MORPHOGENETIC ANALYSIS OF IRAQI AROMATIC RICE (AMBER)

GERMPLASM

A Dissertation

by

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ABSTRACT

The objective of this work was to determine the agronomic, morphological, and molecular diversity characteristics of Iraq's aromatic-rice (*Oryza sativa* L.) cultivars. The first study was conducted for three years (2015, 2016, and 2017) at the Texas A&M AgriLife Research Center in Beaumont. It focused on characterizing and determining the variability of the agronomic and morphological characteristics of Iraq's aromatic rice cultivars. The cultivars varied significantly for the number of days needed to reach 50% heading, plant height, tillers per square meter, flagleaf area, panicle lengths, weights of thousand-grain, and percentage of chalky seed.

The second study was conducted at Beaumont and Eagle Lake in 2017 to characterize and determine the variability of agronomic and morphological characteristics of an $F_{2:6}$ recombinant inbred lines (RIL) population derived from a cross between Amber 33-PI and Antonio rice cultivars. Days to the 50% heading, plant heights, tillers per plant, flag-leaf areas, ligule lengths, panicle lengths, panicles per plant, branches per panicle, the filled grains per panicle, grain yields per plant, chalky seed percentages, number of grains per panicle, sterility percentages, and fertility percentages varied significantly (LSD = 0.05) among locations.

The third study was conducted to study the molecular diversity among 27 aromatic rice cultivars and to map the associated genes of the quantitative trait loci (QTL) in aromatic rice. Data on 21 agronomic and morphological traits were collected from two locations, Beaumont and Eagle Lake, in 2017. All cultivars were genotyped with genotyping-by-sequencing (GBS) to conduct a cluster and principal coordinate analysis (PCoA). Both locations' data were used for the principal component analyses (PCA) of the phenotypic data of aromatic rice cultivars. Most of the genotypic variance within the data was explained by the first three principal components (PCo1 = 45%, PCo2

= 11%, and PCo3 = 6%). The cultivars were divided into five clusters, which included two clusters of Amber rice cultivars, two clusters of Basmati rice cultivars, and one cluster of the U.S. cultivars. To perform the mapping of the QTL, 120 rice lines were included in this study. Eighteen linkage groups were generated, covering all 12 chromosomes of the rice genome in JoinMap. Twenty-six QTL associated with 21 different traits were identified in the Amber 33-PI X Antonio population. Several of these QTLs for aroma and other morphological and agronomic traits had sufficient variation that can result into the development of single nucleotide polymorphic (SNP) markers for marker-assisted selection and marker-assisted breeding to increasing the efficiency of breeding programs focusing on developing aromatic rice cultivars.

DEDICATION

To my father and my mother for their support, love ,and prayer that helped me reach my dreams.

To my lovely wife Alaa and my beautiful daughters Fatimah, Zainab and Aya.

To my big family, my brothers, my sisters, my uncle, and grandfather.

To my beautiful country in the world, Mesopotamia, and IRAQ.

To my committee members, Dr. Amir Ibrahim, Dr. Rodante Tabien, Dr. Wayne Smith, and Dr. Steve Hague.

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To Iraqi people, men, women, children, young, elders, and, especially, the martyrs of Iraq.

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The data analyzed for Chapter II and Chapter III was made possible by Dr. Amir M.H. Ibrahim. All other work conducted for the dissertation was completed by the student independently.

NOMENCLATURE

ANOVA	Analysis of variance
CIM	Composite Interval Mapping
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
FTD	Flame Thermionic Detector
GCMS	Gas Chromatography Mass Spectrometry
KASP	Kompetitive Allele Specific PCR
LOD	Log of Odds
MAB	Marker-Assisted Backcrossing
MAS	Marker-Assisted Selection
MQM	Multiple QTL Mapping
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RADSeq	Restriction site Associated DNA Sequencing
RIL	Recombinant Inbred Lines
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TRC	Toronto Research Chemicals
2AP	2-Acetyl-1-Pyrroline

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Rice (*Oryza sativa L.*) is an important cereal crop, both in terms of directly feeding people and helping the global economy. It is a staple crop for more than 3.5 billion people worldwide (Diagne et al., 2015), and it is a particularly important component of the diet of most people living in Asia. The economy of the rice growing countries is dependent on the supply of rice to feed the growing population. The U.S. is a major exporter of rice, with the global market accounting for about half the annual sales volume of the U.S. produced rice. Six states in the U.S. produce rice. These are Arkansas, California, Louisiana, Mississippi, Missouri, and last but not least, Texas. The United States Department of Agriculture (2018) reported a U.S. production of 5.66 million metric tons and global rice production of 489.50 million metric tons in May 2018, approximately a 0.26% increase from the same month in 2017.

Rice has many characteristics, particularly in terms of grain quality – for example, color, shape, taste, and aroma. Aroma is one of the principal characteristics that determines the quality of rice and it can dictate the price of milled rice. The global demand for aromatic cultivars of rice is increasing, particularly the heirloom aromatic rice. Aromatic rice includes a small subset of the indica and japonica rice groups that are considered of higher quality and thus yields a higher price. There are five groups of rice, namely indica, tropical japonica, temperate japonica, aromatic and aus. In the study done by Garris et al. (2005), aromatic rice is closely linked to temperate japonica and tropical japonica groups as opposed to indica and aus groups. Most of the popular aromatic rice cultivars in the world market have long grains such as Basmati and Jasmine rice cultivars.

Aroma and long grain are important factors for price determination in the domestic and international markets.

Rice is the staple food for most of the Iraqi population. Rice is planted into flooded fields in June and harvested by late November and early December. There are eight Iraqi provinces that produce rice, namely "An Najaf, Al-Qadidiyah, Diyala, Wasit, Al-Muthanna, Dhi Qar, Maysan and Babil" with An Najaf and Al- Qadidiyah being the main sources, producing about more than 70% of Iraqi rice production. Rice is the principal crop in An Najaf and Al- Qadidiyah provinces and important to the regional economy. Rice is the main daily dish of the Iraqi family, where annual consumption is almost 45-kilogram per capita rice consumption. Amber rice is a class of aromatic rice cultivars grown in Iraq for hundreds of years (Younan et al., 2011). It is a domestic rice type in Iraq, generally medium grain, not sticky, soft when cooked and has a distinct aroma in cooked rice. Aromatic rice is commonly used in Iraqi recipes during holidays and special occasions. Being a specialty rice, Amber rice is expensive compared to other types of aromatic rice such as Furat, Daawat, and Yasmin but Iraqi people generally prefer to eat Amber rice (or "Anber" in the local accent). Amber rice, therefore, is simply a high-quality aromatic rice, a very distinct group of rice in Iraqi market. Although Amber rice is popular in Iraq, there are many challenges in growing Amber rice compared to popular high-yielding non-aromatic rice, as Amber rice has lower grain yield, weak straw, susceptibility to insect pest and diseases, poor water use efficiency and low yield. Iraqi consumers can detect variation in aroma among different Amber rice cultivars; however, this group of rice has not been studied in detail in terms of its aroma, and agronomic performance. Diversity analysis has not been done on this group of rice and the genes controlling the aroma trait are still unknown. Evaluating aromatic rice cultivars in terms of genetic diversity is essentially important for Iraqi breeders and the global rice breeding community in general.

Agronomic and morphological characterization are fundamental in providing plantbreeding programs with information about Iraqi aromatic rice cultivars. Most existing studies have focused on investigating Iraqi rice cultivars within and among local cultivars, but it is important to study the differences between Iraqi and other countries' types, including U.S. aromatic rice cultivars. This study will help rice breeders better understand the relationships among aromatic rice cultivars, Amber rice, and the distribution of Amber rice cultivars between the United States and the world rice cultivars. Crop diversity, including within cultivars of rice, is essential for agricultural development and thus increase food production and promote economic growth. Genetic diversity is required for all crops, particularly rice, to infuse breeding programs with multiple gene sources. The amount of genetic diversity within a species is an important factor in understanding the evolutionary status of rice species and the relationships among the indica, japonica, and aromatic rice groups. Although there are many traditional and improved cultivars of rice available in Iraq, no study has produced a complete characterization or systematic analysis on their genetic base and diversity (Younan et al., 2011). This study aims to describe the phenotypic and genetic diversity among Iraqi aromatic rice and improve the understanding of diversity among rice cultivars. In addition, the study aims to determine the genetic control of aroma trait in an Iraqi aromatic rice germplasm.

1.2. Literature Review

1.2.1. Aromatic Rice in Iraq and Globally

Rice is a critical commodity in Iraq, where annual per capita consumption is almost three times that of the United States (50 kg per person compared to 14 kg in the U.S.). In the past 10 years, local rice production has accounted for only 8% to 21% of domestic consumption. In 2016, Iraq ranked among the world's top 10 rice importers (United States Department of Agriculture [USDA], 2016). The sources of Iraq to meet the current demands are importing rice from the U.S. and some Asia countries. Import of Basmati rice from India have increased dramatically in recent years (Napasintuwong, 2012) because of the increase in Iraqi's population and strong demands for high quality aromatic rice. While there is no definitive data showing how much of Iraq's rice consumption is aromatic rice versus non-aromatic rice, but the amount of aromatic rice exported to Iraq and in Iraqi markets provides information on the degree to which aromatic rice is preferred in Iraq. Rice is cultivated as a summer crop in Iraq, particularly in the southern portion, as well as in the valleys of northern Iraq (Rabbani et al., 2008). Use of chemical fertilizer, mechanical harvesting and clean seeds are some of the common production practices done to increase rice yield. Since the early 20th century, many traditional and improved rice cultivars have been cultivated across different regions of Iraq. The most popular cultivar of rice in Iraq is Amber rice, which has been cultivated particularly in the southern region (Chakravarthy and Naravaneni, 2006). Among Amber rice, 'Amber 33' is widely cultivated and command the highest price. Other Amber aromatic cultivars in Iraq include Amber or Anber.

