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QTL Mapping of Panicle Architecture and Yield-Related Traits Between
Two U.S. Rice Cultivars ‘LaGrue’ and ‘Lemont’

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

by

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ABSTRACT

Grain yield in rice consists of multiple yield components such as number of panicles/plant, number of tillers/plant, and number of seeds/panicle. Panicle architecture is an important yield trait yield as the number of seeds on a panicle greatly contributes to the overall panicle yield. A QTL analysis and mapping study on panicle architecture and yield-related traits was performed to identify major QTL and candidate genes associated with the traits. The first objective of the project was to do a yield study evaluating 15 agronomic traits between each of the four U.S. rice cultivars, 'LaGrue', 'Lemont', 'Bengal', and 'Mars', to determine which cultivars should be used in developing a bi-parental population. The results from this study showed LaGrue significantly exceeding Lemont in number of seeds produced on a panicle while Lemont had significantly longer panicles and higher 100 seed weight/panicle. LaGrue and Lemont were therefore chosen for development of a bi-parental population for the second objective of the project. The second objective was to detect major QTL for panicle architecture and yield-related traits. The study was conducted at the University of Arkansas System Division of Agriculture's Rice Research and Extension Center in Stuttgart, AR and the Pine Tree Research Station near Colt, AR. The population was evaluated for plant height, heading date, panicle length, flag leaf length and width, number of primary panicle branches and number of secondary panicle branches. Results from QTL mapping showed two major QTL for plant height co-localized on chromosome 1 that explained a very high amount of phenotypic variation. On Chr. 8, two QTL for flag leaf length and two QTL for panicle length were detected at both locations which were co-localized in a 3.2Mbp region. A candidate gene known as *Semi-dwarf 1 (sd-1)* was found on chromosome1 that gives rice plants a semi-dwarf height and increased lodging resistance. Six candidate genes, *UBP1-5*, *UBP1-8*, Wide and Thick Grain 1 (*WTG1*), *OsSPL16*, *OsSPL14*, and *OsCOL15*, were

found to control panicle development and flag leaf development. The QTL detected would be useful for molecular breeding in the development of elite rice cultivars.

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Rice is one of the most important food crops in the world along with wheat and maize (IRRI, 2019). About 140 million hectares of rice is harvested in the world each year. About half of the world's population relies on rice as part of its diet (Ricepedia,2019). Human consumption of rice accounts for 85% of global production. (IRRI, 2019). In the U.S., Arkansas is the top rice producing state accounting for about 48% of total U.S. rice production (Hardke, 2018). Rice is grown in 40 out of 75 counties in Arkansas but is primarily grown in the eastern half of Arkansas. Rice is one of the top three cash crops for Arkansas farmers and in 2010, rice producers harvested a record 1.785 million acres of rice.

The U.S. rice breeding programs in California, Texas, Arkansas, Louisiana, Mississippi, and Missouri select for higher grain yield as one of the top priorities in the development of elite rice cultivars. Grain yield is a complex quantitative trait that consists of multiple yield components (Xing et al., 2010). Yield components such as number of tillers/plant, number of panicles/plant, number of seeds/panicle, and seed weight/panicle have been found to contribute to overall yield in rice cultivars (Samonte et al., 1998; Devi et al., 2017). Each yield component can be controlled by multiple genes that have a small effect on the phenotype and are greatly affected by the environment (Xing et al., 2010). To determine the genetics behind each yield component, a QTL mapping study is done to look for chromosome regions linked to each yield component. QTL stands for quantitative trait locus which is a region on the chromosome that contains multiple genes that may influence the yield trait of interest. Detecting major QTL for yield traits that are stable across environments would allow researchers to find potential candidate genes that could be used in rice breeding (Collard et al., 2005).

A QTL mapping study was performed using two U.S. rice cultivars ‘LaGrue’ and ‘Lemont’ to look for QTL associated with panicle architecture and yield-related traits. There were three objectives for this project:

- 1) Conduct a parental yield study on four U.S. cultivars, ‘LaGrue’, ‘Lemont’, ‘Bengal’, ‘Mars’, to evaluate yield components between each cultivar and to determine which two varieties would be useful in developing a bi-parental population.
- 2) Conduct a QTL mapping study on panicle architecture and other yield-related traits to detect major QTL in a LaGrue x Lemont bi-parental population.
- 3) Look for candidate genes in the major QTL detected that have been known to control yield traits examined.

Identification of major QTL for these yield traits could be useful for molecular breeding in developing elite rice varieties.

Genealogy of Rice

The *Oryza* genus in rice is part of the grass family and has 22 different species of rice two of which are cultivated species, *Oryza sativa* L. and *O. glaberrima* (Shakiba and Eizenga., 2015). *Oryza sativa* L. is mainly grown throughout the tropical and temperate climates of the world including the U.S. while *O. glaberrima* is found in sub-Saharan Africa (Shakiba and Eizenga., 2015). The *Oryza sativa* L. genome has 12 chromosomes and has the smallest genome among all food crops with a size of 430 million base pairs. In the *Oryza sativa* species, there are two subspecies known as *indica* and *japonica* subspecies. The *indica* subspecies, comprise of two subpopulations, *indica* and *aus*. The *japonica* subspecies is further divided up into three

subpopulations, *aromatic*, *tropical japonica*, and *temperate japonica*. In the southern U.S., the rice cultivars planted belong to the *tropical japonica* subpopulation.

Rice Production in Arkansas

Rice production in Arkansas, U.S.A. began with one acre of rice planted in Lonoke county in 1902 (Hardke, 2018). Since then, Arkansas has become No. 1 in rice production with a record 1.785 million acres of rice harvested in 2010. Rice is primarily grown in the eastern half of the state with rice also grown in the Arkansas River valley between the Ouachita and Ozark mountains; Ouachita river valley from west-central to southern region of Arkansas; and the Red River Valley near the Arkansas-Texas border line. Rice yields in Arkansas have increased considerably since the 1980's mainly due to the release of new cultivars by the Arkansas Agricultural Experiment Station (Hardke, 2018). There are two types of rice cultivars planted in Arkansas based on their seed characteristics, long-grain and medium grain. Long-grain cultivars typically have seeds that have a large kernel length to width ratio of 3.0 to 1 or higher (Hardke et al., 2018). Long-grain rice typically cook dry and fluffy and come as parboiled, quick-cooked or processed for use in rice products. Medium-grain cultivars have a shorter kernel length than long-grain but have a wider kernel width with a kernel length/width ratio between 2.0 and 2.3 ratio. The cooking quality of medium-grain is typically moist and sticky compared to long-grain. This type of rice is typically used in dry breakfast cereals, soups, baby foods, and brewing purposes. Long-grain types typically have intermediate to high amylose content which gives the rice a drier, less sticky texture when cooked. Medium-grain types have low amylose content which gives the rice its sticky texture. From 1984 to 2009, cultivars developed by the University of Arkansas were grown on 42 to 86 percent of Arkansas rice acreage (Hardke et al, 2018). But since 2009, new varieties such as hybrid rice and Clearfield rice developed by private companies

have grown considerably. By 2017, about 64% of Arkansas rice acreage were planted with privately-owned varieties where 42% were hybrid rice and 45% were Clearfield cultivars. Hybrid rice is an important new technology recently introduced to Arkansas rice farmers. Hybrid rice is made by crossing superior rice cultivars and producing F₁ seed. The F₁ seed produced has very high vigor and has a high yield potential compared to conventional inbred varieties, yielding about 10% more. This high vigor allows Arkansas farmers to produce more grain yield per acre than conventional varieties. Rice cultivars released by the University of Arkansas rice breeding program increased rice yield in the U.S. by 29.16kg/ha from 1983 to 2016 (Shew et al., 2018). It is estimated that 4.157 million metric tons of rice were added to the world food supply through genetic gains by the University of Arkansas rice breeding program. This shows that plant breeding is an essential part in increasing crop yield and will be more important as the world population increases.

Stages of Development in Rice

In the development of a rice plant, there are two phases of development, the vegetative phase and reproductive phase (Moldenhauer et al., 2018). The vegetative phase is active tillering of the plant along with increase in plant height and leaf emergence. The tillering stage starts when the plant reaches the V5 stage or when the fifth leaf collar forms on the main stem. The plants will keep producing tillers until it reaches a maximum tillering stage where no tillers are produced before the reproductive stage starts. In early-season cultivars, the reproductive stage may start before maximum tillering has been reached. At maximum tillering, the plant goes through a vegetative lag phase where some of the tillers die off. The potential number of panicles produced per plant is determined at maximum tillering. The reproductive phase is characterized by nine stages R0-R9. The R0-R1 stage is where the panicle begins to develop and begins to

form panicle branches. The two stages are also where the flag leaf begins to develop. The R1 stage is critical for panicle and flag leaf development as the environment can impact development of the traits and impact the potential number of grains that can form on a panicle. R2 and R3 stages are characterized by the formation of the collar on the flag leaf and emergence of the panicle from the flag leaf sheath. The R4 stage is the beginning of flowering which begins at the tip of the panicle and moves down. The environmental conditions at flowering greatly determines the number of filled grains per panicle since it can impact fertilization of the flowers. The R6-R8 stages are the ripening phase where the seed begins to fill and mature. The ripening stage is critical for grain size and weight as the light intensity during this time can influence the amount of carbohydrates that are photosynthesized in the plant. The environmental conditions which occur during the development of a rice plant greatly impacts the potential yield of the rice plant.

Yield Traits in Rice

Yield is one of the major goals in rice breeding. Yield is a quantitative trait which it is determined by multiple traits that have small effects. Yield is greatly affected by the environment unlike a qualitative trait which is affected by the effects of one or two genes (Xu, 2010a) In a segregating population, the phenotypic distribution of yield is usually in a normal or bell-shaped distribution (Xu, 2010a). Grain yield in rice is mostly determined by the number of panicles per plant, number of grains per panicle, and grain weight (Xing et al., 2010). These traits also depend on other important traits i.e. the number of panicles is determined by the number of productive tillers a rice plant produces (Xing et al., 2010) The number of grains per panicle is determined by the number of spikelets per panicle and seed setting rate (Xing et al., 2010). The number of spikelets per panicle is also determined by the number of primary branches and secondary

branches on a panicle (Xing et al., 2010). Grain weight is mainly determined by the grain size which is the length and width of a grain and the degree of filling (Xing et al., 2010). Flowering time allows for setting flowering in optimum conditions for pollination and seed development (Cockram et al., 2007). Plant height is an important trait for yield as tall plants are more susceptible to lodging affecting yield (Sakamoto and Matsuoka., 2008). Semi-dwarf varieties allowed for better lodging resistance to rain and wind without affecting yield (Sakamoto and Matsuoka., 2008) Flag leaves on the rice plant are also an important trait as they provide a source of carbohydrates for grain filling in the panicle (Li et al., 1998). A study by Li et al. (2019) looking at correlations between grain yield and yield components found that grain yield had a positive correlation with number of filled grains per panicle, 1000-grain weight, and number of panicles per unit area (Li et al., 2019). Rice varieties can differ in yield potential with great variation in the combination of yield traits that determine overall yield of a plant (Xing et al., 2010). Because of this, it is important to look at yield components that affect grain yield and develop varieties that have a good combination of those yield traits.

