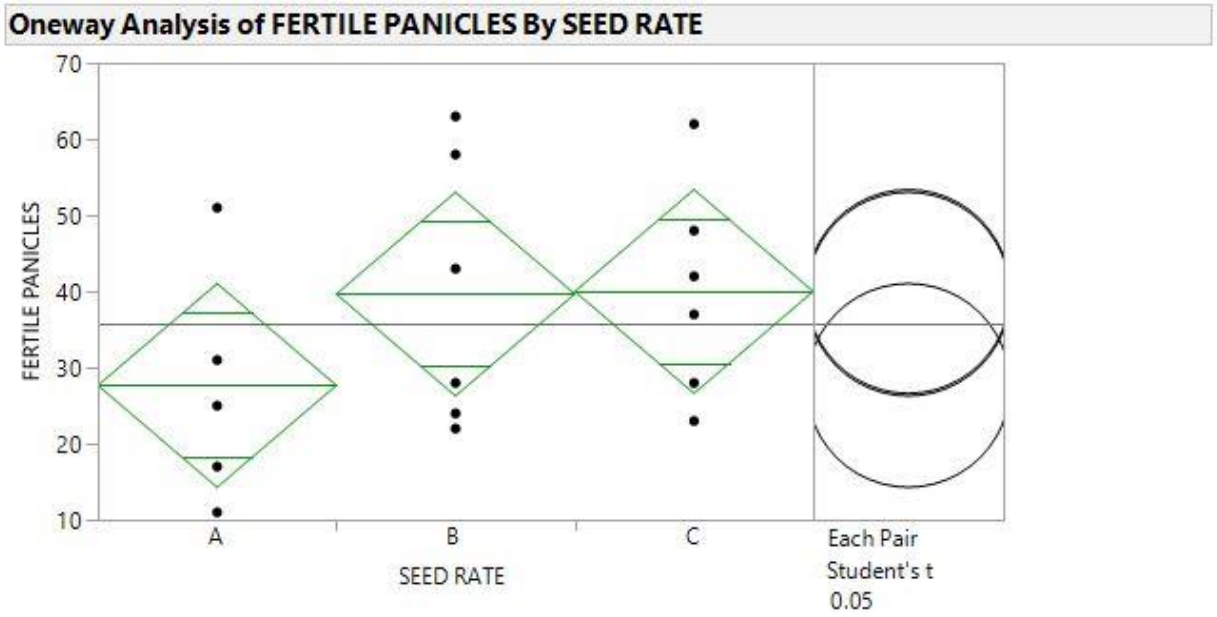


Figure 21. Temperatures during critical sterility inducing growth stages.