Globally, rice has been planted over an area of 162.03 million hectares and produced a yield of 4.51 metric tons per hectare in 2018, which includes non-aromatic and aromatic rice (USDA, 2018). Aromatic rice is also known as fragrant or perfumed rice. It has nutty or popcorn

flavor when cooked. Aroma is one of the most important characteristics in determining rice's quality. Global demand for aromatic rice cultivars is increasing (Diako et al., 2010), and these cultivars can command higher prices than non-aromatic rice not only for their aroma but also for their high nutritional value (Sekhar and Reddy, 1982). People in the Middle East highly prefer long-grain and well-milled rice with a strong aroma, whereas people in Europe prefer long-grain rice without an aroma (Efferson, 1985). Most studies indicate that rice is one of the most important grain crops globally and the principal crop in many countries, especially in Asia. The global population is expected to reach 8 billion by 2030, and rice production must increase by 50% to meet the growing demand (Miah et al., 2013). A significant number of rice cultivars and lines have been developed through varietal improvement and genetic resource conservation, evaluation, and the use of different breeding programs at international and national institutions around the world (Food and Agriculture Organization [FAO], 2000) and some of these are aromatic cultivars. The preference for aroma in rice, however, varies among regions and countries. For instance, aromatic rice is not so popular in the U.S. compared to countries such as Iraq, India, and Pakistan, where it is extremely popular. Aromatic rice is preferred in Iraq, as well as in India, Pakistan, and some other areas of Asia. Most rice-growing countries have their own heirloom aromatic cultivars. For instance, Amber rice cultivars are the popular aromatic rice in Iraq, with Amber 33 in particular being a popular domestic cultivar due to its distinctive aromatic odor (Chakravarty, 1976). As aforementioned, Basmati rice cultivars are popular in India and Paskitan while Jasmine is the prime aromatic in Thailand. India, Pakistan, and Thailand are the best sources for strongly aromatic cultivars such as Basmati-370. Milfor, Sinandoming and Milagrosa are heirloom cultivars in the Philippines (Khush et al., 1979; R. Tabien, personal communication). The demand for aromatic rice increases daily, although aromatic rice has undesirable agronomic traits such as low yield,

susceptibility to disease and pests, and strong shattering (Berner and Hoff, 1986).

1.2.2. Variability in Agronomic, Morphological, and Phenological Characteristics

Agronomic and morphological characterization are fundamental in providing information about Iraqi aromatic rice cultivars to plant breeding programs. The diversity among crops and within cultivars is essential for agricultural development in order to increase food production and promote economic growth. Hossain et al. (2005) studied different aromatic rice cultivars for diverse morphological traits including foliage color, leaf orientation, leaf breadth, awn and panicle types, glume color, and grain shape. They also evaluated agronomic traits such as plant height, fertile tillers per hill, panicle length, spikelets per panicle, grains per panicle, thousand-grain weight, and grain yield but all parameters were not directly associated with aroma. Bisne and Sarawgi (2008) studied 22 morphological, six agronomical, and eight quality characters to evaluate agro-morphological and quality traits of 32 aromatic rice accessions of Badshah bhog group from aromatic rice germplasm of Chhattisgarh, India. The specific genotypes B: 1340, B: 2039, B: 2495, B: 2816, B: 16930 B: 2354, B:1639, B:2094 were identified for good grain quality with higher yield. Aspects related to quality of rice such as the size, shape, and appearance of grain, milling quality, and cooking properties must be considered from the perspective of rice breeders (Dela Cruz and Khush, 2000). Yield is a complex factor, and it depends on many agronomic and morphological traits such as plant height, number of effective tillers, leaf characteristics, panicle size, panicle type, spikelet fertility, grain weight, biological yield, and harvest index. Hossain et al. (2005) evaluated morphological traits as well as agronomic traits of eight aromatic rice cultivars and found the highest grain yielding cultivar from BRRI (Dhan34), the tallest cultivar (Chinigura), the highest number of grains panicle (Dhan 34), and maximum 1000 grain weight observed in BRRI Dhan38 cultivar.

Days to heading (also referred to as "days to flowering") is a key phenological factor of the rice plant. It is one of the critical traits for adapting rice to different environments, and rice cultivars in cropping seasons and multiple regions (Lin et al., 2011). Some studies indicated a correlation between flowering time and whether rice is aromatic or non-aromatic. Mathure et al. (2011) characterized seven aromatic rice germplasm and evaluated the correlation between its agronomic and quality traits. Results showed a negative correlation between aroma and days to 50% flowering and between aroma and filled grains per panicle.

Plant height is another key agronomic trait related to rice yield potential and harvest index (Yang and Hwa, 2008). Plant height and days to heading are two traits related to rice's yield potential. Cultivars with shorter plant height can avoid wind and rain damage – avoiding lodging and increasing yield (Lin et al., 2011). Thick rice culm common in shorter plant type contributes to lodging resistance (Duan et al., 2004). Lodging in relation with plant height is one of the key factors limiting the yield potential of both inbred and hybrid rice cultivars, receiving close attention from rice breeders and potentially causing severe yield loss. Anjali et al. (2014) studied genetic diversity of 50 aromatic rice cultivars, noting the diversity among the cultivars in plant height trait, the higher differences in the mean values were observed for plant height.

Tillering is a key component of grain yield and one of the major agronomic traits for grain production since tiller number per plant determines panicle number (Zhu et al., 2011). Tillering (or the degree of branching) determines the shape of rice plants. There are two types of tillers: primary tillers grown from the main stem and secondary tillers grown from primary tillers (Kirby and Appleyard, 1981). Saha et al. (2015) analyzed yield components and the aroma of small-grain aromatic rice and reported the minimum number of tillers per plant observed in the 'Begun Bichi' cultivar and the maximum in the 'Tilkapur' cultivar.

Increasing the percentage of productive tillers per plant plays an important role in determining rice's grain yield. The number of productive tillers is affected by factors such as planting density, temperature, water supply, and light. This number can be determined at the vegetative phase or reproductive phase (Golam et al., 2011), with the important stage being the maximum tiller-number stage (Wang et al., 2007). One study found no observed correlation between aroma and productive tillers among aromatic rice (Mathure et al., 2011). The number of productive tillers per plant is an important trait in determining the diversity among rice plants. Wang et al. (2007) found that the maximum tiller-number stage is the most important stage in identifying the number of panicles. Additionally, Jaiswal et al. (2007) studied variability and association studies in indigenous aromatic rice, reporting the highest genotypic coefficient of variation (GCV) for the number of panicle-bearing tillers and grain yield per plant. A study by Medhi et al. (2004) on the extent of genetic variation in indigenous scented rice cultivars of Assam indicated there was considerable variation among the aromatic rice cultivar for all traits studied; GCV and phenotypic coefficient of variation (PCV) estimates were high for effective tillers, grains per panicle, and grain yield per plant.

The leaf is the organ of photosynthesis, and research has found the flag leaf is a main source photosynthesis product for grain filling. (Prakash et al., 2011). The leaf area, length, width and angle determine the size and shape of a leaf in rice, and there is a high correlation between leaf area and length, as well as between leaf area and width (Peng et al., 2008). Leaf area is an important factor in biometrical observation to evaluate plant growth in the field (Kumar and Sharma, 2010), being measured in experiments on physiological characteristics such as photosynthesis, transpiration, respiration, and plant water consumption. Additionally, a plant's leaf area and leaf number play a significant role in some cultural practices such as irrigation and fertilization (Cirak

et al., 2008). Four modern rice cultivars namely 'Binashail', 'BRRI Dhan32', 'Ukunmadhu' and 'Kataribhough' were found generally had higher Total Dry Matter (TDM), Leaf Area Index (LAI) and Leaf Area Ratio (LAR) (Baset Mia and Shamsuddin, 2011). Davood et al. (2009) showed the rice's flag leaf area could be a key factor in increasing rice grain yield. Mall et al.'s (2005) study estimating genetic variability in rice found highly significant differences and wide variation among the 35 cultivars for many traits, such as flag leaf length and width. Some agronomic traits such as area, size, and shape of the flag leaf affect photosynthesis to a certain extent and thus influence rice yield production (Yue et al., 2006). The flag leaf has played an important role in rice's grain yield by increasing grain weight by 41% to 43% (Yoshida, 1972).

Patil et al. (2009) studied the variability of rice germplasm accessions used for wild rice eradication, finding that genotype showed significant variability for panicle length and the number of filled grains per panicle. Mall et al.'s (2005) study estimating genetic variability in rice found highly significant differences and wide variation among the 35 cultivars for many traits such as the number of panicles per plant, number of spikelets per panicle, and panicle length. Additionally, Pinson (1994) observes that high levels of sterility in Amber F_2 populations might hinder the use of its novel aroma gene in Amber rice. Saha et al. (2015) found significant variation among aromatic rice cultivar in panicle length.