QTL Mapping for Yield Traits

The genetics behind yield traits in rice is usually studied through a QTL mapping study. The word QTL stands for Quantitative trait locus which is a region of a chromosome that is associated with a trait of interest. (Xu, 2010c) A QTL is usually detected through a marker-based study which is looking for associations or linkages between DNA markers and a yield trait (Xu, 2010c). The DNA markers typically used for study are called SNP and SSR markers. SSR (short sequence repeats) are markers used to analyze the locus linked to a trait of interest in a region (Xu, 2010b). The main advantage of SSR markers are that there is a high level of allelic variation in the region. A disadvantage of SSR markers is the need of polyacrylamide gels for their

resolution, and extensive manual labor in examining SSR markers (Xu, 2010b). SNP stands for Single Nucleotide Polymorphism where there is a single nucleotide difference in a chromosome region between two DNA sequences (Xu, 2010b). SNP markers are very abundant throughout the genome making them excellent for high-resolution genotyping of populations (Gonzaga et al., 2015). The strength of the linkage between a QTL and DNA markers is measured using an LOD score or otherwise known as Logarithm of the odds (Xu, 2010c). The LOD score is the measure of the likelihood that the QTL is linked to the position against the likelihood that it is unlinked to the position (Xu, 2010c). A QTL is usually detected if the LOD threshold goes beyond a certain LOD threshold such as 2 or 3. QTL mapping for yield traits are usually determined through mapping methods that are able to detect multiple QTL close to each other on a chromosome such as Composite Interval Mapping and Inclusive Composite Interval Mapping.

In QTL mapping, studies often use a bi-parental population by crossing two parents together and evaluating the progeny for traits (Xu et al. 2017). When choosing parents for a study, it is important that the two parents differ in terms of the yield traits being evaluated such as one parent has a higher number of grains per panicle than the other (Xu et al. 2017). The simplest mapping population to use is a F_2 or BC_1 population by selfing the F_1 plants or crossing the F_1 with either of the parents (Collard et al. 2005). The advantages of using these populations is that they are easy to construct and are able to estimate additive and dominant effects of QTL. The disadvantage of F_2 populations are that they are temporary populations that cannot be propagated indefinitely or replicated to be used multiple times in different environments. A second disadvantage is that only a few recombination events have occurred in the development of the population (Mulualet et al. 2016). DNA markers that are far away from each other on the chromosome can appear to be close together due to limited recombination between chromosomes

which can limit localization of detected QTL on the chromosome (Mulualet et al., 2016).

Recombinant Inbred Lines (RIL) are lines that are formed by repeated selfing from F₂ derived lines for several generations until alleles are fixed or homozygous (Mulualet et al. 2016). Each RIL line contains a unique set of recombinants from the parental lines (Shakiba and Eizenga, 2015) The main advantage of RIL lines are that they can be propagated indefinitely and can be used many times in different locations and years (Collard et al., 2005). A downside to RIL populations is the time it takes to develop a population for QTL mapping (Collard et al. 2005) A backcross inbred line (BIL) or lines are where the F₁ plant from the initial cross was backcrossed back to one of the parents known as a recurrent parent two or three times and then selfed for several generations until the lines are fixed (Shakiba and Eizenga, 2015). The lines would contain chromosome segments from the other parent known as a donor parent. Main advantages of BIL lines are the method of creating them is straightforward and can detect major QTL and single genes in the population. A disadvantage is limited success in identifying QTL with low heritability and identifying minor QTL. An advanced backcross population (AB-QTL) is when the F₁ lines are backcrossed to the recurrent parent two or three times and then selfed once to create BC₂F₂ or BC₃F₂ populations. A chromosome segment substitution line population (CSSL) is a set of near isogenic lines that cover the entire genome of the donor parent (Shakiba and Eizenga, 2015). The lines are backcrossed to the recurrent parent four or five times before being selfed to achieve homozygosity. The lines homozygous for the donor region are selected to create a CSSL library. CSSL lines can be used to fine map both major and minor QTLs and validate interactions between regions. Near Isogenic lines (NIL) are lines that contain a single chromosome segment from the donor parent (Shakiba and Eizenga, 2015) NIL's are different from CSSL in which that the lines only have a small segment that contains a gene of interest.

They are often used to fine map QTL's identified in populations such as RIL and BIL's and to look for genes that are within the QTL of interest. A downside is that it is labor intensive requiring more backcrosses than CSSL's and BIL's to incorporate one segment into a line. There are many different methods that researchers can use to search for genes underlying yield traits. QTL mapping is an important method for researchers in determining the genetics behind yield traits in rice.

Current Research in QTL Mapping

Many QTL studies have been conducted over the years to identify regions that contain potential yield traits. Sun et al. (2017) conducted an QTL analysis for panicle structure by creating four backcross populations with an *indica* hybrid restorer line 'HR1128' assigned as the donor parent and the *japonica* cultivar 'Nipponbare' designated as the recurrent parent. The authors reported twelve QTLs for grain number, panicle length, number of primary panicle branches, and number of secondary branches on chr. 6. The QTL's were tightly linked to three chromosome intervals between the SSR markers RM7213 to RM19962, RM20000 to RM20210, and RM412 to RM20595. Ujiie et al. (2014) used two sets of chromosome segment substitution lines (CSSL) through reciprocal crossing between two *japonica* lines 'Koshihikari' and 'Nipponbare'. They found a region on chr. 1 associated with panicle number, total grain number, and yield that increased each trait by 37%, 40%, and 35%, respectively, compared to Koshihikari. Moreover, they found that an increase in panicle number increased leaf area per plant that could improve source capacity by increasing photosynthate production. Zhu et al. (2011) detected a QTL for panicle number on chr.1 using an introgression line "C3074" derived from a set of 112 CSSL lines that were developed from Nipponbare, as the donor parent, and *indica* line 'Guangluai 4' as the recurrent parent. The C3074 line had significantly fewer

panicles compared to Guangluai 4. The QTL was fine mapped to a 34.4kb region on the long arm of chr. 1 and had a negative impact on panicle length, plant height, grain number per panicle, and grain yield per plant compared to Guangluai 4. Tian et al. (2015) reported there are two QTLs for flag leaf width and grain number per panicle that were co-localized on chr. 1. Through their investigation on this region, they detected a pleiotropic effect by increasing grain number per panicle and flag leaf width. Zhang et al. (2015) found two QTLs for panicle length on chr.s 6 and 8 by using four backcross populations resulting from the cross Nipponbare, designated as the recurrent parent, and *indica* line ‘WS3’, assigned as the donor parent. They found that the QTL on chr.6 had a larger effect with longer panicles and increased number of primary and secondary branches than the QTL on chr. 8. Han et al. (2017) found five major QTLs for heading date and plant height under different day-length conditions using a RIL population derived from *indica* line ‘Zenshan 97’ and *japonica* line ‘Xizang 2’. The populations were planted in different environments under long day conditions (day-length>13.5hrs) and short day conditions (day-length<12.5 hrs) for 3 years. Two major QTL’s for heading date on chromosomes 7 (*qHd7.1,7.2*) and one on chromosome 8 (*qHd 8.1*) were detected under long day conditions in all 3 years. Two major QTL for plant height on chromosome 1 and 7 were detected in all 3 years. Significant interaction was found between *qHd 7.2* and *qHd8* in all three years that explained 7.6%-15% heading variation.

Genes Controlling Yield Traits

Through these QTL studies, researchers have been able to find genes that are important for identification of yield traits. Genes such as *FRIZZY PANICLE* (FZP) on chr. (7), *LAX PANICLE* (LAX) on chr. 1, *ABBERANT PANICLE ORGANIZATION1* (APO1) on chr.6, *ABBERANT PANICLE ORGANIZATION 2/RFL* (APO2/RFL) on chr. 6, *TAWAWAI* (TAW1) on

chr. 10, *OsSPL18* on chr. 9, *OsSPL14* on chr.8, *SD1* on chr. 1, *Screw flag leaf 1* (SFL1) on chr. 10, and *Narrow and Rolled leaf 1* (NRL1) on chr.10 are important for panicle development and grain yield (Bai et al., 2016; Fujishiro et al., 2018; Ikeda-Kawakatsu et al., 2009; Yoshida et al., 2013; Yuan et al., 2019; Dong et al., 2010; Rao et al., 2008; Qiao et al., 2010; Alamin et al., 2017; Hu et al., 2010; funRiceGenes., 2019; Rice Genome Annotation Project., 2019). The *FZP* gene controls development of panicle branches and spikelets and is a negative regulator of *APO2* (Bai et al. 2016). Low levels of expression for *FZP* was found to increase panicle branching and yield (Fujishiro et al., 2018). The *FZP* gene also controls genes that are involved in floral development on the panicle. The *APO1* gene controls the timing of spikelet development from panicle branch development (Ikeda-Kawakatsu et al., 2009) Overexpression of *APO1* has been found to delay spikelet development and increase number of branches on the panicle which also increased the number of spikelets per panicle. The *TAW1* gene also has been found to regulate the timing of spikelet development and control branching in panicle development (Yoshida et al., 2013). The *OsSPL18* gene regulates grain weight and grain number per panicle in rice (Yuan et al., 2019). The gene has been found to control grain size, panicle length, grain number per panicle, 1000-grain weight, and number of secondary branches per panicle. The gene also controls another gene *DEP1* that is also involved in panicle development. The *OsSPL14* gene is also known Wealthy Farmers Panicle is known to increase yield as well as provide an ideal plant type (Dong et al., 2010). Increased expression of the gene caused plants to experience reduced tillering, increased panicle branching and grain yield. The *APO2/RFL* gene is a regulator gene that controls flowering and panicle development by regulating genes involved in those processes such as *LAX* and *FZP* (Rao et al., 2008). *SD-1* is a gene that is known to cause semi-dwarfness in rice plants (Qiao et al. 2011). The gene encodes an enzyme involved in gibberellin

synthesis (Sasaki et al. 2002a). Loss of function in the gene causes semi-dwarfism in rice without reducing grain yield and improving lodging resistance (Sasaki et al., 2002b). *SFL1* was found to control flag leaf length and width as well as plant height and panicle length (Alamin et al., 2017). The *NRL 1* gene controls for leaf development, flag leaf length and plant height (Hu et al., 2010). Finding new variation within known rice genes or new novel genes would be beneficial in yield improvement in rice varieties.

Parental Lines

“LaGrue” (PI-568891) is a high-yielding, short season, long-grain rice cultivar which was developed by the Arkansas Agricultural Experiment Station in cooperation with the USDA-ARS. The cultivar was released in 1993 by the Agricultural Experiment Stations of the University of Arkansas, University of Florida, Louisiana State University, Mississippi State University, University of Missouri, Texas A&M University, and the USDA-ARS (Moldenhauer et al., 1994). LaGrue originated from a cross between three parents: “Bonnet 73” (Clor 9654), “Nova 76” (Clor 9948), and “Newrex” (Clor 9969) (BN73/NV76//BN73/3/NWRX). LaGrue is a short-statured cultivar with an average height of 108 cm and is susceptible to blast and sheath blight.

Lemont is an early maturing, semi-dwarf, long-grain cultivar developed by USDA-ARS in cooperation with the Texas Agricultural Experiment Station, Texas Rice Improvement Association, Texas Rice Research Foundation; Louisiana Agricultural Experiment Stations, and the Mississippi Agricultural and Forestry Experiment Station (Bolligh et al., 1985). Lemont is resistant to panicle blight, moderately resistant to rice blast, brown spot and straighthead, and very susceptible to sheath blight. These two rice lines have been chosen because they have been used as parents in the development of elite rice varieties in the University of Arkansas rice breeding program. Another reason is that they do not share a common parent with each other

which provides a high degree of genetic diversity between the two lines. Using these two lines in the study will help uncover genetic variation associated with yield traits that could be used in rice breeding.