Oneway Anova

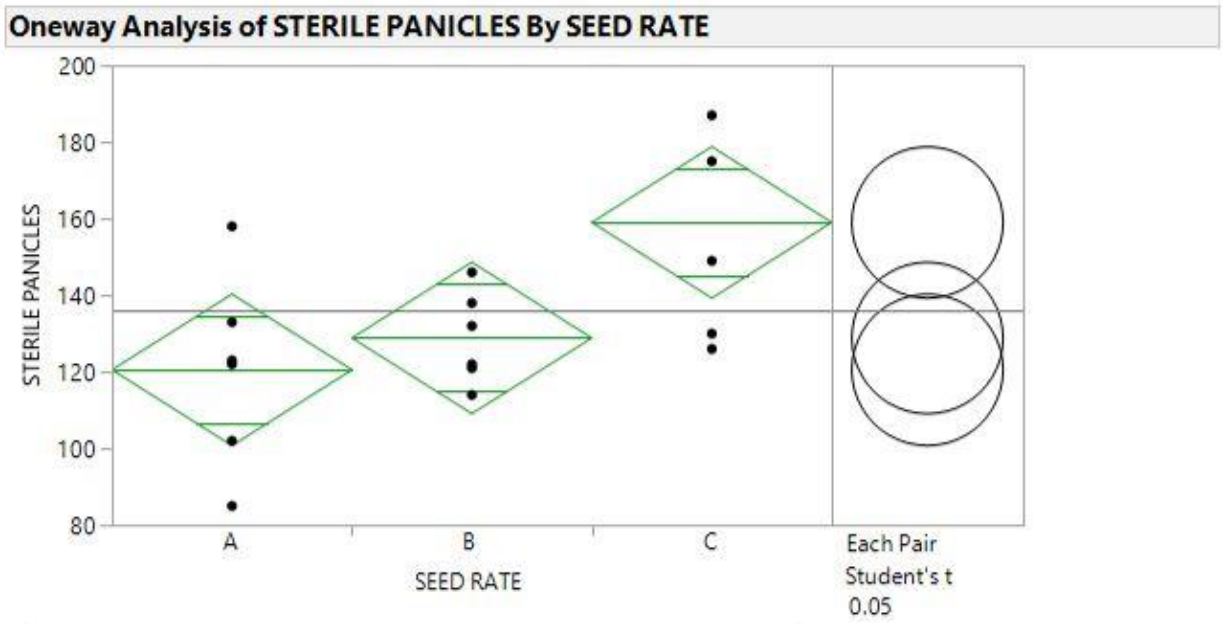
Summary of Fit

Rsquare	0.143202
Adj Rsquare	0.028963
Root Mean Square Error	15.37241
Mean of Response	35.77778
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	592.4444	296.222	1.2535	0.3138
Error	15	3544.6667	236.311		
C. Total	17	4137.1111			

Figure 22. Tillers with fertile panicles by seeding rate analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha.



Oneway Anova

Summary of Fit

Rsquare	0.388659
Adj Rsquare	0.307147
Root Mean Square Error	22.72199
Mean of Response	136.1111
Observations (or Sum Wgts)	18

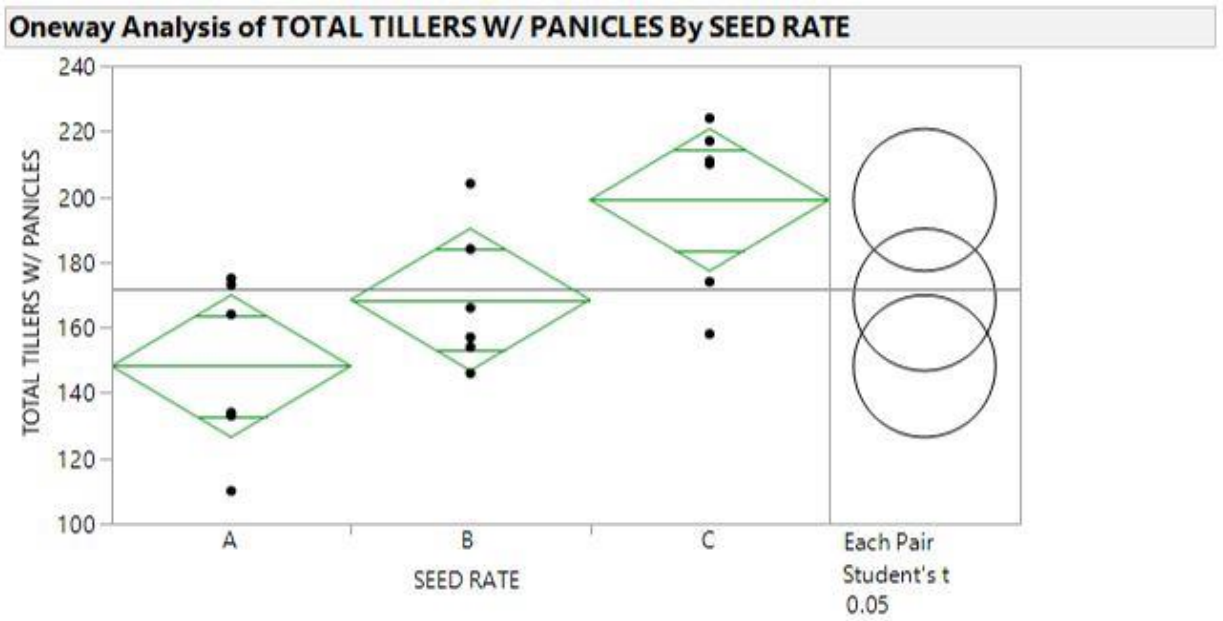
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	4923.444	2461.72	4.7681	0.0250*
Error	15	7744.333	516.29		
C. Total	17	12667.778			

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
C	A	38.50000	13.11855	10.5385	66.46152	0.0102*
C	B	30.16667	13.11855	2.2051	58.12819	0.0363*
B	A	8.33333	13.11855	-19.6282	36.29486	0.5348

Figure 23. Tillers with sterile panicles by seeding rate analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha. Ordered differences report shows significant differences between seed rates A and C, and between seed rates Band C.