It is important to note that aromatic rice is generally low yielder compared with nonaromatic rice. Rice yield is a quantitative trait influenced by many agronomic and environmental factors. For example, fertility and spikelets per panicle are key components of rice grain yield (Zong et al., 2012), and some studies have shown a correlation between filled grains per panicle and the type of rice (aromatic or non-aromatic). Mathure et al. (2011) studied the characterization of aromatic rice germplasm and the correlation between its agronomic and quality traits. Looking at 88 aromatic cultivars, the authors found that aroma was negatively associated with filled grains per panicle for some of these cultivars and noted would be the best strategy in increasing yield, improving length of panicle and increasing number of productive tillers in medium or mild scented cultivars.

Grain yield per unit area is very low for aromatic rice when compared to non-aromatic rice due to tall plant habit and late maturity, which is the genetic base of rice and is an essential requirement for aromatic rice breeding program. Souroush et al. (2004) conducted genetic and phenotypic variability and cluster analysis for quantitative and qualitative traits of rice, evaluating 36 cultivars and determining the relationship between yield components and grain yield and they found a highly significant difference among the rice cultivar for all traits studied. Chauhan (1996) found substantial genetic variability for grain yield and spikelets per panicle when studying 11 morpho-agronomical characteristics. Additionally, a study by Saha et al. (2015) analyzing yield components and the aroma of small-grain aromatic rice found variations among the genotypes for the number of panicles per plant. Golam et al. (2010) found the highest grain elongation ratio are not same except for two of the aromatic rice after evaluating 10 outstanding genotypes for aroma. The length and width of the rice seed vary, sometimes even within a cultivar (IRRI, 2009).

Thousand-grain weight is also essential in identifying rice cultivar. Individual plants' mass of grains allows for an accurate assessment of a population's yield (Verica et al., 2013). For example, Sarawgi et al. (2009) evaluated 126 rice cultivars on 22 qualitative and quantitative traits such as thousand-grain weight and yield per plant. Aromatic rice cultivars have different yield potential related to a significant number of morphologic traits including 1000 grain weight. Baset et al. (2011) indicated that physio-morphological attributes, yield, and yield-contributing characteristics differed among the aromatic rice cultivar. They found that aromatic fine-rice cultivar had smaller grains, and lower yield, biomass production, and harvest index compared to the modern rice cultivar Dhan32 and Binasail of Bangladesh. Thousand- grain weight is a useful trait in calculating seeding rates and harvest losses (Anonymous, 2007) but it is important in increasing grain yield. Patil et al. (2009) found that genotype showed significant variability for 100- grain weight and grain yield per plant and these traits were correlated.

Millers' preference is for aromatic rice cultivar with high percentage of milling, and they often will pay a premium price for such cultivar. Milling is done by passing clean rough rice through a shelling device to remove the hulls from the grains. A significant number of studies have found a correlation between milling rice or milling quality and characteristics such as grain length, width, and thickness. Zheng et al. (2007) reported that grain-milling quality was negatively associated with grain length and the length-to-width ratio. Another study on paddy, brown, and milled rice grains from 408 rice lines showed a wide range of grain morphology and relationships among milling quality, grain weight, and chalkiness (Xie et al., 2013).

Rice seeds' length and width are the two principal quantitative milling and classification traits. The size and shape of seeds are stable varietal properties that can be used to identify rice cultivar (Rickman et al., 2006), and rice cultivar are -classified as long, medium, or short grain using the rough kernel dimension ratio (Slaton et al., 2000). Sinha et al. (2015) evaluated 55 traditional rice cultivars of West Bengal, and found a wide variation in grain characters, like grain size and shape. The variation of grain length of cultivar ranged from 5.6 to 11.2 mm, grain width from 1.8 to 4 mm, kernel length from 3.95 to 8.3 mm, and kernel breadth from 1.6 to 3.1 mm. The length to width ratio of grain varied from 2.15 to 4.45 while kernel length to width varied from 1.56 to 4.11. In a study on quality characteristics of 12 short-grain scented rice, Kumari et al. (2013) found longest kernel length of 7.07 millimeters in the 'NDR 6265' cultivar and widest

kernel width of 1.81 millimeters in the 'NDR 625' cultivar. In general, it was found that seed length is more variable and important than seed width, thickness, or shape.

Chalkiness is related to grains' quality and is complex, being affected by a broad range of environmental factors such as water, light, and temperature, in addition to genotype. In the marketplace, chalkiness is a major factor in classifying rice. China has two types of chalky rice: the white-belly rice kernel having an opaque area on grains' ventral side and the white-core rice kernel having an opaque area in the grains' center (Qiao et al., 2011). Zhang et al. (2014) indicate the difference in mechanisms of white-belly rice kernels (WBRK) and white-core rice kernels (WCRK) aimed to lower chalky grain rate. In a study on variation in yield and physicochemical quality traits among mutants of the japonica rice cultivar 'Wuyujing', Kumari et al. (2013) reported that the chalky-grain percentage was significantly and negatively correlated with milled rice yield and positive correlated with the degree of milling. Grain chalkiness decreases rice's value in global markets because of grain breakage during milling and its quality as a food product (Ishimaru et al., 2016).

1.2.3. Molecular Diversity in Aromatic Rice

Genetic diversity is required for all crops for improvement of yield, end-use quality and tolerance to biotic and abiotic stress, helping breeders select and develop superior recombinants by providing necessary gene sources associated with these traits (Naik et al., 2006). The amount of genetic diversity within *Oryza sativa* species is an important factor in understanding the evolutionary status of rice species. Studies have shown a distinct difference between the Indica and Japonica rice groups based on molecular markers (Wang and Tanksley, 1989; Nakano et al., 1992). An essential component in germplasm characterization and conservation is an assessment of genetic diversity based on these markers. Studies have shown a distinct difference between the

Indica and Japonica rice groups based on molecular markers (Wang and Tanksley, 1989; Nakano et al., 1992). The Asian cultivated rice species *Oryza sativa L*. is spread and planted in many parts of the world and is more diverse than African cultivated rice, *Oryza glaberrima L*. (Sarla and Swamy, 2005). Several groups of DNA markers can be used in assessment of diversity. For many purposes, diversity and genome mapping, and varietal identification, microsatellites have been used among other DNA markers (Teixeira da Silva, 2005). Tu Anh et al. (2018) studied the phenotypic variation and genetic diversity in 15 rice mutants and four rice cultivars using Simple Sequence Repeat (SSR) markers. They found that the variation among groups was 34%, while the variability of different plant yield characteristics in rice found that yield traits can be detected by their correlation with grain yield in aromatic rice and that it can be helpful to understand and find higher aromatic rice cultivar (Tahir et al., 2002). However, the Asian rice species *Oryza sativa L*. is spread and planted in many parts of the world and is more diverse than *Oryza glaberrima L*. (Sarla and Swamy, 2005).

Day to flowering is mostly affected by genetic diversity, which has created a broad difference in this trait among aromatic rice cultivar. Patil et al.'s (2009) study on the variability among rice accessions used in wild rice found that genotype exhibited significant variability for days to 50% flowering, flag leaf length, and plant height. A study estimating the genetic variability in rice found highly significant differences and wide variation among the 35 cultivars for plant height and days to 50% flowering (Mall et al., 2005). Many studies have reported on different genes' effect on heading time in rice (Okumoto and Tanisaka, 1997), with this trait being controlled by many identified genes such as Se-1-Se-7 and Ef-1 (Poonyarit et al., 1989).

The plant height of rice is regulated by many genes and affected by the environment. A thorough understanding of the system mechanism of plant height has helped breeding programs develop shorter, and high-yielding rice (Ashikari et al., 2005). The International Rice Research Institute (1967) reported in the early 1960s the sd-1 gene, first identified in the Chinese cultivar Dee-geo-woo-gen (DGWG) and crossed with Peta (tall phenotype) to develop the semi-dwarf cultivar IR8, the miracle rice that triggered the rice green revolution. Most aromatic rice are tall and may the not have sd-1 gene.

Tillering is one of the traits highly correlated with yield. Diversity among agronomic traits is related to plants' genomic diversity, and the genetic explanation behind tiller number has become a focus in rice genetic and breeding research (Liu et al., 2010). Tillers of different cultivars show various spatial orientations at different stages, giving rise to morphologically different plant types or architectures. Liu et al. (2017) reported that ORF4 was a strong candidate gene for *ts1* and *ts1* might play a role in regulating rice tillering through MOC1 and HTD1 associated pathway. Ten years ago, a series of EMS induced reduced culm number (rcn) mutants and their responsible genes have been described in rice. The gene RCN8 have been mapped on the short arm of chromosome 6 and the gene RCN9 have been mapped on the long arm of chromosome 1 (Jiang et al., 2006). Mall et al. (2005) reported highly significant differences and wide variation among 35 cultivars for the number of tillers per plant.