Conclusion

The state of Arkansas is number one in rice production and increases in overall grain yield can be attributed to the University of Arkansas rice breeding program. Achieving higher yield in new rice varieties is one of the major goals in rice breeding. Yield is a quantitative trait that is affected by several yield components and the environment. There have been many QTL studies conducted over the years to look for genes that are associated with yield traits. However, there is limited information on the genetic background of yield in U.S. rice varieties. Genetic variation is important in plant breeding and it can lead to greater yield gain through better combinations of yield components. LaGrue and Lemont are two high-yielding rice varieties that are being used as parents in breeding to develop new rice varieties. Finding QTL associated with yield traits could be used for marker assisted selection in rice breeding.

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Chapter 2

COMPARISON OF YIELD TRAITS BETWEEN FOUR U.S. RICE CULTIVARS

ABSTRACT

Developing high yield rice varieties is the foremost goal in the rice breeding. The overall yield of a cultivar is determined by the performance of multiple yield components such as number of panicles and number of seed/panicle. A yield study was conducted between four U.S. rice cultivars; ‘LaGrue’, ‘Lemont’, ‘Bengal’, and ‘Mars’; to compare the yield components between each cultivar and to determine which two cultivars would be suitable in the development of a bi-parental population. Each cultivar was planted at the University of Arkansas System Division of Agriculture’s Rice Research and Extension Center in Stuttgart, Arkansas and evaluated for 15 yield components. Results showed that LaGrue produced more seed than the other rice cultivars. Although Lemont had the lowest number of seed, it had significantly higher 100 grain weight and longer panicle length than the others. These results show that LaGrue and Lemont would be suitable parents for the development of a bi-parental population for a QTL mapping study.

INTRODUCTION

Higher yield production is the foremost goal in rice breeding programs. The state of Arkansas is the number one rice producer in the U.S. producing about 49% of the total U.S. rice and accounting for 49% of total rice acres planted in the U.S. (Hardke, 2019) In 2018, rice farmers harvested 1,422,000 acres of rice with an average yield of 166.7 bu/acre which is the third highest state average yield recorded in the Arkansas (Hardke, 2019). Yield is a quantitative trait that is controlled by multiple yield components such as number of productive tillers, number of seeds per panicle, panicle length, number of spikelets per panicle and seed weight per panicle,

and 1000-seed weight per panicle (Samonte et al., 1998; Akinwale et al., 2011; Devi et al., 2017). A quantitative trait is controlled by multiple genes that have a small effect on the phenotype and are greatly affected by the environment (Xu, 2011). The trait distribution is usually a normal or bell distribution in a segregating bi-parental population (Xu, 2011). Panicle architecture is also important in grain yield as the number of primary and secondary branches determine the amount of spikelets that form on the panicle (Xing et al., 2010). Rice cultivars differ greatly in grain yield with each cultivar having different combinations of yield components (Xing et al., 2010). Each cultivar can differ in their yield components for example, some cultivars may have more seeds per panicle than others but have a lower number of productive tillers (Xing et al., 2010).

Four rice cultivars, LaGrue (PI-568891), Lemont (PI 475833), Bengal (PI 561735), and Mars (Clor 9945), are used as parents in development of U.S. elite rice varieties. LaGrue and Lemont are both parental lines for the rice cultivars “Lakast” (PI 674613), “Roy J” (PI 660665), and “CL172” (PI674614). Bengal is a parental line for Titan (PI 680613), CL272 (PI 677004), and Jupiter (PI 639742). Mars is not a parental line for any released rice cultivars in the past 15 years. LaGrue is a short-statured, long-grain cultivar with an average plant height of 115 cm, and weak straw making it susceptible to lodging (Moldenhauer et al., 1994; Wilson et al., 2009). LaGrue has a heading date of 93 days and an average grain yield of 8,624 kg/ha with average milling yields of 57-70 (% whole kernel rice / % total rice) (Wilson et al., 2009). The length of the rice kernel is 7.6 mm with a width of 2.1 and a length/width ratio of 3.6 mm (GRIN-USDA, 2019). LaGrue is susceptible to blast but moderately susceptible to sheath blight and straighthead (Moldenhauer et al., 1994). Lemont is a semi-dwarf, long grain cultivar with an average plant height of 74 cm, and a heading date of 84 days (Bolligh et al., 1985). It has an erect plant type

with excellent lodging resistance and no awns on the seed (GRIN-USDA, 2019). Grain seed has an average length from 6.61mm to 7.50mm and an average length/width ratio over 3.0 (GRIN-USDA, 2019). Lemont is resistant to rice blast races IH-1, IB-1, IB-54, and IG-1, resistant to panicle blight, moderately resistant to brown spot and straighthead disease, moderately susceptible to narrow brown leaf spot, and very susceptible to sheath blight (Bolligh et al., 1985). Bengal is an early maturing, high yielding, medium-grain cultivar with a plant height of 95 cm and a heading date of 89 days (Linscombe et al., 1993; Wilson et al., 2009). It has an intermediate plant type and is moderately susceptible to lodging (Wilson et al., 2009; GRIN-USDA, 2019). Grain yields average 8,574 kg/ha with an average milling yield of 63-70% (Wilson et al., 2009). Grain size for Bengal is 5.51-6.60mm in length and 2.6mm in width with a length/width ratio of 2.1-3.0 (GRIN-USDA, 2019). The cultivar is susceptible to blast races IB-1 and IB-49 and resistant to races IG-1, IH-1, IB-54, IC-17, and ID-13 (Linscombe et al., 1993). Bengal is also moderately susceptible to sheath blight, resistant to narrow brown leaf spot, moderately resistant to leaf smut, and susceptible to straighthead (Linscombe et al., 1993). Mars is a short season, medium grain cultivar with a plant height of 92 cm and has good lodging resistance (Johnston et al., 1979; GRIN-USDA, 2019). Grain length is 5.51-6.60 mm and length/width ratio is 2.1-3.0. The cultivar has an erect plant type with a heading date of 84 days (GRIN-USDA, 2019). Mars is resistant to blast races IH-1 and IG-1, moderately susceptible to brown spot, and susceptible to straighthead.

There is little information about the agronomic traits associated with yield in these cultivars. The objectives of this study were to evaluate the yield components of each cultivar, and to determine which two lines should be used as parents in the development of a bi-parental population for further study into yield components.

MATERIALS AND METHODS

Parental Material

Four *tropical japonica* cultivars of LaGrue, Lemont, Bengal, and Mars were used for this study. These cultivars were from the University of Arkansas System Division of Agriculture' hybrid rice breeding program, located in Rice Research and Extension Center, Stuttgart, AR. For eliminating any possible seed contamination, several plants from each cultivar were grown in the greenhouse to eliminate any possible seed contamination, and leaf samples from each single plant from each cultivar were collected and screened via molecular markers. Seeds from the molecularly verified plants were collected and used for the study.

Field Study

The four cultivars were evaluated in a complete randomized design (CRD) study with three replications and two planting dates 18 May and 6 June 2017 (figure 1 and 2). Each planting date was planted in a separate bay. The cultivars were planted in seven row 3.7x1.5 m plots. The planting depth was 1 cm and spacing within rows was 20.3cm apart. The germination date for the first planting was 26 May and 15 June for the second planting. The first planting was flooded on 20 June and the second planting on 11 July. Preflood nitrogen application of urea was applied at a rate of 56 kg/ha for each bay. Field management consisted of pulling weeds by hand from the bays and no diseases were reported. Ten plants from each plot in the first planting date were randomly collected for 15 agronomic traits including number of tillers/plant, number of panicles/plant, 1000 seed weight/plant, number of seeds/panicle, number of blank seed per panicle, seed weight per panicle, 100 seed weight/panicle, seed weight/plant, number of blank seed/plant, total number of seeds/plant, number of primary panicle branches/panicle, panicle

length, number of spikelets/panicle, and number of spikelets/plant. The panicles were dried in a grain drier prior to data collection. The plots in the second planting date were harvested to obtain yield/plot for each cultivar. The plots were hand harvested by creating bundles for each plot and then threshing each bundle using a mechanical thresher. The seed were dried to 15% moisture and weighed in grams.

Statistical Analysis

Yield traits were analyzed using JMP Pro 14 software (SAS Institute Inc., Cary, NC). ANOVA analysis followed by Student's T-test was carried out to compare yield traits between each cultivar. One hundred seed weight/panicle and 1000 seed weight/plant were calculated using the formulas below:

$$100 \text{ seed weight/panicle: } \left(\frac{\text{seed}_{\text{panicle}}^{\text{weight}}}{\text{number of filled}_{\text{panicle}}^{\text{seeds}}} \right) \times 100$$

$$1000 \text{ seed weight/plant: } \left(\frac{\text{seed}_{\text{plant}}^{\text{weight}}}{\text{number of filled}_{\text{plant}}^{\text{seeds}}} \right) \times 1000$$

RESULTS

Parental Yield Trait Evaluation

Filled seeds/plant: The number of filled seeds/plant (fs/pl) were calculated by taking the sum of the number of seeds counted for each plant. The ANOVA average for number of filled seeds/plant from ANOVA analysis from highest to lowest were LaGrue (1813 fs/pl)>Bengal (1427 fs/pl)>Mars (1333 fs/pl)>Lemont (1279 fs/pl) (Table 1). LaGrue had a significantly higher number of seed than Lemont and Mars producing an average of 1813 fs/pl (Table 1). Lemont produced the lowest number of filled seeds/plant producing an average of 1279 fs/pl. In addition,

Bengal, Mars, and Lemont were not significantly different in terms of number of seeds produced. Even though LaGrue was not significantly higher than Bengal, LaGrue still produced about 386 more seed per plant than Bengal.

Spikelets produced/plant: The number of spikelets per plant (sp/pl) were calculated by taking the sum of the number of spikelets counted on each panicle from each plant. The ANOVA analysis for average number of spikelets per plant from highest to lowest were LaGrue (2077 sp/pl)>Bengal (1873 sp/pl)>Mars (1734 sp/pl)>Lemont (1408 sp/pl) (Table 1). LaGrue was significantly higher than Lemont in spikelet production but was not significantly higher than Bengal and Mars. Bengal and Mars were similar in number of spikelets produced and were not significantly different from each other.

Number of blank spikelet/plant: This factor is calculated by taking the difference between the number of spikelets and the number of filled seed for each panicle on a plant and calculating the sum from all panicles. The ANOVA average for number of blank spikelets/plant (bsp/pl) from highest to lowest were Bengal (445 bsp/pl)>Mars (401 bsp/pl)>LaGrue (264 bsp/pl)>Lemont (129 bsp/pl) (Table 1). Bengal and Mars had significantly higher number of blank spikelets/plant than LaGrue and Lemont (Table 1). Lemont was the lowest in number of blank spikelets being significantly lower than the three other cultivars. LaGrue was the third lowest in number of blank spikelets/plant and was also significantly different from Bengal, Mars, and Lemont. Bengal and Mars were similar in amount of blank spikelets produced and were not significantly different from each other.

Seed weight/plant: Seed weigh/plant (g/pl) is calculated by taking the sum weight of all the filled seed produced from a single plant. The ANOVA average for seed weight/plant from highest to lowest were LaGrue (43.4g/pl)>Bengal (37.5g/pl)> Lemont (34.1g/pl)> Mars

(31.2g/pl) (Table 1). LaGrue had significantly higher seed weight/plant than Mars. Despite no significant differences, LaGrue's seed weight/plant was 9.3g and 5.9g greater than Lemont and Bengal, respectively (Table 1). Mars had the lowest seed weight/plant even though it was not significantly lower than Bengal and Lemont. The results revealed that Lemont was the third lowest in seed weight/plant despite being the lowest producer in filled seed/plant.