Oneway Anova

Summary of Fit

Rsquare	0.456559
Adj Rsquare	0.3841
Root Mean Square Error	24.96709
Mean of Response	171.8889
Observations (or Sum Wgts)	18

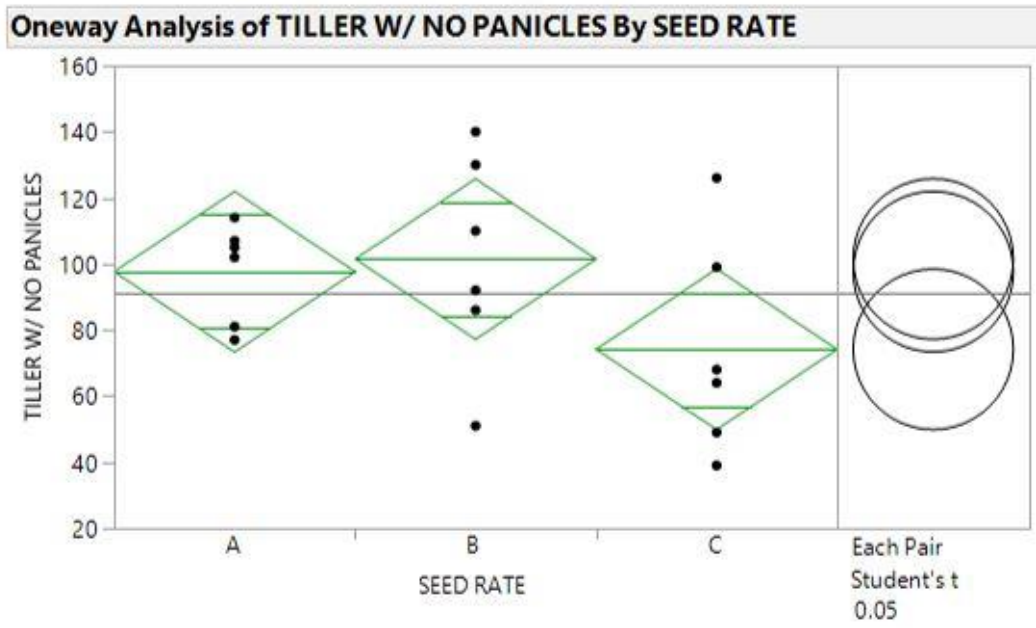
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	7855.444	3927.72	6.3009	0.0103*
Error	15	9350.333	623.36		
C. Total	17	17205.778			

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
C	A	50.83333	14.41476	20.1090	81.55766	0.0031*
C	B	30.50000	14.41476	-0.2243	61.22432	0.0515
B	A	20.33333	14.41476	-10.3910	51.05766	0.1788

Figure 24. Total tillers with panicles produced by seeding rate analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha. Ordered differences report shows significant differences between seed rates A and C.