The literature has not definitively identified how leaf area, size, and shape are controlled from the perspective of leaf development (Moon and Hakes, 2011). Some genes associated with leaf size and shape have been determined via map-based cloning in rice plants. A genome-wide association study (GWAS) was performed for 29 leaf traits related to leaf size, shape, and colour at three growth stages using high-throughput leaf scoring (HLS) on a panel of 533 rice cultivars, and 9 associated loci contained known leaf-related genes, such as Nal1 for controlling the leaf width, and a total of 73 and 177 new loci were detected for traits associated with leaf size and leaf shape, respectively (Yang et al., 2015). Previous studies (Farooq, et al., 2010; Wang et al., 2011; Yue et al., 2006) showed some quantitative trait loci (QTL) have been mapped for leaf size and leaf shape.

Through linkage mapping in the recombinant inbred line population derived from a cross between the cultivars Xiushui79 (short panicle) and C-bao (long panicle), four QTLs for panicle length (PL) were detected on chromosomes 4, 6, and 9. Also, ten SSR markers associated with panicle length were detected on chromosomes 2, 3, 5, 6, 8, 9, and 10 in the natural population consisting of 540 cultivars (Liu at el., 2016). Qiao at el. (2008) found four QTLs for panicle length located on chromosomes 1, 3, 5, and 10, which explained 6.8 to17.8 percentage of the phenotypic variances. Yamamoto at el. (2001) found six QTLs for culm length, four QTLs for days to heading and four QTLs panicle length were detected for the traits.

One major contributor to genetic divergence in rice is the aroma of milled rice. However, rice fragrance (aroma) demonstrates a complex inheritance pattern. Because aroma is a polygenic quantitative trait significantly affected by environment, it is difficult to identify genes that determine it (Pachauri et al., 2010). The BADH2 gene located on chromosome 8 in aromatic rice is significantly associated with aroma, and the BADH1 gene located on chromosome 4 may have same biochemical function and play a role in expressing aroma in rice (Singh et al., 2010). However, some genetic studies on the inheritance of aroma in rice have shown that a recessive gene or genes control aroma (Dong et al., 2000). Pinson (1994) discusses how aroma is controlled by a single recessive gene in Jasmine 85 and PI 457917 and controlled by two genes in Amber rice and Dragon Eyeball 100. Additionally, Lin (1991) reported that digenic segregation for aroma.

1.2.4. Evaluating Aroma in Rice

Aroma is of great interest to rice breeders and there are various techniques to identify and estimate aroma. Aroma in rice can be detected in different parts of the plant such as seeds, stems and leaves. Historically, chewing grains has been used to evaluate rice's aroma (Dhulappanawar, 1976). Nagaraji et al. (1975) used a simple technique, heating leaf tissue in water, to note the aroma. A popular laboratory method used to estimate aroma is by cooking milled rice from an individual plant or line (Choudhury and Ghosh, 1978; Ghose and Butany, 1952). Dehulled rice grain treated with KOH and snipped by a panel is another laboratory method to evaluate aroma. Using test panels, detached leaves enhanced by KOH, dehulled mature rice seeds enhanced by KOH, and smelling small test tube-cooked rice samples were used to test aroma in rice (Berner and Hoff, 1986; Sood and Siddiq, 1978). The rice leaf tissues of aromatic plants have characteristic aromas (Nagaraji et al., 1975; Leung et al. 1998) and can be used to detect aroma compounds in the tissue of young rice plants. Gas chromatography-mass spectrometry (GC-MS) is one of the most important and accurate methods to estimate aromatic substances. This instrumental technique is used to evaluate 2-acetyl-1-pyrroline (2AP) in aromatic rice. The aroma in scented rice depends on the concentration of 2AP in milled rice grain, and it varies with genetic and environmental conditions (Nadaf et al., 2006). More than 100 different volatile compounds have been associated with aromatic rice (Yajima et al., 1979), with 2AP being the major compound distinguishing aromatic from non-aromatic rice (Lorieux et al., 1996).

1.2.5. Genetics, Inheritance of Aroma

Aroma in grain rice was reported to be control by a single recessive gene (Huang et al., 1994; Sood and Siddiq, 1978; Dong et al., 2000;). However, aroma in grain rice was also reported to be controled by a dominant gene (Jodon, 1944), or found to be digenic or trigenic

(Dhulappanavar, 1976; Kadam and Patankar, 1938; Nagaraju et al., 1975; Reddy and Sathyanarayanaiah, 1980; Lin, 1991)). Aroma is controlled by two genes in Amber and Dragon Eyeball, and by a single recessive nuclear gene in Jasmine 85 and PI467917 and reported by Pinson (1994). The *fgr* gene is responsible for rice fragrance and it was reported by Chen et al. (2006). Mapping study of Chen et al. (2006) and Amarawathi et al. (2008) identified recessive allele (badh2) on chromosome 8 as a candidate gene for aroma. Many of the aromatic rice cultivars from different isozyme groups share the same 8 bp deletion in intron 7 of badh2 gene (Bradbury et al., 2005). Another BADH1 gene located on chromosome 4 was reported to have contributory role in aroma expression in rice (Singh et al., 2010). Nayak and Acharya (2004) indicated that the inheritance of aroma in rice is regulated between various genetic interactions and the environment.

1.2.6. Mapping Quantitative Trait Loci and Associated Genes

Genetic differences for a quantitative trait might be controlled by one or few major genes or by the collective effects of many genes known minor genes or quantitative trait loci (QTLs). Using only traditional phenotypic evaluation, it is challenging to identify QTLs without linked DNA markers. Molecular markers help identify QTLs and is essential to mapping the genome of plants and thus improving crops through breeding programs (Asins, 2002).

Mapping QTL is a common strategy for discovering the genes associated with many important quality-related traits for rice. Plant breeders used different methods to develop populations for QTL mapping, such as double haploid lines (DHLs), backcross (BC), F₂ population, and recombinant inbred lines (RILs). RILs are inbred generation derived by selfing individual F₂. Generation advancement in RIL development is through single seed descent plants and further. In general, RILs are used in breeding programs to develop traditional QTL mapping population that can be re-evaluated as needed. The key advantage of RILs is their ability to produce

homozygous lines and reproduce without genetic change occurring in the population. Association mapping and linkage analysis are the two most commonly used methods for QTL mapping. A common strategy of QTL mapping is to use recombinant inbred lines (RILs), which are usually established by many generations of inbreeding derived from F_1 population to F_6 or F_7 populations (Takuno et al., 2012).

One of this study's main objectives is to construct a linkage map based on single nucleotide polymorphisms (SNPs) for aromatic rice. Genotyping-by-sequencing (GBS) is a novel application used to sequence multiplexed samples that combine molecular marker discovery and genotyping. It offers a highly simplified library production procedure more amenable to use with larger numbers of individuals (Elshire et al., 2011). He et al. (2014) write that GBS has been used successfully for the study of genomic diversity, genetic linkage analysis, molecular marker discovery, and genomic selection in plant breeding programs. SNP genotyping technologies use expensive reagents and detection equipment but promise accurate and high-throughput results (Hayashi et al., 2004). However, several researchers have developed a number of inexpensive allele-specific SNP genotyping assays (Ye et al., 2001; Soleimani et al., 2003; Zhang et al., 2003; Chiapparino et al., 2004; Bundock et al., 2006).

Many mapping studies have been conducted in recent years to identify QTLs for aroma (Ahn et al., 1992; Tian et al., 2005; Amarawathi et al., 2008; Ahamadi et al., 2008). In 1992, the first mapping of grain aroma gene in rice took place (Ahn et al., 1992); 12 years later, scientists identified the gene controlling 2AP that was responsible for grain aroma (Vanavichit et al., 2004; 2005). The 2AP accumulation in aromatic rice phenotype could be regulated exclusively by genes or by a combination of genes and environmental conditions. Genetic mappings of grain aroma of aromatic rice were reported as a qualitative trait based on many sensory tests. Aromatic compounds

from various aromatic rice cultivars specifically the amount of 2-Acetyl-1-pyrroline (2AP) differed quantitatively (Fitzgerald et al., 2008; Goufo et al., 2010). The aroma of rice consists of more than 200 aromatic compounds such as 2-acetyl-1-pyrroline (2AP), acids, esters, phenols, pyridines, hydrocarbons, alcohols, aldehydes, ketones, pyrazines, and other compounds (Maga, 1984; Paule and Power, 1989). Several studies mentioned the major QTL mapped on chromosome 8 that coincided with the chromosome 8 location of badh2 gene based on sensory test data (Chen et al. 2006; Lorieux et al., 1996). However, the two minor QTLs were identified on chromosomes 4 and 12 (Lorieux et al., 1996).

Genetic diversity has created a broad difference in days to heading among rice cultivar. Wei et al. (2010) found DTH8, a QTL that regulates days to flowering and plant height in rice. Nemoto et al. (2016) reported biological interactions between Ghd7 and Hd1, which together repress early heading date 1 (Ehd1). Many genes affected cell division, and the elongation and development of apical meristem have higher effect on plant height. Zhu et al. (2008) found that a number of QTL alleles affecting stem length, strength, and thickness in rice are related to lodging resistance. Leaf area and the number of leaves determine a plant's photosynthetic potential and play major roles in determining plants' grain yield, stress responses, and disease resistance (Pérez-Pérez et al., 2010). QTLs for flag leaf size traits have also been mapped in chromosome 4 ,6, 9,12 in diverse populations, such as F_2 , DHLs, and RILs (Wang et al., 2009; Jiang et al., 2010).