1000 seed weight/plant: 1000 seed weight/plant is usually determined by grain size and the amount of filling within the seed (Xing et al., 2010). The ANOVA average of 1000 seed weight/plant from highest to lowest were Lemont (26.4g/plant)> Bengal (26.1g/plant)> LaGrue (24.0g/plant)> Mars (23.3g/plant) (Table 1). Lemont and Bengal significantly had the highest 1000 seed weight/plant than LaGrue and Mars (Table 1). There were no significant differences between Lemont and Bengal. Likewise, there were no significant differences between LaGrue and Mars.

Panicle Length: Panicle length (cm) was measured by measuring each panicle from the base of the rachis to the tip of the top panicle branch and calculating the average length for each plant. The average panicle length from ANOVA for the cultivars from highest to lowest were Lemont (20.8 cm)>Bengal and Mars (20.1 cm)>LaGrue (19.9 cm) (Table 1). Lemont had significantly longer panicle length than LaGrue (Table 1). LaGrue had the shortest panicle length but was not significantly different from Bengal and Mars. Lemont was not significantly different in panicle length from Bengal and Mars.

Number of Primary Panicle Branches/Panicle: Primary panicle branches (ppb/pan) are branches that grow from the main rachis of the panicle. The branches were calculated by counting the number of branches growing from the main rachis and calculating the average for each plant. The ANOVA average for number of primary panicle branches from highest to lowest

were LaGrue (11.9ppb/p)>Mars (11.2 ppb/pan)> Bengal (11.1 ppb/pan)> Lemont (10.8 ppb/pan) (Table 1). LaGrue significantly had the highest number of primary panicle branches/panicle than Lemont, Bengal, and Mars (Table 1). Lemont significantly had the lowest number of primary branches compared to LaGrue but not significantly different with Bengal and Mars. There were no significant differences between Bengal and Mars in number of primary panicle branches.

Number of Filled Seeds/Panicle: The number of filled seeds/panicle were calculated by calculating the average for each plant. The ANOVA average for number of filled seeds/panicle (fs/pan) from highest to lowest were LaGrue (122 fs/pan)> Bengal (113 fs/pan)>Mars (111 fs/pan)>Lemont (89 fs/pan) (Table 1). LaGrue significantly had the highest number of seeds/panicle compared to Lemont but was not significantly higher than Bengal and Mars (Table 1). Lemont significantly had the lowest number of seeds/panicle compared to LaGrue, Bengal, and Mars. Bengal and Mars had about the same amount of seeds/panicle produced and were not significantly different.

Number of Spikelets/Panicle: Number of spikelets were calculated by taking the sum of filled seeds and blank spikelets counted for each panicle and calculating the average for each plant. The ANOVA average for number of spikelets/panicle (sp/pan) from highest to lowest were Bengal (148 sp/pan)>Mars (147 sp/pan)> LaGrue (139 sp/pan)> Lemont (98 sp/pan) (Table 1). Lemont significantly had the lowest number of spikelets/panicle than LaGrue, Bengal, and Mars. There were no significant differences between LaGrue, Bengal, and Mars in number of spikelets/panicle.

Number of Blank Spikelets/Panicle: Number of blank spikelets/panicle (bsp/pan hereafter) is calculated by taking the difference between number of spikelets and number of seed produced on a panicle and calculating the average for each plant. The ANOVA average for

number of blank spikelets/panicle from highest to lowest were Bengal (36 bsp/pan)> Mars (35 bsp/pan)> LaGrue (17 bsp/pan)> Lemont (9 bsp/pan) (Table 1) Lemont significantly had the lowest number of blank spikelets/panicle compared to LaGrue, Bengal, and Mars. LaGrue significantly differed from Lemont, Bengal, and Mars having the third lowest number of blank spikelets/panicle. Bengal and Mars have the highest number of bsp/pan being significantly higher than LaGrue and Lemont.

100 Seed Weight/Panicle: Like 1000 seed weight/plant, 100 seed weight/panicle is usually determined by grain size and the degree of filling in a rice seed. It was calculated by taking the average of 100 seed weight/panicle for each plant. The ANOVA average of 100 seed weight/panicle from highest to lowest were Lemont and Bengal (2.6g/panicle)> LaGrue (2.4g/panicle)> Mars (2.3g/panicle) (Table 1). Lemont had significantly higher 100-seed weight/panicle compared to LaGrue and Mars but has the same weight as Bengal (Table 1). LaGrue was the third lowest being significantly different from the three cultivars. Mars has the lowest 100 seed weight being significantly lower than LaGrue, Bengal, and Lemont.

Seed Weight/Panicle: Seed weight/panicle was calculated by taking the average seed weight per panicle for each plant The ANOVA average for seed weight/panicle from highest to lowest were Bengal (3.0g/panicle)> LaGrue (2.9g/panicle)> Mars (2.6g/panicle)> Lemont (2.4g/panicle) (Table 1). LaGrue and Bengal significantly had the highest seed weight per panicle compared to Lemont and Mars. Lemont significantly had the lowest seed weight/panicle compared to LaGrue and Bengal but was not significantly different compared to Mars. Mars had the third lowest seed weight/panicle and was significantly lower than LaGrue and Bengal.

Number of Tillers/plant: The ANOVA average for number of tillers/plant from highest to lowest were Lemont (15tillers/plant)>LaGrue (14 tillers/plant)>Mars (12 tillers/plant)> Bengal

(11 tillers/plant) (Table 1). Bengal significantly had the lowest number of tillers/plant compared to Lemont (Table 1). Lemont had the highest number of tillers/plant but was only significantly higher than Bengal. There were no significant differences between LaGrue, Lemont, and Mars in number of tillers produced.

Number of Panicles/Plant: The ANOVA average for number of panicles/plant from highest to lowest were Lemont (14 panicles/plant)> LaGrue (14 panicles/plant)> Mars (12 panicles/plant)> Bengal (11 panicles/plant) (Table 1). Lemont significantly had a higher number of panicles/plant than Bengal but was not significantly different between LaGrue and Mars. Likewise, Bengal was not significantly different between LaGrue and Mars.

Plot Yield/Plot: The ANOVA average for plot yield/plant from highest to lowest were LaGrue (2004.5g/plot)> Bengal (1883.4g/plot)> Lemont (1693.2g/plot)> Mars (1572.9g/plot) (Table 2). LaGrue had the highest plot yield compared to Bengal, Lemont, and Mars having a percent yield increase of 6%, 15.5%, and 21.5%, respectively. Mars was the lowest yielding cultivar producing an average of 1572.9 grams/plot.

DISCUSSION

The results from the parental study show that LaGrue has the highest overall yield and highest seed count among the cultivars. LaGrue significantly excelled all three cultivars in number of primary panicle branches/panicle. (Table 1). Despite having the shortest panicle length on average, LaGrue had significantly higher number of filled seeds/plant and seed weight/panicle than Mars and Lemont but was not significantly different to Bengal (Table 1). LaGrue also had significantly higher number of filled seeds and spikelets/panicle compared to Lemont. Bengal and Mars had more spikelets per panicle than LaGrue but produced a lower

number of seed per panicle. Despite having higher 100 and 1000 seed weight, Bengal had lower seed weight overall compared to LaGrue. It can be assumed that the Bengal and Mars low seed production compared to LaGrue is due to having a higher amount of blank spikelets/panicle than LaGrue which reduced the number of seeds/panicle for the cultivars. Blank spikelets on a panicle may be due to environmental conditions or genetics in the cultivars. Studies have shown that number of seeds and seed weight/panicle contribute greatly to the overall yield of the cultivar. Research by Akinwale et al. (2011) and Samonte et al. (1998) showed strong positive correlations between grain yield and number of seeds and seed weight/panicle (Samonte et al., 1998; Akinwale et al., 2011). Sun et al. (2017) showed that number of primary panicle branches greatly contributed to increasing number of seeds/panicle in four backcross populations (Sun et al., 2017). A QTL study by Zhou et al. (2013) showed LaGrue having a higher number of seeds/panicle than another cultivar called Guanghui 116 (Zhou et al., 2013). The researchers found a major QTL from LaGrue on Chr. 4 that explained 10.66% of phenotypic variation in the population (Zhou et al., 2013). Overall, the results show that LaGrue's high-yielding potential stems from producing a high number of seeds/panicle than other cultivars and would make an excellent parent for use in RIL development.

Lemont significantly had the lowest number of seed/panicle, number of spikelets/panicle and number of primary branches/panicle but significantly excelled all three cultivars in panicle length (Table 1). Lemont significantly excelled LaGrue and Mars in 100-seed weight/panicle and 1000-seed weight/plant but was not significantly different from Bengal. Lemont also had significantly lower seed weight/panicle compared to LaGrue and Bengal. The lower number of seeds/panicle on Lemont is probably due to compensating for increased grain size for 100 seed weight/panicle. Research by Kato. 1989 showed that grain size is negatively

correlated with number of seed on a panicle. The degree of grain filling was shown to have a negative effect on the number of seeds produced on a panicle. (Kato., 1989). Research by Guo et al. (2018) found that a gene called *Grain Size Number 1* (GSN1) on chr. 5 that was found to regulate the relationship between grain size and grain number (Guo et al., 2018). Reduced expression was found to increase grain size but reduce grain number while increased expression increased grain number but reduced grain size (Guo et al., 2018). It was also found that the gene also controls panicle branching which also determined the number of spikelets on a panicle (Guo et al., 2018). Despite having a significantly longer panicle than other cultivars, it did not help with the yield potential of Lemont. Studies have shown that having a longer panicle can increase the amount of primary and secondary branches on a panicle which can increase the number of spikelets produced and the number of seeds/panicle (Peng et al., 2014; Jang et al., 2018). This shows that there are probably genes involved that control both panicle architecture, seed size, and grain number in Lemont. But despite this, Lemont was the third lowest yielding cultivar in seed weight/plant and plot yield. Mars was the lowest yielding cultivar despite having higher number of seed/panicle than Lemont. This is probably due with Lemont having a higher 100 seed weight and 1000 seed weight than Mars.

Mars had the lowest 100 and 1000 seed weight and a higher number of blank spikelets/panicle which contributed to reducing the yield potential of Mars. Lemont would make an excellent parent in the development of an RIL population due to its low production of seed but also having greater grain size and panicle length.

CONCLUSION

Grain yield of a rice cultivar is determined by the combination of yield components such as number of panicles and number of seed produced (Xing et al., 2010) A parental yield study

was conducted to compare yield traits among four rice cultivars to see which variety performs the best. The results showed that panicle characteristics of the cultivars were the main factors in determining overall grain yield. LaGrue was the highest yielding rice cultivar outperforming all three cultivars in the number of seeds produced per panicle, number of seeds produced per plant, and number of primary panicle branches. Lemont produced the lowest number of seed but had significantly higher 100 grain weight per panicle and longer panicle length than the other rice cultivars. In the development of a bi-parental population for RIL development, it is important to choose two parents that have significant contrasts between two or more traits of interest (Mulualet al., 2016). An example in Peng et al. (2014) used two parents, 93-11 and PA64 in a QTL mapping study of panicle structure (Peng et al., 2014) The parent 93-11 has significantly higher number of spikelets per panicle and secondary branches per panicle than PA64 which would help in detecting QTL for those traits. Based on the results from the study, we chose LaGrue and Lemont to be used in a bi-parental population for QTL mapping due to LaGrue having significantly higher number of seeds/panicle, number of primary panicle branches/panicle, number of spikelets/panicle, and seed weight/panicle made it a good choice to combine with Lemont which had significantly higher 100 seed weight/panicle and longer panicles. Using these two varieties would hopefully allow researchers to look at the genetics behind yield components in U.S. rice cultivars.