Oneway Anova

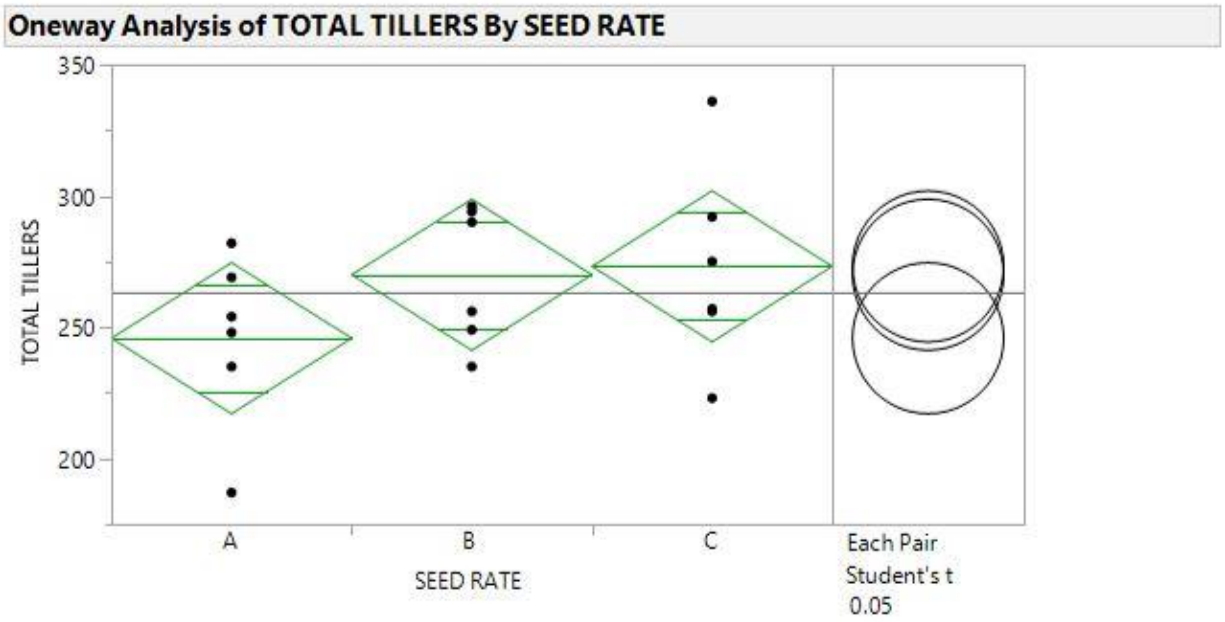
Summary of Fit

Rsquare	0.183505
Adj Rsquare	0.074638
Root Mean Square Error	27.92092
Mean of Response	91.11111
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	2628.111	1314.06	1.6856	0.2186
Error	15	11693.667	779.58		
C. Total	17	14321.778			

Figure 25. Tillers with no panicles produced by seeding rate analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha.



Oneway Anova

Summary of Fit

Rsquare	0.140657
Adj Rsquare	0.026078
Root Mean Square Error	33.05316
Mean of Response	263
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
SEED RATE	2	2682.333	1341.17	1.2276	0.3208
Error	15	16387.667	1092.51		
C. Total	17	19070.000			

Figure 26. Total tillers by seeding rate analysis. Seed rate A = 52.9 kg/ha, seed rate B = 103.2 kg/ha, and seed rate C = 211.8 kg/ha.

CHAPTER 3: CONCLUSIONS

This study proves that Arkansas EGMS lines 236s, 801s, 805s, and 811s are applicable for hybrid seed production in Arkansas. They are TGMS (thermos-sensitive genic male sterile) with a sterility threshold that matches or is below an average rice growing season environment in Arkansas, meaning that they have a high chance of becoming sterile which enables for crossing with the male (pollen donor) parent for hybrid seed production without any seed contamination from self-pollination. Line 236s may be less successful due to the higher sterility threshold; however, it can be sterile in average Arkansas rice growing conditions. These EGMS lines can be planted during the average, optimal seeding date recommendation (April 25th) made for conventional rice varieties in Arkansas. A seeding rate of 103.2 kg/ha is shown to be the most effective for maximum sterile panicle production, while using fewer EGMS seed than needed.

Potential EGMS lines GSOR 1, GSOR 2, GSOR 3, and GSOR 4 were shown to not fully possess any EGMS gene(s) which may be due to the loss of the gene(s) over time. There are still plants within these lines that expressed sterility, but they must be selected and isolated in different environmental conditions to verify the genetic source of sterility and to produce pure seed. It is from this study that it may be determined that the genetic source(s) of the GSOR lines may be PGMS, but further evaluations must be conducted.

ACKNOWLEDGEMENTS

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I would like thank my fellow workers – Daniel Wood, Thomas Coleman, and Adam Rice – for their assistance in field preparation, plant management, and data collection. With all of your help I was able to provide the data used to solve the issues proposed for this project.

Lastly, I would like to thank my family and friends. My sweet, beautiful wife has been along my side the whole time, and I greatly thank her for that. My parents, brother and his family, grandmothers, and my friends have all expressed their support, and I greatly thank them for that. I hope to celebrate my graduation near the time I welcome my son, Nolan. This is for you son. Through the collaboration and assistance from my committee members, support from my family and friends, and through my faith is this all possible. Thank you all.