The number of grains per panicle is one of the major components determining grain yield. Saha et al. (2015) found significant variation among aromatic rice cultivar in terms of the number of grains per panicle. Studies have found three QTLs controlling grain number per panicle in rice (Liu et al., 2010; Ahamadi et al., 2008). Spikelets per panicle QTLs have been mapped and found on chromosomes 5 and 10 (Tan et al., 2008). Hori et al. (2012) found two QTLs with significant

genetic effects on grain chalkiness, detected on the long arms of chromosomes 8 and 11.

1.3. References

- Abacar, J.D., L. Zhao-miao, Z, Xin-cheng, D. Cheng-qiang, T. She, L. Zheng-hui, W. Shao-hua, and D. Yan-feng. 2016. Variation in yield and physicochemical quality traits among mutants of Japonica rice cultivar Wuyujing 3. Rice Sci. 23: 33–41.
- Ahamadi, J., M.H. Fotokian, and S. Fabriki-Orang. 2008. Detection of QTLs influencing panicle length, panicle grain number and panicle grain sterility in rice (Oryza sativa L.). J Crop Sci Biotech. 11(3): 163-170.
- Ashikari, M., H. Sakakibara, S. Lin, T. Yamamoto, T. Takashi, A. Nishimura, E.R. Angeles, Q. Qian, H. Kitano, and M. Matsuoka. 2005. Cytokinin oxidase regulates rice grain production. Science 309: 741-745.
- Anjali, K., S. Vanisree, Ch. Surender Raju, and M. Sreedhar. 2014. Genetic diversity studies in aromatic rice (Oryza Sativa L.) germplasm. Madras Agric. J. 101 (7-9): 207-211.
- Anonymous, 2007. Albert Agriculture and Food; Using 1,000 Kernel Weight for Calculating Seeding Rate and Harvest Losses; Available on line at http://www1.agric.gov.ad/\$department/deptdocs.nfs/all/agdex8.
- Ahn, S. N., C.N. Bollich, and S.D. Tanksley. 1992. RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84: 825–828.
- Amarawathi, Y., R. Singh, A. K. Singh, V.P. Singh, T. Mohapatra, T.R. Sharma, and N.K. Singh. 2008. Mapping of quantitative trait loci for basmati quality traits in rice. Mol. Breed. 21: 49-65.
- Asins, M. 2002. Present and future of quantitative trait locus analysis in plant breeding. Plant Breed. 121: 281–291.
- Baset Mia, M.A. and Z.H Shamsuddin. 2011. Physio-morphological appraisal of aromatic fine rice (Oryza sativa L.) in relation to yield potential. Inter. J. Bot.7: 223-229.
- Berner, D.K. and B. J. Hoff. 1986. Inheritance of scent in American long grain rice. Crop Sci. 26: 876-878.
- Bisne, R. and A.K. Sarawgi. 2008. Agro-morphological and quality characterization of badshahbhog group from aromatic rice germplasm of Chhattisgarh. Bangladesh J. Agr. Res. 33(3): 479-492.

- Bundock P.C., M.J. Cross, F.M. Shapter, and R.J. Henry. 2006. Robust allele-specific polymerase chain reaction markers developed for single nucleotide polymorphisms in expressed barley sequences. Theor. Appl. Genet. 112: 358-65.
- Bullard, R.W. and G. Holguin. 1977. 'Volatile components of unprocessed rice (Oryza sativa)'. J. Agric. Food Chem. 25: 99.
- Bradbury, L.M.T., T.L. Fitzgerald, R.J. Henry, Q. Jin, and D.L.E. Waters. 2005. The gene for fragrance in rice. Plant Biotechnol. J. 3: 363–370.
- Chen, S., J. Wu, Y. Yang, W. Shi and M. Xu. 2006. The *fgr* gene responsible for rice fragrance was restricted within 69kb. Plant Sci. 171: 505-514.
- Chakravarthy, B.K. and R. Naravaneni. 2006. SSR marker based DNA finger printing and diversity study in rice (Oryza Sativa L.). African J. Biotech. 5(9): 684-688.
- Cirak, C., M.S. Odabas, A.K. Ayan, B. Saglam, and K. Kevseroglu. 2008. Estimation of leaf area in selected Hypericum species. Acta Bot. Hun., 50(1–2): 81–91.
- Chiapparino E., D. Lee, and P. Donini. 2004. Genotyping single nucleotide polymorphisms in barley by tetra-primer ARMS-PCR. Genome 47: 414-20.
- Choudhury, D. and A.K. Ghosh. 1978. Evaluation of agronomic and physiochemical characteristics of five scented rice varieties. Indian J. Agric. Sci...48: 573-528.
- Davood, B.T., G. Ali, A. P. Hemmal, and N. Morteza. 2009. Flag feaf morphophysiological response to different agronomical treatment in a promising line of rice (Oryza sativa L.). American-Eurasian J. Agric and Environ. Sci, 5(3): 403-408.

Dhulappanavar, C.V. 1976. Inheritance of scent in rice. Euphytica. 25: 659-662.

- Dong, J.Y., E. Tsuzuki, and H. Terao. 2000. Inheritance of aroma in four rice cultivars (Oryza sativa L.). IRRI. International Rice Research Notes 25:2.
- Duan, C.R., B.C. Wang, P.Q. Wang, D.H. Wang, and S.X. Cai. 2004. Relationship between the minute structure and the lodging resistance of rice stems. Colloid Surface B 35: 155-158.
- Dela-cruz, N. and G. S. Khush. 2000. Rice grain quality evaluation procedures. In R. K. Singh, U. S. Singh, & G. S. Khush (Eds.), Aromatic Rice (pp. 15–28). New Delhi, Calcutta: Oxford and IBH Publishing Co. Pvt. Ltd.
- Diako, C., E. Sakyi-Dawson, B. Bediako-Amoa, F. K. Saalia, and J. T. Manful. 2010. Consumer perceptions, knowledge and preferences for aromatic rice types in Ghana. Nat. Sci. 8(12): 12-19.

- Doyle, J.J. and J.E. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.
- Efferson, J.N. 1985. 'Rice quality in world markets'. In: Rice Grain Quality and Marketing: Papers presented at the International Rice Research Conference 1-5 June 1985, Manila: International Rice Research Institute.
- Elshire, R. J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, and S. E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoSONE 6: e19379. doi:10.1371/journal.pone.0019379
- Farooq, M., A.G. Tagle, R.E. Santos, L.A. Ebron, D. Fujita, and N. Kobayashi. 2010. Quantitative trait loci mapping for leaf length and leaf width in rice cv. IR64 derived lines. J. Integr. Plant Bio. 52: 578–584.
- Fitzgerald, M. A., N. R. S. Hamilton, M. N. Calingacion, H. A. Verhoeven, and V. M. Butardo. 2008. Is there a second fragrance gene in rice? Plant Biotech. J. 6: 416–423.
- Food and Agriculture Organization, FAO .2000. Rice Information Volume 2, January.
- Fitzgerald, M.A., N.R.S. Hamilton, M.N. Calingacion, H.A. Verhoeven, and V.B Butardo. 2008. Is there a second fragrance gene in rice? Plant Biotech. J. 6: 416–423.
- Garris, A. J., T. H. Tai, J. Coburn, S. Kresovich, and S. McCouch. 2005. Genetic structure and diversity in Oryza sativa L. Genetics 169: 1631–1638.
- Ghose, R.L.M. and W.T. Butany. 1952. Studies of the Iinheritance of some characteristics in rice (Oryza sativa L.). Indian J.Genet.Plant Breed. Vol. 12: 26-30.
- Golam, F., Y.Y. Hui, A. Masitah, N. Afnierna, A. M. Nazia, K. Norzulaani, and O. Mohamad. 2011. Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment, Aust. J. Crop Sci. 5(11): 1318-1325.
- Golam, F., K. Norzulaani, A. H. Jennifer, B. Subha, M. Zulqarnain, M. Osman, A. M. Nazia, M. Zulqarnian, and O. Mohammad. 2010. Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment. Afr. J. Agr.c. Res. 5(12): 1515-1522.
- Goufo, P., S. Wongpornchai, and X. Tang. 2010. Decrease in rice aroma after application of growth regulators. Agron.r Sustain. Dev.t doi:10.1051/agro/2010011.
- Hayashi K, N. Hashimoto, M. Daigen, and I. Ashikawa. 2004. Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. Theor. Appl. Genet. 108: 1212-20.

- He, J., X. Zhao, A. Laroche, Z. X. Lu, H. Liu, and Z. Li. 2014. Genotypingby-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front. Plant Sci. 5:484. doi: 10.3389/fpls.2014.00484
- Hori, K., T. Kataoka, K. Miura, M. Yamaguchi, N. Saka, T. Nakahara, Y. Sunohara, K. Ebana, and M. Yano. 2012. Variation in heading date conceals quantitative trait loci for other traits of importance in breeding selection of rice. Breed. Sci. 62: 223–234.
- Hossain, M. F., M. S. U. Bhuiya, M. Ahmed, and M. H. Mian. 2009. Effect of harvesting time on the milling and physiochemical properties of aromatic rice. J. Agr. Sci., 42 (20): 91-96.
- Hossain, M. F., M. S. U. Bhuiya, and M. Ahmed. 2005. Morphological and agronomic attributes of some local and moden aromatic rice varieties. Oryza, 34(3): 201-208.
- Huang, N., S. R. McCouch, T. Mew, A. Parco, and E. Guiderdoni. 1994. A rapid technique for scent determination in rice. Rice Genet. Newsl. 11: 134–137.
- International Rice Research Institute, IRRI. 2009. Introduction to Seed Management; Available at http://www.knowledgebank.irri.org/qualityseed.
- International Rice Research Institute. 1972. Annual Report for 1971. Los Baños, Philippines. 238 p.