TABLES AND FIGURES

Table1: ANOVA analysis of yield traits between each cultivar.

Trait	LaGrue	Lemont	Bengal	Mars
No. filled seeds/plant	1812.9 ^a	1279.2 ^b	1427.4 ^{ab}	1332.6 ^b
No. spikelets/plant	2077.1 ^a	1408.3 ^b	1872.7 ^{ab}	1733.9 ^{ab}
No. blank spikelets/plant	264.2 ^b	129.1 ^c	445.2 ^a	401.3 ^a
Seed weight/ plant, g	43.4 ^a	34.1 ^{ab}	37.5 ^{ab}	31.2 ^b
1000 seed weight/Plant	24.0 ^b	26.4 ^a	26.1 ^a	23.3 ^b
Panicle Length, cm	19.9 ^b	20.8 ^a	20.1 ^{ab}	20.1 ^{ab}
No. primary panicle branches/panicle	11.9 ^a	10.8 ^b	11.1 ^b	11.2 ^b
No. filled seeds/panicle	121.5 ^a	88.8 ^b	113.4 ^a	111.3 ^a
# spikelets/panicle	138.9 ^a	98.0 ^b	148.1 ^a	147.4 ^a
No. blank spikelets/panicle	17.4 ^b	9.2 ^c	36.2 ^a	34.7 ^a
100-seed weight/panicle, g	2.4 ^b	2.6 ^a	2.6 ^a	2.3 ^c
Seed weight/panicle, g	2.9 ^a	2.4 ^b	3.0 ^a	2.6 ^b
No. tillers/Plant	14.0 ^{ab}	14.6 ^a	11.4 ^b	12.3 ^{ab}
No. panicles/Plant	13.8 ^{ab}	14.4 ^a	11.1 ^b	12.0 ^{ab}

Significance of 0.05 given by abc subscript

Table 2: Evaluation of plot yield between rice cultivars

Cultivar	Average yield per plot	Yield difference compared to LaGrue	Yield increase
	g		%
LaGrue	2004.47	0	0
Bengal	1883.37	-121.1	6
Lemont	1693.17	-311.3	15.5
Mars	1572.87	-431.6	21.5

Figure 1: 18 May planting date. Photo by author

Figure 2: 6 June planting date. Photo by author

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Chapter 3

QTL ANALYSIS OF PANICLE ARCHITECTURE AND YIELD RELATED TRAITS BETWEEN 'LAGRUE' AND 'LEMONT'

ABSTRACT

Grain yield in rice is a quantitative trait controlled by several yield components such as number of tillers/plant, plant height, number of seeds/plant, and flowering time. Panicle architecture is another important yield component that greatly contributes to overall yield of a rice cultivar. Traits such as number of primary and secondary panicle branches, panicle length, and number of spikelets greatly affect the amount of seed produced on a panicle. A QTL mapping study was conducted on a bi-parental population between 'LaGrue' and 'Lemont' at two locations in Arkansas. Seven yield traits such as plant height, number of primary panicle branches, number of secondary panicle branches, panicle length, and flag leaf traits were measured. The results showed two major QTL for plant height located near each other on chr.1. Two major QTL for flag leaf length and two for panicle length were co-localized in a region on the long arm of chr. 8. Candidate gene analysis of the QTL regions found the semi-dwarf gene *sd-1* on chr.1 for plant height which caused semi-dwarfism in rice plants without reducing grain yield. Six candidate genes were found for the QTL on chr.8 that were found to control panicle development as well as grain size and flag leaf traits. The mapping population would be developed into a RIL population to allow for further genetic studies and verify the major QTL detected.

INTRODUCTION

The genetics underlying yield traits are complex. Each yield trait can be controlled by multiple genes that have a small effect on yield (Mulualet et al., 2016). Yield traits are also heavily influenced by the environment in that some yield traits may perform better at one location and perform poorly in another location (Xing et al., 2010). Panicle architecture is an important trait contribution to overall grain yield in rice (Xing et al., 2010). The number of seeds on a panicle depend upon the number of spikelets that form on a panicle. The number of spikelets also depend upon the number of primary and secondary branches that form on a panicle. Other traits such as plant height, heading date, and flag leaf traits are also important for overall yield in rice (Cockram et al., 2007; Sakamoto and Matsuoka., 2008; Li et al., 1998) Most QTL studies are done using bi-parental populations. The bi-parental population is formed by crossing two parents with significantly dissimilar traits (Shakiba and Eizenga,2015). A Recombinant Inbred Line (RIL) population is a population where individual lines have been selfed through several generations until there is uniformity within the line (Shakiba and Eizenga, 2015). In an RIL population, each line consists of a unique combination of chromosome segments from each parent. An advantage of an RIL population is that the lines are nearly homozygous for all the alleles and can be used for multiple generations (Collard et al., 2005). The RIL population can be planted at multiple locations over multiple years to look for QTL that are stable across different environments and years.

A number of QTL studies have been conducted to identify the genetics behind yield components. Navea et al. (2017) used a RIL population derived from temperate *japonica* line ‘TR22183’ and *indica* ‘Dasanbyeon’ to identify QTL related to panicle length and other yield-related traits under different water and phosphorus fertilizer conditions. The study was conducted

during the wet and dry season and were grown under different P fertilizer treatments. They found six major QTL for panicle length on chr. 1, 2, 4, 9, and 11 that were co-localized with previous detected QTL for number of seeds/panicle, panicle length, panicle branching, number of spikelets/panicle, grain number/panicle, and grain weight. Tian et al. (2015) did a genetic mapping study of a QTL that controls flag leaf width and grain number. They used a F₂ population derived from *indica* line ‘HP’ and *japonica* ‘Nipponbare’. They detected a two major QTL for flag leaf width and grain number in the same region on chr. 1 that contributed the most to phenotypic variation for each trait. They then conducted backcrossing with Nipponbare to fine map the region containing the QTL’s using a BC₅F₂ population. They narrowed the QTL region down to a 1,137-kb region. Han et al., (2017) detected major QTL’s for heading date and plant height under multiple environments. They used a RIL population between *indica* ‘Zhenshan 97’ and *japonica* ‘Xizang 2’, studied over multiple years and in different locations under short day (day length <12.5h) and long day conditions (day length >13.5h). The analysis identified QTL for heading date and one for plant height co-localized on chr. 7. where previous QTL for grain yield were also located. QTL for heading date and plant height were also discovered on chr. 7 that were co-localized with previous QTL for tiller number. From QTL mapping studies similar to these, researchers were able to find gene that control panicle architecture and other yield associated traits such as APO1, sd-1, Ghd7, NRL1, and DEP1 (Ikeda-Kawakatsu et al., 2009; Spielmeyer et al., 2002; Xue et al., 2008; Hu et al., 2010; Xu et al., 2016). In the present study, a QTL mapping analysis was done on a bi-parental population derived from a cross between two U.S. rice cultivars ‘LaGrue’ and ‘Lemont’ to examine traits related to panicle architecture and yield. There is little information about the genetics of yield in U.S. rice cultivars. The results

from this experiment will add knowledge about the genetics of yield in the U.S. rice germplasm and uncover potential QTL and candidate genes that could be used in rice breeding.

The objectives of this experiment are to 1) look for QTL associated with yield traits 2) look for candidate genes within major QTL detected and 3) Develop an RIL population from the cross LaGrue x Lemont.

MATERIALS AND METHODS

Bi-Parental Population Development

A bi-parental mapping population was developed by crossing LaGrue with the cultivar Lemont. Crosses were made in the summer 2016 and F₁ seed grown in the Spring 2017 in a greenhouse. The F₁ plants were checked for true/false F₁ using simple sequence repeat (SSR) markers at the molecular genetics lab located at the University of Arkansas System Division of Agriculture's Rice Research and Extension Center (RREC) near Stuttgart, Arkansas. DNA from F₁ leaf tissue were extracted using a rapid high-throughput extraction method (Xin et al., 2003). SSR analysis was carried out using an ABI 3500 Capillary Electrophoresis DNA Analyzer (Thermo Fisher Scientific, Waltham, MA). Seed from each F₁ plant population were harvested and F₂ seed were planted in the greenhouse in the spring 2018. The F₂ plants in the greenhouse were then tissue sampled for genotypic analysis and seed from each plant was harvested separately to create F_{2:3} families for the phenotypic study.

Phenotypic Evaluation of F_{2:3} Families

In the summer of 2018, 322 F_{2:3} families from the LaGrue × Lemont population were planted in panicle rows at two locations; RREC in Stuttgart, Arkansas and the University of Arkansas Division of Agriculture's Pine Tree Research Station (PTRS) near Colt, Arkansas. Ten

seed were planted in each row in an RCBD design with three replications for each family. Each replication was planted separately in a block. The panicle rows were 152 cm long with 41 cm spacing between rows. Ten replications of each parent were used in each replication as a control. The lines were planted at PTRS on 10 July and RREC on 11 July. Due to field conditions, about 280 lines in Pine Tree and 200 lines in Stuttgart were evaluated and grain yield was not recorded. The $F_{2:3}$ families were evaluated in the field for plant height and heading date. Plant height (PH) was measured from the base of the plant to the tip of the main flag leaf. Heading date (HD) was recorded when 50% of the panicles partially emerged. Two panicles from one plant were sampled from each row to evaluate flag leaf length (FLL), flag leaf width (FLW), number of primary branches (PBN), number of secondary panicle branches (SBN), and panicle length (PL).

Genotypic Analysis

The LaGrue x Lemont bi-parental population were genotyped using single nucleotide polymorphism (SNP) and SSR markers. Leaf tissue from each F_2 plant were freeze dried and sent to Eurofins Scientific Inc. to be genotyped using an Infinium 7K Rice SNP chip. For SSR marker analysis, DNA extraction on parental lines were done using cetyltrimethylammonium bromide (CTAB) method (Williams and Ronald., 1994). The lines were genotyped using 313 SSR markers to look for polymorphic markers between the parents using an ABI 3500 Capillary Electrophoresis DNA Analyzer. DNA from F_2 plants were extracted using the same method CTAB method and were used to run SSR markers that were polymorphic between the two parents.

QTL Mapping and Candidate Gene Analysis

QTL mapping was done using ICI mapping software (Meng et al., 2015). The markers were ordered onto a linkage map using Kosambi function. QTL mapping was performed using Inclusive Composite Interval Mapping of additive and dominant QTL function with a LOD threshold of 2.5. QTL with a LOD score of 3.0 or higher were declared for major QTL. The rice genome database Oryzabase was used to search for potential candidate genes within the major QTL detected.

Statistical Analysis

Data analysis on F_{2:3} families and parental controls was done using JMP Pro 14 software and SAS 9.4 (SAS Institute Inc., Cary, NC). ANOVA analysis on parental controls was done using JMP. ANOVA and MANOVA analysis of F_{2:3} population was carried out using PROC GLM in SAS to get genotypic and environmental effects on traits and correlations between each trait. The genotype, environment and genotype x location interaction were treated as fixed effects with Rep(Location) treated as random effects. The mean, range, standard deviation (SD), standard error (SE), and coefficient of variation (CV) of the population was calculated using tabulate function in JMP. The distributions of each trait were done using the distribution function in JMP. Broad Sense Heritability for each trait was calculated using the formula below:

$$\text{Broad Sense Heritability: } H^2 = \left(\frac{\sigma^2_G}{\sigma^2_P} \right) \times 100 = \left(\frac{\sigma^2_G}{\sigma^2_G + \left(\frac{\sigma^2_{GE}}{L} \right) + \left(\frac{\sigma^2_E}{bL} \right)} \right) \times 100$$

Where σ^2_G : genotypic variance; σ^2_P : phenotypic variance; σ^2_{GE} : genotypic x environment; σ^2_E : variance associated with error; L: number of locations or environments; b: number of replications or blocks. The σ^2_G , σ^2_{GE} , and σ^2_E were obtained using the formulas below:

$$\sigma^2G=(MSG-MSG_E)/bL$$

$$\sigma^2GE=(MSG_E-MSE)/b$$

$$\sigma^2E=MSE$$

Where MSG is mean square genotype, MSG_E is mean square genotype x location, and MSE is mean square error. The mean square estimates were taken from the ANOVA table in SAS.

Linkage between DNA markers and yield traits were analyzed using simple t-test in JMP to determine the parental origin of major QTL that had a positive effect on yield traits.

RESULTS

Phenotypic Analysis of Parental Control and F_{2:3} Population

The ANOVA analysis revealed that the average mean of plant height was 99.1 cm with a range from 66.5 to 136.5 cm, standard deviation (SD hereafter) of 11.7, standard error (SE hereafter) of 0.33, and CV of 11.8 indicating wide variation for the trait (Table 1). The genotype and environment had a large effect on the environment with genotype having a larger significant effect on plant height (Table 3). The genotype x environment interaction did not have a significant effect on plant height. The broad sense heritability for the trait was 0.7 indicating that the trait is highly heritable.

Days to heading had an average of 79.4 days with heading date ranging from 69 to 94 days. The SD for the trait is 4.8 with an SE of 0.14 and CV of 6.0 which indicates lower variation in days to heading than plant height (Table 1). The genotype and location and genotype x environment interaction influenced days to heading with genotype having a larger effect with a

p-value of 0.0001 (Table 3). The heritability for days to heading was 0.21 that indicate significant genotype x environment interaction.

Flag leaf length had a mean of 27.5 cm with a range of 16.4-46.8 cm. Flag leaf length had a SD of 4.4, SE of 0.13, and a CV of 16.2 (Table 1). The trait had a larger CV than Plant height and days to heading indicating that there is more variation in flag leaf length than the two traits. Results from ANOVA showed no genotypic, environment, or genotype x location effects on flag leaf length (Table 3). Heritability for the trait was the lowest among the traits with a broad sense heritability of 0.08.

Flag leaf width had a mean of 1.2 cm with a range of 0.6-1.9 cm. The trait had a SD of 0.2, SE of 0.0059, and a CV of 16.9 (Table 1). The trait had a very small SE indicating that the sample mean is very close to the actual mean within the population. Flag leaf width has a CV like flag leaf length indicating that variation is similar between the two. There were no significant effects by genotype, location, or genotype x Location effect on the trait (Table 3). Flag leaf length had a heritability of 0.19 indicating very low heritability for this trait in the population.

The mean of panicle length in the population was 23.3 with a range of 16.8-31.8. The SD was 2.4 with a SE and CV of 0.068 and 10.1, respectively (Table 1). The trait had a very small SE showing that the mean of the sample is very close to the actual mean of panicle length in the population. The CV of the trait shows less variation in the trait than flag leaf length and flag leaf width but more variation than days to heading. Location had a significant effect on panicle length indicating that panicle length was longer in one location than the other (Table 3). Broad sense heritability for the trait was low with a heritability of 0.17.

Primary Panicle Branch has a mean of 23.1 with a range of 12.0-34.0 branches. The trait has a SD of 3.5 with a SE of 0.1 (Table 1). The trait has a CV of 14.9 indicating more variation in the trait than plant height and days to heading but less variation than flag leaf length and flag leaf width. Number of primary panicle branches did not significantly differ in genotype, location, or genotype x location (Table 3). The trait had a low heritability with a broad-sense heritability of 0.27.

For secondary branch number, the mean number of secondary branches in the population was 37.7 with a range of 14-68.5 branches (Table 1). The SD for number of secondary branches was 8.6 with a SE and CV of 0.25 and 22.7, respectively, which indicates a wider variation in the trait than all the other traits measured from the population. Secondary Branch Number was not affected by the genotype, location, or genotype x location effects (Table 3). The broad sense heritability for the trait was low with a heritability of 0.17.

Parental analysis showed significant differences in plant height between LaGrue and Lemont. For the other traits, there were no significant differences between the two parents.

Correlations Among the Traits in the Population

MANOVA analysis shows strong significant correlations between the traits Panicle Length, Flag Leaf Length, Primary Branch Number, and Secondary Branch Number. Flag Leaf Length has strong correlations with Panicle Length ($r=0.57$), Primary Branch Number ($r=0.41$), and secondary branch number ($r=0.43$) (Table 2). Panicle Length also has strong correlations with Primary Branch Number ($r=0.47$), and Secondary Branch Number ($r=0.61$). Primary and secondary branch number are strongly correlated ($r=0.63$) since an increase in primary branches can increase the number of secondary branches on a panicle. The correlations among the traits

show that an increase in flag leaf length can lead to an increase in panicle length. This increase in panicle length can also lead to more primary and secondary branches on the panicle which can lead to more seed on a panicle. Interestingly, panicle length has a stronger correlation with secondary branch number than primary branch number. This is probably due to increases in the length of the primary branch as panicle length increases leading to more secondary branches. The results show that there might be genes that control both flag leaf size and panicle development in the population.

QTL Mapping for Yield Traits

A total of 27 QTL were detected from the population. Four QTL for flag leaf length were detected with two QTL on chr. 2 population and two QTL on chr. 8 (Table 4). The two QTL *qFLL8-1* and *qFLL8-2* on chr. 8 are major QTLs that were co-localized in the same chromosome region. Both QTL are linked to the Lemont allele which confers an increase in flag leaf length and explained 14.16% of the phenotypic variation in the population. One major QTL for Flag Leaf Width *qFLW2* was detected on chr.2 with the positive parental allele from Lemont.

Five QTL for Heading Date were detected on chr. 1,2,7, and 8 with two QTL on chr. 7 (Table 4), one of which, *qHD8*, had the highest PVE explaining about 10.98% of the phenotypic variation and was co-localized with the QTL for panicle length *qPL8-2*. All of these QTL except for *qHD7-2* were detected in Pine Tree. Three of the QTL, *qHD2*, *qHD7-2*, and *qHD8*, were major QTL with *qHD8* having the highest LOD of 7.47. All the major QTL were linked to the Lemont allele which delayed heading date.

Six QTLs for Panicle Length were detected on chr. 1,2,7, and 8 with two QTLs on chr. 2 and two on chr. 8 (Table 4). Of the six QTL associated with panicle length, five were major QTL

with two QTLs, *qPL8-1* and *qPL8-2*, detected near each other on chr. 8. Both QTL explained about 20.62% of phenotypic variation in the population. Each of the QTL were detected in one test site, respectively, and at different locations on chr. 8 but were detected in a region about 3.2mbp in length on the long arm of chr.8. The base pair distance between the two QTL is about 486,144 bp. The QTL *qPL8-2* is colocalized with the two major QTL for flag leaf length *qFLL8-1* and *qFLL8-2*. LaGrue was the major contributor to increased panicle length in *qPL1* and *qPL2-2* while Lemont was the major contributor in *qPL2-1*, *qPL8-1*, and *qPL8-2*.

Three QTLs for Secondary Branch Number were detected on chr.1 and 9 with two QTL on chr.1 (Table 4). The QTL *qSBN1-2* had the highest PVE with a LOD score of 7.57 and explained about 16.25% of phenotypic variation. The two QTL *qSBN1-1* and *qSBN1-2* were detected near each other with a 175,913 bp distance between the two QTL. The major QTL detected, *qSBN1-2* and *qSBN9*, had a positive allele from LaGrue that increased the number of secondary branches per panicle. One major QTL for Primary Branch Number was detected on chromosome1 with a LOD of 3.41 and explained 8.65% of phenotypic variation. The parental origin of the positive allele was LaGrue.

Seven QTL were detected for Plant height on chr. 1,2,8, and 11 with four QTL on chr.1 (Table 4). Two QTL, *qPH1-3* and *qPH1-4*, were co-localized in the same location at both Stuttgart and Pine Tree with both having very high LOD values of 55.49 and 21.03, respectively. The QTL together explained 86.14 % of phenotypic variation. This shows that there might be a major gene that controls plant height in the region. Six of the seven QTL are major QTL with five QTL (*qPH1-2*, *qPH1-3*, *qPH1-4*, *qPH8*, *qPH11*) coming from LaGrue that increased plant height. Interestingly, the QTL *qPH2* came from Lemont that increased plant height despite Lemont being a semi-dwarf cultivar.

Identification of Candidate Genes within Major QTL

Using the online rice database Oryzabase, a total of 14 candidate genes were found with four genes on chr.1 and seven genes on chr.8 controlling plant height, panicle development, and flag leaf size. The genes on chr.1 are semi-dwarf 1 (*sd-1*), Monoculm 2 (*MOC2*), *DCL3a*, and Rolled and Erect Leaf 1 (*REL1*) (Table 5). The semi-dwarf *sd-1* gene is a defective 20-oxidase GA biosynthetic enzyme involved in the synthesis of gibberellin located in the two QTL *qPH1-3* and *qPH1-4* (Sasaki et al., 2002a). The gene causes a semi-dwarf height in rice like Lemont without affecting grain yield. The gene was very important in the development of high yielding cultivars in Asia during the 1960's (Sasaki et al., 2002b). Monoculm 2 encodes a gene for fructose-1,6-bisphosphatase 1 located near *qPH1-2*. The *MOC2* gene was found to control plant height and the number of tillers in rice (Koumoto et al., 2013). A mutant *moc2* was found to inhibit the growth of tiller buds that reduced plant height, tillering, leaf size and growth rate. The mutant gene was found to reduce sugar content in the leaves as well as the size of panicles and grain number. The *DCL3a* gene is a Dicer-like 3 homolog located near *qPBN1* that produces 24-nt small interfering RNA (siRNA) that target miniature inverted repeat transposable elements to control gene expression (Wei et al., 2013). Knockdown of *DCL3a* was found to cause reduced plant height, smaller panicles, and reduced number of primary and secondary branches. The knockdown gene altered the expression of 859 genes with 679 genes up-regulated and 180 genes down-regulated. The genes upregulated were related to metabolic processes including cell wall organization, defense response, GA catabolic process, and other functions. The *REL1* gene located near *qPH1-2* has no known function and was found to control leaf morphology and plant height (Chen et al., 2015) The *rell* mutants showed a significant reduction in plant height, panicle length, and number of tillers. The mutants also exhibited leaf rolling and bending.

Knockdown of expression of the *rel1* mutant gene was found to restore the wild type phenotype while over expression of the wild-type *REL1* gene induces the same mutant phenotype as the *rel1* mutant.