International Rice Research Institute. 1967. Annual Report for 1966, 59-82.

- Ishimaru, T., H. Hirabayashi, K. Sasaki, C. Ye, and A. Kobayashi. 2016.Breeding efforts to mitigate damage by heat stress to spikelet sterility and grain quality. Plant Prod. Sci. 19: 12–21.
- Jaiswal, H. K., A. K. Shrivastava, and A. Dey. 2007. Variability and association studies in indigenous aromatic rice. Oryza, 44(4): 351-353.
- Jiang, S.K., X.J. Zhang, C. Huang, Y.N. Xing, Z.J. Xu, and W.F. Chen. 2010. Comparison of genetic linkage map and QTLs controlling flag leaf traits based on F2 and F2:6 populations derived from japonica rice. Chin. J. Rice Sci. 24: 372-378.
- Jiang, H., L. Guo, D. Xue, D. Zeng, G. Zhang, G. Dong, M. Gu, and Q. Qian. 2006. Genetic analysis and gene-mapping of two reduced-culm-number mutants in rice. J. Integr. Plant Biol. 48(3): 341-347.
- Jodon, N. E. 1944. The inheritance of flower fragrance and other character in rice. J.Amer. Soc. Agron. 27: 910–921.
- Kadam, B. S. and V. K. Patankar. 1938. Inheritance of aroma in rice. Indian J.Genet. and Breed. 40: 327–329.

- Khush, G. S., C. M. Paule, and M. N. DeLa Cruz. 1979. Rice quality evaluation and improvement at IRRI. In: Proceedings of the workshop on chemical aspects of rice grain quality, International Rice Research Institute. Los Banos, Laguna, Philippines: 21-31.
- Kirby, E. J. M. and M. Appleyard. 1981. Cereal development guide. Cereal Unit, National Agricultural Centre, Stoneleigh, UK.
- Kumar, R. and S., Sharma. 2010. Allometric model for nondestructive leaf area estimation in clary sage (Salvia sclarea L.). Photosynthetica, 48(2): 313–316.
- Kumari, S., R.N. Kewat, R.P. Singh, and P. Singh. 2013. Studies of quality characteristics in short grain scented rice (Oryza sativa L.) varieties accessions. Trends Biosci., 6 (2): 177-179.
- Kobayashi, S., Y. Fukuta, S., Morita, T., Sato, M., Osaki, and G. S. Khush. 2003. Quantitative trait loci affecting flag leaf development in rice (Oryza sativa L.). Breed. Sci. 53 (3): 255-262.
- Kohnaki, M.E., G. Kiani, and G. Nematzadeh. 2013. Relationship between morphological traits in rice restorer lines at F3 generation using multivariate analysis. Int. J. Adv. Biol. Biomed. Res. 1(6): 572-577.
- Leung, A., J.D. Craske, and M. Wootton. 1998. Volatile aroma compounds in green tissues of Australian rice plants at different stages of maturity and different levels of nitrogen fertilization. 48th Australian Cereal Chemistry Conference: 278-281.
- Lorieux, M., M. Petrov, N. Haung, E. Guiderdoni, and A. Ghesquiere. 1996. Aroma in rice: Genetic analysis of a quantitative trait. Theor. Appl. Genet. 93: 1145-1151.
- Lin, S. 1991. Rice Aroma: Methods of Evaluation and Genetics. In: Rice Genetics II, (Eds.) International Rice Research Institute, Manila, Philippines.
- Liu L., F. Meng, Y. He, M. Zhu, Y. Shen, and Z. Zhang. 2017. Fine mapping and candidate gene analysis of the tiller suppression gene ts1 in rice. PLoS ONE 12(1): e0170574. doi:10.1371/journal.pone.0170574.
- Liu, E., Y. Liu, G. Wu, S. Zeng, T. G. Tran Thi, L. Liang, Z. Dong, D. She, H. Wang, I.U. Zaid, and D. Hong. 2016. Identification of a candidate gene for panicle length in rice (Oryza sativa L.) via association and linkage analysis. Front. Plant Sci. 7: 596. <u>http://doi.org/10.3389/fpls.2016.00596</u>
- Liu, G.F., H.T. Zhu, S.W. Liu, R.Z. Zeng, Z.M. Zhang, W.T. Li, X.H. Ding, F.M Zhao, and G.Q. Zhang. 2010. Unconditional and conditional QTL mapping for the developmental behavior of tiller number in rice (*Oryza sativa L*.). Genetica.138 (8): 885-893.

- Liu, T., D. Shao, and M.R. Kovi. 2010. Mapping and validation of quantitative trait loci for spikelets per panicle and 1,000-grain weight in rice (*Oryza sativa L*.). Theor. Appl. Genet. 120: 933-942.
- Maga, J. A. 1984. Rice product volatile: a review. J. Agr.Food Chem. 32: 924–970.
- Mathure, S., A. Shaikh, N. Renuka, K. Wakte, N. Jawali, R. Thengane, and A. Nadaf. 2011. Characterization of aromatic rice (Oryza sativa L.) germplasm and correlation between their agronomic and quality traits. Euphytica 179(2): 237-246.
- Mathure, S., N. Jawali, and A. Nadaf. 2010. Diversity analysis in selected non-basmati scented rice collection (Oryza sativa L.). Rice Sci. 17(1): 35–42.
- Mall, A.K., J.D.P. Babu, and G.S. Babu. 2005. Estimation of genetic variability in rice. J. Maharashtra Agr. Univ. 30(2): 166-168.
- Medhi, K., P. Talukdar, P. K. Barua and I. Baruah. 2004. Extent of genetic variation in indigenous scented rice varieties of assam. Indian J. Plant Genet. Resour 17(1): 27-29.
- Miah, G., M.Y. Rafii, M.R. Ismail, A.B. Puteh, H.A. Rahim, R. Asfaliza, and M.A. Latif. 2013. Blast resistance in rice: A review of conventional breeding to molecular approaches. Mol. Biol. Rep. 40: 2369-2388.
- Moon, J and S. Hake. 2011. How a leaf gets its shape. Curr. Opin. Plant Biol. 14:24-30.
- Murai, M. and T. Kinoshita. 1986. Diallel analysis of traits concerning yield in rice. Jpn. J. Breed. 36: 7-15.
- Nagaraji, M., D. Chaudhry, and M.J.B.K. Rao. 1975. A simple technique to identify scent in rice and inheritance pattern of scent. Curr. Sci. 44: 599-602.
- Naik, D., A. Sao, S.K. Sarawagi, and P. Singh. 2006. Genetic divergence studies in some indigenous scented rice (Oryza sativa L.) accessions of Central India. Asian J Plant Sci. 5: 197-200.
- Nakano, M, A. Yoshimura, and N. Iwata. 1992. Phylogenetic study of cultivated rice and its wild relatives by RFLP. Rice Genet. Newsl. 9: 132-134.
- Napasintuwong, O. 2012. Survey of Recent Innovations in Aromatic Rice. 131th EAAE Seminar Innovation for Agricultural Competitiveness and Sustainability of Rural Areas, Prague, September 18-19.
- Nemoto, Y., Y. Nonoue, M. Yano, and T. Izawa. 2016. Hd1, a CONSTANS ortholog in rice, functions as an Ehd1 repressor through interaction with monocot-specific CCT-domain protein Ghd7. Plant J. 86: 221–233. doi: 10.1111/tpj.13168

- Pachauri, V., M.K. Singh, A.K. Singh, S. Singh, N.A. Shakeel, V.P. Singh, and N.K. Singh. 2010. Origin and genetic diversity of aromatic rice varieties, molecular breeding and chemical and genetic basis of rice aroma. J Plant Biochem. Biotechnol. 19(2): 127-143.
- Paterson, A.H., M. Freeling, and T. Sasaki. 2005. Grains of knowledge: genomics of model cereals. Genome Res. 15: 1643-1650.
- Patil, S. G., V. N. Sahu, and P.A. Deokar. 2009. Study of variability of rice germplasm accessions used for wild rice eradication. Inter. J. Plant Sci. Muzaffarnagar, 4(2): 535 537.
- Paule, C. M. and J. J. Powers. 1989. Sensory and chemical examination of aromatic and nonaromatic rices. J.Food Sci. 54: 343–346.
- Peng, S., S. G. Khush, P. Virk, Q. Tang, and Y. Zou. 2008. Progress in ideotype breeding to increase rice yield potential. Field Crop Res. 108: 32-38.
- Pérez-Pérez, J.M., D. Esteve-Bruna, and J.L. Micol. 2010. QTL analysis of leaf architecture. J.Plant Res. 123: 15–23.
- Prakash, M., A. Anandan, and S. B. Kumar. 2011. Varietal variations in flag leaf area and yield in mutant lines of PY5 rice. Karmataka J. Agric. Sci. 24(4): 525-526.
- Pinson, S. R. M. 1994. Inheritance of aroma in six rice cultivars. Crop Sci., 34: 1151-1157.
- Poonyarit, M., D.J. Mackill, and B.S. Vergara. 1989. Genetics of photoperiod sensitivity and critical day length in rice, Crop Sci. 29: 647-652.
- Qiao, J., Z. Liu, S. Deng, H. Ning, X. Yang, Z. Lin, G. Li, Q. Wang, S. Wang, and Y. Ding. 2011.Occurrence of perfect and imperfect grains of six japonica rice cultivars as affected by nitrogen fertilization. Plant Soil, 349: 191-202.
- Qiao B., X. Zhu, Y. Wang, and D. Hong. 2008. Mapping QTL for three panicle exsertion-related traits in rice (Oryza sativa L.) under different growing environments. Acta Agron. Sin. 34: 389–396.
- Rabbani, M., Z. Pervaiz, and M. Masood. 2008. Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (Oryza sativa L.) using RAPD markers. Electronic J. Biotechnol. 11(3).

http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v11n3-3/11

Reddy, P. R. and K. Sathyanarayanaiah. 1980. Inheritance of aroma in rice. Indian J. Plant Breed. 40: 327.

- Rickman, J. F., M. Bell, and D. Shires. 2006. Seed Quality. Available at http// Www.knowledgebank.irri.org.
- Robin, A. H. K. and P. S. Saha. 2015. Morphology of lateral roots of twelve rice cultivars of Bangladesh: dimension increase and diameter reduction in progressive root branching at the vegetative stage. Plant Root.9: 34-42.
- Saha, P., M. Islam, M. Islam, and M. Salam, 2015. Analysis of yield components and aroma of small grain aromatic rice (Oryza sativa L.) in Bangladesh. The Agriculturists 13(2): 17-24.
- Sarawgi, A. K., A. Tirkey, and R. K. Verma. 2009. Evaluation studies for qualitative quantitative characters in indigenous rice germplasm, Poster presented In: National seminar on designing crops for the changing climate, October 30-31, Ranchi, Birsa Agriculture University: 27-28.
- Sarla, N. and B.P.M. Swamy. 2005. Oryza glaberrima: a source for improving Oryza sativa. Curr. Sci. 89 (6): 955-963.
- Selvaraj, C.I., P. Nagarajan, K. Thiyagarajan, M. Bharathi, and R. Rabindran. 2011. Genetic parameters of variability, correlation and path-coefficient studies for grain yield and other yield attributes among rice blast disease resistant Genotypes of rice (Oryza sativa L.). Afr. J. Biotechnol. 10(17): 3322-3334.
- Sinha, A.K., G.K. Mallick, and P.K. Mishra. 2015. Diversity of grain morphology on traditional rice varieties (Oryza sativa L.) of lateritic region of West Bengal. World J. Agric. Sci. 11: 48-54.
- Singh, A., P.K. Singh, R. Singh, A. Pandit, A.K. Mahato, D.K. Gupta, K. Tyagi, A.K. Singh, N.K. Singh, and T.R. Sharma. 2010. SNP haplotypes of the BADH1 gene and their association with aroma in rice (Oryza sativa L.) Mol. Breed. 26: 325–338.
- Slaton, N., K. Moldenhauer, J. Gibbons, M. Blocker, C. Wilson Jr., R. Dilday, J. Robinson, and B. Koen. 2000. Grain Characteristics of Rice Varieties. In: Cooperative Extension Service Rice Information, University of Arkansas. 1-8.
- Soleimani, V.D., B.R. Baum and D.A. Jhonson .2003. Efficient validation of single nucleotide polymorphisms in plants by allele-specific PCR, with an example from barley. Plant Mol. Biol. Rep. 21: 281-288.
- Sood, B.G and E.A. Siddiq. 1978. A rapid technique for scent determination in rice. Indian J. Gen. Plant Breed. 38: 268-271.
- Sourosh, H. R., M. Mesbah, A. Hossainzadeh, and R. Bozorgipour. 2004. Genetic and phenotypic variability and cluster analysis for quantitative and qualitative traits of rice. Seed Plant. 20(2): 167-182.

- Talukdar P.R., S. Rathi, K. Pathak, S. Chetia, A.R. Baruah, and R.N. Sarma. 2017. QTL analysis in aromatic rice of Assam, India. J. Rice Res. 5: 186. doi:10.4172/2375-4338.1000186.
- Tan, L., P. Zhang, F. Liu, G. Wang, S. Ye, Z. Zhu, Y. Fu, H. Cai, and C. Sun. 2008. Quantitative trait loci underlying domestication and yield-related traits in an Oryza sativa x Oryza rufipogon advanced backcross population. Genome. 51: 692-704.
- Tahir, M., D. Wandan, and A. Zada. 2002. Genetic variability of different plant yield characters in rice. Sarhad J. Agric. 18(2): 22-27.
- Takuno S., R. Terauchi, and H. Innan. 2012. The power of QTL mapping with RILs. PLoS ONE 7(10): e46545. doi:10.1371/journal.pone.0046545.
- Teixeira da Silva, J.A. 2005. Molecular markers for phylogeny, breeding and ecology in agriculture. In: Thangadurai, D., Pullaiah. T., and Tripathy, L. (Eds) Genetic Resources and Biotechnology, Regency Publications, New Delhi, India (Vol III): 221-256.
- Tu Anh, T.T., T.D. Khanh, T.D. Dat, and T.D. Xuan. 2018. Identification of phenotypic variation and genetic diversity in rice (Oryza sativa L.) mutants. Agriculture. 8 (2): 30.

United States Department of Agriculture (USDA).2018. https://apps.fas.usda.gov/psdonline/circulars/production.pdf.

United States Department of Agriculture (USDA).2016. https://ipad.fas.usda.gov/highlights/2016/12/Iraq/Index.htm.

- Vanavichit, A., W. Kamolsukyurnyong, S. Wanchana, S. Wongpornchai, S. Ruengphayak, T. Toojinda, and S. Tragoonrung. 2004. Discovering genes for rice grain aroma. In: Proc. 1st International Conference on Rice for the Future, 31 August–3 September, 2004:71–80. Kasetsart University, Bangkok, Thailand.
- Vanavichit, A., S. Tragoonrung, T. Theerayut, S. Wanchana, and W. Kamolsukyunyong. 2005. Transgenic rice plants with reduced expression of Os2AP and elevated levels of 2-acetyl-1-pyrroline. United States Patent, Patent No. US 7,319,181 B2.
- Verica, I., M. Natalija, A. Dobre, and A. Danica. 2013. Inheritance of grain weight per plant in rice, Sci. Technol. 3(6): 29-33.
- Wang, Z.Y. and S.D. Tanksley. 1989. Restriction fragment length polymorphism in Oryza sativa L. Genome. 32: 1113-1118.
- Wang, F., F. Cheng, and G. Zhang. 2007. Difference in grain yield and quality among tillers in rice genotypes differing in tillering capacity. Rice Sci. 14(2): 135-140.

- Wang, Y., L.R. Cheng, T.Q. Zheng, Y. Sun, Z. Zhou, J. Yang, Z.J. Xu, and J.L. Xu. 2009. Response of main effect QTL for plant height and flag leaf width to artificial selection in rice. Chin. J. Rice Sci. 23: 363-370.
- Wang, P., G. Zhou, H. Yu, and S. Yu. 2011. Fine mapping a major QTL for flag leaf size and yield–related traits in rice. Theor. Appl. Genet. 123: 1319–1330.
- Wei, X., J. Xu, H. Guo, L. Jiang, S. Chen, C. Yu, Z. Zhou, P. Hu, H. Zhai, and J. Wan. 2010. DTH8 suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiol. 153: 1747–1758.
- Xie, L., S. Tang, N. Chen, J. Luo, G. Jiao, G. Shao, X. Wei, and P. Hu. 2013. Rice grain morphological characteristics correlate with grain weight and milling quality. Cereal Chem. 90: 587-593.
- Yajima, I., T. Yanai, M. Nakamura, H. Sakakibara, and T. Habu. 1979. Volatile flavor components of cooked rice kaorimai (scented rice, O. sativa japonica). Agr. Bio. Chem.: 2425-2429.
- Yamamoto, T, F. Taguchi-Shiobara, Y. Ukai, T. Sasaki, and M. Yano. 2001. Mapping quantitative trait loci for days-to-heading, and culm, panicle and internode length in a BC1F3 population using an elite rice variety, Koshihikari, as the recurrent parent. Breed Sci. 51: 63–71.
- Yang, W., Z. Guo, C. Huang, K. Wang, N. Jiang, H. Feng, G. Chen, Q. Liu, and L. Xiong. 2015. Genome-wide association study of rice (Oryza sativa L.) leaf traits with a high-throughput leaf scorer. J. Exp. Bot. 66(18), 5605–5615. <u>http://doi.org/10.1093/jxb/erv100</u>.
- Yang, X.C. and C.M. Hwa. 2008. Genetic modification of plant architecture and variety improvement in rice. Heredity. 101: 396–404.
- Lin, Y.R., S.C. Wu, S.E. Chen, T.H. Tseng, C.S. Chen, S.C. Kuo, H.P. Wu, and Y.C. Hsing. 2011. Mapping of quantitative trait loci for plant height and heading date in two inter-subspecific crosses of rice and comparison across Oryza genus. Bot. Studies. 52: 1-14.
- Ye, S., S. Dhillon, X. Ke, A.R. Collins, and I.N. Day. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res. 29: e88-8.
- Younan, H.Q., A.K. Al-kazaz, and B.K. Sulaiman. 2011. Investigation of genetic diversity and relationships among a set of rice varieties in Iraq using random amplified polymorphic DNA (RAPD) analysis. Jordan J. Biol. Sci. 4: 249-256.
- Yoshida, S. 1972. Physiological aspects of grain yield. Ann. Rev. Plant Physiol. 23: 437-464.
- Yue, B., W.Y. Xue, L.J. Luo, and Y.Z. Xing. 2006. QTL Analysis for flag leaf characteristics and their relationships with yield and yield traits in rice. Acta Genet. Sin. 33: 824-832.