The genes found on chr.8 are *UBP1-5*, *UBP1-8*, Wide and Thick Grain 1 (*WTG1*), *OsSPL16*, *OsSPL14*, *OsCOL15*, and *OsMADS7* (Table 5). *UBP1-5* and *UBP1-8* are ubiquitin carboxyl-terminal hydrolase genes (Ke et al., 2018) The two genes were found to be upregulated at three different panicle development stages; formation of higher-order branches, differentiation of glumes, and differentiation of floral organs. *WTG1* is a gene that encodes an otubain-like protease with deubiquitination activity (Huang et al., 2017). Knockdown of expression of the gene in the *wtg1* mutant caused an increase in grain size in thickness and length and 1000 seed weight per panicle. The mutant panicles were short, thick, and dense but had an increase in the number of primary and secondary branches which also gave the mutant higher number of seed than the wild type. The mutant also had flag leaves that were significantly wider than the wild type but no difference in flag leaf length. Knockdown of gene expression also caused a decreased in number of tillers per panicle but also increased grain yield per plant (Wang et al., 2017). The gene was found to interact with another gene known as *OsSPL14* which also controls plant architecture and grain number (Wang et al., 2017). The *WTG1* gene controls the expression of *OsSPL14* through cleaving K63-linked ubiquitin chains and promote the degradation of *OsSPL14*. A decrease in expression of *WTG1* caused an increase in expression of *OsSPL14* which gave an ideal plant type in rice such as few unproductive tillers, increased number of seed per panicle, and increased lodging resistance. *OsSPL16* is a squamosa promoter-binding protein transcription factor associated with grain size and 1000 grain weight (Wang et al. 2012). Overexpression of the gene has been found to reduce the length-to width ratio in the grain and

reduce panicle branching. A gene found in basmati rice was found to increase the amount of slender grains and increase grain quality but also caused a reduction in grain yield. Another version of the gene from a high-yielding cultivar was found to increase grain yield by producing short and wide grains but had significantly higher 1000 grain weight. The gene was also found to negatively regulate another gene involved in grain size *GW7*. The *GW7* gene also produces slender grains and better yield quality without penalizing yield unlike the *OsSPL16* gene from basmati rice. Upregulation of *OsSPL16* or downregulation of *GW7* formed wider and shorter grains than non-transgenic rice. The results show that *OsSPL16* regulates grain size through regulation of *GW7*. The gene *OsSPL14*, otherwise known as Wealthy Farmer's Panicle, encodes a SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14 protein and is located in the QTL *qPL8-1* (Miura et al., 2010). The gene is found to induce an ideal plant architecture in rice having few unproductive tillers, heavier panicles, and greater lodging resistance. Higher expression of the gene was found to increase the number of primary panicle branches per panicle and increase grain yield (Miura et al., 2010). Knockdown in expression of the gene was found to reduce plant height, increase tiller number, and reduce grain number (Dong et al., 2010). The gene is directly regulated by *osmiR156* controlling plant architecture (Dong et al., 2010). Overexpression of *osmiR156* reduced the expression of *OsSPL14*. Mutations in the target region for *osmiR156* in *OsSPL14* reduced regulation by the miRNA gene which caused an increase in the number of primary and secondary branches, number of seed per panicle, 1000-grain weight, and reduced lodging. Introduction of *OsSPL14* into four elite indica varieties was found to increase grain yield and increase flag leaf length of the cultivars (Kim et al. 2018). *OsCOL15* is a CONSTANS-like transcription factor located in the QTL *qHD8* that regulates flowering time (Wu et al., 2018). The gene exhibits a diurnal expression pattern where the expression level increases 2 hours after

dawn and peaks 2 hours before dusk before quickly decreasing with minimum levels at dawn. This expression pattern was observed under short day conditions and long day conditions. When put under continuous dark conditions, the expression level increased showing that the gene may be induced by a long-night treatment. *OsCOL15* acts as a floral inhibitor delaying flowering time in rice. An increase in gene expression was shown to delay the days to heading under both short day and long day conditions. The gene was shown to influence flowering by upregulating flowering gene *Ghd7* and inhibit expression of *RID1*. It was also shown that overexpression also inhibited other floral genes such as *Ehd1*, *Hd3a*, *RFT1* which are regulated through *Ghd7* and *RID1*. *OsMADS7* (or otherwise known as *OsMADS45*) is a MADS-box class-E class gene which participates in regulation of floral development (Wang et al., 2013). Overexpression of the gene causes early flowering and reduced plant height in rice. It also caused a reduction in panicle length, tiller number, number of spikelets per panicle, and grain yield. Two flowering genes *RFT1* and *Hd3a* were upregulated showing that they may have contributed to the early flowering. Two other genes *OsMADS14* and *OsMADS18* were upregulated early in rice development which may have contributed to shortening the vegetative growth period which resulted in flowering much earlier than the wild type.

RIL Development of Bi-Parental Population

Due to conditions in the field, one plant from each line were transplanted into the greenhouse from the field. The plants were ratooned and allowed to produce seed for the next generation. Two panicles were harvest from each plant. About 97 lines in the F₃ generation that were not planted in the summer 2018 were planted in a winter nursery in Puerto Rico. Two panicles from each line were harvested and shipped back to the RREC. The F₄ seed from a single panicle from each line were planted in the greenhouse to produce F₅ seed. In the summer 2019,

79 F₅ lines and 274 F₄ lines were planted in panicle rows with 15 seed in each row. Three panicles from each line will be harvested to advance the generation. We plan to have a F₇ or F₈ RIL population for use in further studies.

DISCUSSION AND CONCLUSION

Results from the experiment showed major QTL for plant height, flag leaf length, and panicle length detected at Stuttgart and Pine Tree that were colocalized in the same region on chr.1 and 8. Two major QTL for plant height, *qPH1-3* and *qPH1-4*, were colocalized in the same location on chr. 1 and explained a very high amount of phenotypic variation in the population. The trait highly heritable in the population having a broad sense heritability of 0.70. A candidate gene semi-dwarf 1 (*sd-1*) was found in the QTL regions that control plant height. The gene is a defective 20-oxidase GA biosynthetic enzyme involved in the synthesis of gibberellin (Sasaki et al. 2002a). The gene causes a semi-dwarf height in rice without affecting grain yield. The semi-dwarf height also decreases the chance of lodging in rice increasing the potential yield of rice cultivars. The gene was very important in Asia in the development of high-yielding rice cultivars with reduced susceptibility to lodging (Sasaki et al. 2002b). The gene very likely came from Lemont since the cultivar is considered a semi-dwarf in height. Two other candidate gene *MOC2* and *REL1* were found near *qPH1-2* and were involved in the control of plant height as well as number of tillers, panicle size, and grain yield (Koumoto et al., 2013; Chen et al., 2015).

On chr. 8, major QTL for panicle length and flag leaf length were co-localized in a 3.2Mbp region. The QTL for flag leaf length *qFLL8-1* and *qFLL8-2* were colocalized in the same region on chr. 8. One QTL for panicle length *qPL8-2* was in the same region as the QTL *qFLL8-2*. The other QTL *qPL8-1* was located nearby upstream of the other QTL but was not in

the same region as *qPL8-2*. All of the major QTLs which increased flag leaf length and panicle length came from Lemont. Five potential candidate genes for the traits *UBP1-5*, *UBP1-8*, Wide and Thick Grain 1 (*WTG1*), *OsSPL16* and *OsSPL14* were found in these QTL regions. The genes *UBP1-5*, *UBP1-8* were found to be upregulated during panicle development (Ke et al., 2018). Wide and Thick Grain 1 (*WTG1*) is a gene that affect grain size, panicle architecture, 1000 seed weight, and flag leaf width (Huang et al., 2017). The gene was found to interact with another gene in the region *OsSPL14* or otherwise known as Wealthy Farmer's Panicle (Wang et al., 2017) *OsSPL14* induces an ideal plant architecture such as increased number of primary and secondary branches, increased number of seeds per panicle, increase in length of flag leaf length and reduced lodging. Reduced gene expression of *WTG1* was found to increase the expression of *OsSPL14* giving an ideal plant type in rice (Huang et al., 2017). *OsSPL16* controls grain size, panicle branching, and 1000 grain weight and may control grain size through regulation of *gw7* (Wang et al. 2015; Wang et al., 2012). Correlation analysis showed that flag leaf length is strongly correlated with panicle length and number of primary and secondary branches. This relationship shows that genes that control both panicle and flag leaf development such as *OsSPL14* and *WTG1* may be potential targets for molecular breeding. One major QTL for flag leaf width was found on chr. 2 that came from Lemont that increased leaf width

Five QTL for heading date were detected on chr. 1,2,7, and 8 with one major QTL *qHD8* explaining 10.98% of phenotypic variation in the population. All the major QTL for heading date came from Lemont which increased days to heading. Two candidate genes for heading date were detected in *qHD8*; *OsCOL15* and *OsMADS7*. *OsCOL15* is a floral inhibitor that delays the days to heading in rice (Wu et al., 2018). The gene influences heading date by upregulating the flowering gene *Ghd7* and inhibiting *RID1* which in turn influences other genes such as *Ehd1*,

Hd3a, and *RFT1*. *OsMADS7* is another flowering gene that participates in regulation of floral development (Cui et al., 2010). The gene has been found to control flowering as well as plant height, panicle length, tiller number, number of spikelets per panicle, and grain yield (Wang et al., 2013). The gene was found to control four other genes, *RFT1*, *Hd3a*, *OsMADS14*, and *OsMADS18* which may contribute to flowering and vegetative growth (Wang et al., 2013). Since the gene is found to control panicle length and number of spikelets per panicle, it could be considered a candidate gene for yield traits since it is found in the same regions as *qPL8-2* and *qFLL8-2*. Surprisingly, the heritability for heading date was low with a broad-sense heritability of 0.21.

Three QTL for Secondary Branch Number were found on chr. 1 and 9. Two QTL, *qSBN1-1* and *qSBN1-2*, were found close together on chr. 1 with *qSBN1-2* having the highest LOD and was responsible for 16.25% of phenotypic variation in the population. One major QTL for Primary Branch Number was found on chr. 1. The major QTL for primary and secondary branch number *qPBN1*, *qSBN1-2*, and *qSBN9* were found to come from LaGrue which increased the number of panicle branches. This was expected since LaGrue produces a significantly higher number of seeds than Lemont. One candidate gene *DCL3a* was found near *qPBN1* that was found to control both primary branch number and secondary branch number (Wei et al., 2013).

Improving grain yield in rice is one of the important goals in rice breeding programs. Overall grain yield in rice is dependent of several yield components such as plant height, number of seeds per panicle, and 1000 seed weight (Xing et al., 2010). The detection of major QTL for yield components can be beneficial for breeding of high-yielding rice varieties. Results from this experiment showed major QTL for plant height, flag leaf length, and panicle length that were colocalized in regions on chr. 1 and 8. A major gene for plant height *sd-1* was found in the QTL

that gives increased lodging resistance without reducing yield. Six potential candidate genes for flag leaf length and panicle length; *UBP1-5*, *UBP1-8*, Wide and Thick Grain 1 (*WTG1*), *OsSPL16*, *OsSPL14*, and *OsMADS7*; were found on chr. 8 that could be used in breeding programs. The bi-parental population will be developed into an RIL population to be used in further mapping experiments. Using an RIL population would allow us to verify the major QTL found and to look for additional QTL that could be used for rice breeding.

TABLES AND FIGURES

Table1: Yield Analysis of Parental Controls and descriptive statistics of F_{2:3} Population

Trait	LaGrue	Lemont	F _{2:3} Population				
	Mean	Mean	Mean (cm)	Range (cm)	Standard Dev. (SD)	Standard Err. (SE)	CV
Plant Height (cm)	101 ^a	90 ^b	99.1	66.5-136.5	11.8	0.33	11.8
Days to Heading	79.8 ^a	82 ^a	79.4	69-94	4.8	0.14	6
Flag Leaf Length (cm)	26.3 ^a	28.3 ^a	27.5	16.4-46.8	4.4	0.13	16.2
Flag Leaf Width (cm)	1.2 ^a	1.3 ^a	1.2	0.6-1.9	0.2	0.0059	16.9
Panicle Length (cm)	23 ^a	23.9 ^a	23.3	16.8-31.8	2.4	0.068	10.1
Primary Branch Number	23.2 ^a	23.3 ^a	23.1	12.0-34.0	3.5	0.1	14.9
Secondary Branch Number	36.6 ^a	34 ^a	37.7	14-68.5	8.6	0.25	22.7

Significance of 0.05 given by abc subscript

Table 2: Correlations between yield traits in F_{2:3} population

Trait	Plant Height	Days to heading	Flag Leaf Length	Flag Leaf Width	Panicle Length	Primary Branch Number	Secondary Branch Number
Plant Height	1	0.052489	0.191061	0.167098	0.105132	0.121957	0.165395
		0.1925	<.0001	<.0001	0.0089	0.0024	<.0001
Days to heading	0.052489	1	0.1798	0.018196	0.270397	0.15137	0.241675
	0.1925		<.0001	0.6517	<.0001	0.0002	<.0001
Flag Leaf Length	0.191061	0.1798	1	0.189338	0.574084	0.41541	0.433789
	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
Flag Leaf Width	0.167098	0.018196	0.189338	1	0.029408	0.223711	0.111571
	<.0001	0.6517	<.0001		0.4655	<.0001	0.0055
Panicle Length	0.105132	0.270397	0.574084	0.029408	1	0.475573	0.610198
	0.0089	<.0001	<.0001	0.4655		<.0001	<.0001
Primary Branch Number	0.121957	0.15137	0.41541	0.223711	0.475573	1	0.635593
	0.0024	0.0002	<.0001	<.0001	<.0001		<.0001
Secondary Branch Number	0.165395	0.241675	0.433789	0.111571	0.610198	0.635593	1
	<.0002	<.0001	<.0001	0.0055	<.0001	<.0001	

Table 3: ANOVA analysis of yield traits in F_{2:3} population

Trait	Genotype	Location	Genotype x Location	Mean Square Err.	Heritability
Plant Height	209.82** <.0001	19829* 0.0084	62.91 0.4254	61.65	0.7
Days to Heading	17.87** 0.0001	5196.40* 0.0087	14.24 0.0436	11.62	0.21
Flag Leaf Length	20.88 0.2616	3.49 0.7404	19.063 0.5834	19.62	0.08
Flag Leaf Width	0.038 0.1153	1.39 0.3199	0.030 0.8263	0.033	0.19
Panicle Length	4.95 0.0504	688.65* 0.0007	4.14 0.5429	4.21	0.17
Primary Branch Number	11.65 0.3092	227.44 0.2513	9.67 0.8612	11.1	0.27
Secondary Branch Number	78.38 0.0516	545.34 0.2764	64.58 0.6002	66.81	0.17

Effects with P-values less than 0.01 given *

Effects with P-values less than .0001 given **

Table 4: List of QTL detected and parental origin of positive allele for major QTL

QTL	Parental origin of positive allele	Location	LeftMarker	RightMarker	BP Position	LOD	PVE(%)	Add.	Dom.
<u>qFLL2-1</u>		ST	1981019	c2p19270352	19154391- 19270353	2.86	6.216	-0.74	-0.833
<u>qFLL2-2</u>		ST	SNP- 2.28645110.	2369745	28650980- 31295132	2.58	5.914	0.345	1.196
<u>qFLL8-1</u>	Lemont	PT,ST	SNP- 8.26142013.	9030959	26144728- 27300243	3.53	5.97	-1.04	0.0874
<u>qFLL8-2</u>	Lemont	PT,ST	SNP- 8.26142013.	9030959	26144728- 27300242	3.16	8.191	-1.12	0.5048
<u>qFLW2</u>	Lemont	ST	SNP- 2051794	SNP- 2.21778435.	21329057- 21784305	4.01	9.207	-0.05	-0.028
<u>qHD1</u>		PT	SNP- 1.34680507.	SNP- 1.34834470.	34681551- 34835514	2.92	3.84	-0.1	-1.077
<u>qHD2</u>	Lemont	PT			22089558- 23396915	4.18	5.773	-0.98	-0.231
<u>qHD7-1</u>	Lemont	PT	7094244	rd7002048	5185191- 6855960	3.05	4.83	-0.29	1.1291
<u>qHD7-2</u>		ST	SNP- 7.21232810.	id7004163	21233804- 23427756	2.75	3.385	-1.87	-2.703
<u>qHD8</u>	Lemont	PT	SNP- 8.26142013.	9030959	26144728- 27300243	7.48	10.98	-1.22	-0.379

Table 4 cont.

QTL	Parental origin of positive allele	Location	LeftMarker	RightMarker	BP Position	LOD	PVE(%)	Add.	Dom.
<u>qPL1</u>	LaGrue	ST	SNP-1.37415410.	1212517	37416454-37692801	5.27	12.2	0.649	0.3076
<u>qPL2-1</u>	Lemont	PT	SNP-2.18507632.	SNP-2.18584949.	18513502-18590819	3.03	4.652	0.413	-0.07
<u>qPL2-2</u>	LaGrue	ST	SNP-2.11601520.	id2005538	11601525-12154391	4.06	9.025	0.468	-0.312
<u>qPL7</u>		PT	rd7002219	id7004930	24113175-25945760	2.85	5.32	-0.47	-0.129
<u>qPL8-1</u>	Lemont	PT	SNP-8.26142013.	9030959	26144728-27300243	5.1	9.103	-0.56	-0.252
<u>qPL8-2</u>	Lemont	ST	id8006881	8980373	24803160-25658584	5	11.52	-0.57	-0.231
<u>qPBN1</u>	LaGrue	ST	SNP-1.39395295.	SNP-1.39465827.	39396339-39466871	3.41	8.659	0.717	0.4043
<u>qPH1-1</u>		PT	id1004164	id1005141	5134796-6667544	2.55	1.646	-1.15	-1.816
<u>qPH1-2</u>	LaGrue	ST	id1023347	SNP-1.37300662.	37030733-37301706	3.31	4.308	-0.01	3.2339
<u>qPH1-3</u>	LaGrue	PT	1226391	rd1000365	38258929-38361942	55.5	52.75	9.809	0.0026
<u>qPH1-4</u>	LaGrue	ST	SNP-1.38422515.	SNP-1.38536811.	38423559-38537885	21	33.4	6.606	-0.249
<u>qPH2</u>	Lemont	ST	2051794	SNP-2.21778435.	21329057-21784305	3.15	4.086	-2.27	-0.061
<u>qPH8</u>	LaGrue	PT	SNP-8.26090329.	SNP-8.26142013.	26093044-26144728	4.24	2.583	2.128	0.2226
<u>qPH11</u>	LaGrue	PT	SNP-11.7010315.	rd11003092	7014571-10086551	3.14	4.154	-0.63	-4.612
<u>qSBN1-1</u>		PT	1226391	rd1000365	38258929-38361942	2.83	4.35	1.768	-0.348
<u>qSBN1-2</u>	LaGrue	ST	SNP-1.38536811.	1237300	38537855-38652270	7.57	16.25	3.139	0.9015
<u>qSBN9</u>	LaGrue	PT	rd9002719	SNP-9.17707021.	17416860-17708023	3.05	4.764	1.876	-0.017

Table 5: List of candidate genes for yield traits

Gene Id	Gene	Location	Trait	Description
LOC_Os01g66100	sd-1	Chr 1: 38382382 - 38385504	Plant height	semi dwarf 1 gene
LOC_Os01g64660	Monoculm 2, MOC2	Chr 1: 37519523 - 37522613	Plant height	CYTOSOLIC FRUCTOSE- 1,6-BISPHOSPHATASE 1
LOC_Os01g68120	DCL3A	Chr1: 39605717 - 39595681	Primary Branch Number	Endoribonuclease Dicer homolog 3a
Os01g0863500	REL1	Chr1: 37365499-37366446	Plant height	Rolled and Erect leaf 1, unknown function
LOC_Os08g41580, Os08g0527600	UBP1-5	Chr 8: 26268141-26263393	Panicle	ubiquitin carboxyl-terminal hydrolase
LOC_Os08g41630, Os08g0528100	UBP1-8	Chr 8: 26299397 - 26287372	Panicle	ubiquitin carboxyl-terminal hydrolase family protein
LOC_Os08g42540	OTUB1, WTG1	Chr 8: 26887363 - 26882955	Panicle,flag leaf	ubiquitin thioesterase otubain-like
LOC_Os08g41940	GW8,OsSPL16	Chr 8: 26501167 - 26506218	Panicle	SBP-box gene family member
LOC_Os08g39890	Wealthy farmers panicle, IPA 1,WFP,OsSPL14	Chr 8: 25278696-25274449	Panicle,flag leaf	SBP-box gene family
LOC_Os08g42440	OsCOL15	Chr8: 26797181 - 26792824	heading date	CCT/B-box zinc finger protein
LOC_Os08g41950	OsMADS7,S45	Chr 8: 26507753 - 26512261	heading date	MADS-box family gene with MIKCC type-box

Figure 1: Distribution of Plant Height **Figure 2:** Distribution of Days to Heading **Figure 3:** Distribution of Flag Leaf Length **Figure 4:** Distribution of Flag Leaf Width

Figure 5: Distribution of Panicle Length

Figure 6: Distribution of Primary Branch Number

Figure 7: Distribution of Secondary Branch Number

Figure 8: Linkage Map and QTL location in Pine Tree

Figure 9: Linkage Map and QTL location in Stuttgart

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OVERALL CONCLUSION

Selecting for higher grain yield is one of the main objectives for rice breeding programs in the U.S. A parental yield study of four U.S. rice cultivars determined that LaGrue is the highest yielding cultivar and produced the most seed while Lemont was the lowest producer of seed but had longer panicles and heavier grain size. We chose LaGrue and Lemont as parents in the development of a bi-parental population for use in a QTL mapping study of panicle architecture and yield-related traits. The F₂ plants from the population were genotyped and F₂:₃ families were evaluated in a field study at two locations for seven yield traits. Strong correlations were found between flag leaf length, panicle length, number of primary panicle branches, and number of secondary panicle branches. QTL mapping found a total of 20 major QTL including major QTL's for plant height on chr.1 that accounted for a large variation of plant height in the population. Two QTL's for flag leaf length and panicle length were co-localized in a region on chr. 8. Candidate gene analysis of major QTL regions found one major gene for plant height *sd-1* on chr.1 that caused semi-dwarfism in rice plants without affecting grain yield. On chr. 8, six candidate genes were found to be previously mapped in the QTL regions for flag leaf length and panicle length. The genes *WTG1*, *OsSPL14*, *OsSPL16*, *UBP1-5*, *UBP1-8*, and *OsMADS7* were found to be involved in panicle development and flag leaf development. The bi-parental population will be further developed into a RIL population to be used to confirm the QTL detected and be used in future genetic studies. The QTL and candidate genes detected would be useful in marker-assisted selection in development of future elite rice cultivars.