- Zhang, W., M.C. Gianibelli, W. Ma, L. Rampling, and K.R. Gale. 2003. Identification of SNPs and development of allele-specific PCR markers for γ-gliadin alleles in Triticum aestivum. Theor. Appl. Genet. 107: 130-138.
- Zhang, X.C., A.A. Md, Z.M. Lin, Z.H. Liu, G.H. Li, Q.S. Wang, S.H. Wang, and Y.F. Ding. 2014. Analysis of variations in white-belly and white-core rice kernels within a panicle and the effect of panicle type. J. Integr. Agric. 13: 1672-1679.
- Zheng, T. Q., J. L. Xu, Z. K. Li, H. Q. Zhai, and J. M. Wan. 2007. Genomic regions associated with milling quality and grain shape identified in a set of random introgression lines of rice (Oryza sativa L.). Plant Breed. 126: 158-163.
- Zhu, J., Y. Zhou, Y. Liu, Z. Wang, Z. Tang, C. Yi, S. Tang, M. Gu, and G. Liang. 2011. Fine mapping of a major QTL controlling panicle number in rice. Mol. Breed. 27(2): 171-180.
- Zong, G., A. Wang, L. Wang, G. Liang, M. Gu, T. Sang, and H. Han. 2012. A pyramid breeding of eight grain-yield relate quantitative trait loci based on marker-assistant and phenotype selection in rice (Oryza sativa L.). J. Genet. Genomics. 39(7): 335-350.
- Zhu, L.H., D.B. Zhong, J.L. Xu, S.B. Yu, and Z.K. Li. 2008. Differential expression of lodging resistance related QTLs in rice (Oryza sativa L.). Plant Sci. 175: 898–905.

CHAPTER II

DETERMINING THE DIVERSITY OF THE AGRONOMIC, MORPHOLOGICAL, AND MOLECULAR CHARACTERISTICS OF IRAQ'S AROMATIC-RICE GERMPLASM

2.1. Introduction

Rice (Oryza sativa L.) is a very important commodity in Iraq. It is the staple food for the majority of the country's population, and its annual consumption is almost 50 kilograms rice per Iraqi individual when compared with a U.S. individual consuming about 14 kilograms rice of the same age and gender. In the last 10 years, Iraqi's rice production has met only 8% to 21% of the domestic consumption. In addition, Iraq in 2016 was ranked among the top 10 countries that imported the most rice (USDA, 2016). Using farm machinery, rice is cultivated as a summer crop in Iraq, especially in the southern and northern areas (Rabbani et al., 2008), and since the early 20th century, many traditional and improved rice cultivars have been planted throughout the regions of Iraq. Most rice-growing countries have unique aromatic heirloom cultivars. In Iraq, the most popular cultivar of rice is Amber, which is grown mostly in the southern region (Chakravarthy and Naravaneni, 2006). Amber, together with other aromatic and non-aromatic rice cultivars have been grown in Iraq for hundreds of years (Younan et al., 2011). Amber is considered a very popular domestic type of rice in Iraq, and this has to do with the aroma of the rice that they consume as a staple food. The Iraqi people prefer to eat rice that has a strong aroma, especially Amber or Anber in the local accent. However, the price of Amber is high when compared with other aromatic cultivars of rice, such as Furat, Daawat, and Yasmin. Therefore, Amber is simply an aromatic and high quality rice. Higher prices are charged for Amber rice for two reasons: its aroma and high nutritional value (Sekhar and Reddy, 1982). However, growing Amber poses

numerous problems, such as lower yields, lodging, and inefficient water use but, simply it has a high-quality aromatic rice, a very distinct group of rice in Iraqi market.

One of the major contributors to the genetic divergence of rice is the aroma, which is a primary characteristic for determining high-quality rice, and the global demand for aromatic rice cultivars is increasing. The cultivars of aromatic rice compose a small group among indica and japonica groups, but it is a special group of rice and considered higher quality, which has long grain, good flavor, easy cooking and high nutritional value. These kinds of rice have long been popular in the Asia, and are becoming more popular in Middle East, Europe and the United States (Singh et al., 2000). Aromatic rice is closely linked to temperate japonica and tropical japonica groups as opposed to indica and aus groups (Garris et al., 2005).

In the context of increasing food production and promoting economic growth, understanding the diversity among the crops and within cultivars is essential to develop high-yield crops. The agronomic and morphological characterizations are integral to providing information about Iraqi aromatic rice cultivars for plant-breeding programs. The grain qualities of rice such as the size, shape, and appearance of the grain, as well as the milling quality and cooking properties should also be considered from the rice breeders' point of view (Dela Cruz and Khush, 2000). Yield is a complex factor that depends on many agronomic and morphological traits—such as plant heights, number of productive tillers, number of panicles, number grains per panicle and number of filled grains per panicle, leaf characteristics, panicle sizes and type, spikelet fertility, grain weight, biological yield, and harvest index, Hossain et al. (2005) recorded diverse morphological traits such as foliage color, leaf orientation, leaf breadth, awn and panicle types, glume color and grain shape, and agronomic traits such as plant height and fertile tillers per hill, panicle length, spikelets per panicle, grains per panicle, thousand grain weight and grain yield, and reported that all the parameters varied significantly in different aromatic rice cultivars.

Rice yield is a quantitative trait influenced by many agronomic and environmental factors. For instance, fertility and spikelets per panicle are key components of rice grain yield (Zong et al., 2012). Mathure et al. (2011) studied the characterization of aromatic rice germplasm and the correlation between its agronomic and quality traits and found that aroma was negatively associated with days to 50% flowering and filled grains per panicle. Additionally, a study by Saha et al. (2015) analyzing yield components and the aroma of small-grain aromatic rice found variations among the genotypes for the number of panicles per plant. Many rice cultivars and lines have been developed through breeding, conserving genetic resources, conducting evaluations, and implementing different breeding programs at international and national institutions throughout the world (FAO, 2000). Genetic diversity is required for all crops in improvement programs, and it aids breeders in selecting and developing superior recombinants by providing the necessary gene sources (Naik et al., 2006). The amount of genetic diversity within a species is an important factor for understanding the evolutionary status of rice species. Previous studies have shown that indica and japonica rice groups are distinctly different, based on molecular markers (Wang and Tanksley 1989; Nakano et al., 1992). Tu Anh et al. (2018) studied the phenotypic variation and genetic diversity in 15 rice mutants and four rice cultivars using Simple Sequence Repeat (SSR) markers. Analysis showed that full grain number per plant trait was the most relevant factor contributing to grain yield per plant trait, and grain length to grain width ratio was the key parameter affecting amylose content of rice grains. Mgonja et al. (2017) studied 190 African rice cultivars using a genotyping-by-sequencing (GBS)-based diversity analysis and an association mapping of blast resistance (R) genes and quantitative trait loci (QTLs). The results of diversity analysis of the

cultivars were clustered into three groups based on the 184K single nucleotide polymorphisms generated by GBS. They also identified markers linked to the regions associated with blast resistance (RABRs) and 14 highly resistant cultivars. An essential component of germplasm characterization and conservation is an assessment of genetic diversity. For many purposes such as diversity, genome mapping, and varietal identification, SSR are used among the DNA markers (Teixeira da Silva, 2005). Dramé et al. (2013) used SSR markers on both *O. glaberrima* and *O. sativa* cultivars from West Africa and obtained that these markers were highly polymorphic and were able to distinguish the genetic groups of African rice. Tahir et al. (2002) studied the genetic variability of different plant-yield characteristics in rice, and they found that the yield traits were correlated with grain yield in aromatic rice, and it can be helpful to understand and find highly aromatic rice cultivars.

Though many traditional and improved cultivars of rice are available in Iraq, no study has completely characterized or systematically analyzed their genetic base and diversity (Younan et al., 2011). The results of the current study provide insight into the diversity among Iraq's aromaticrice cultivars. This study aims to delineate the differences among aromatic-rice cultivars of Amber. An evaluation of these aromatic rice cultivars in the context of their agronomic, morphological, and genetic diversity should prove valuable to global breeding programs and especially for Iraqi rice breeders. Also, an attempt was made to classify the extent of genetic diversity of Iraqi aromatic rice with the U.S. rice and Basmati rice cultivars for agronomic, morphological, certain yield and quality traits in aroma rice cultivars for ultimate use in a breeding program.

2.2. Materials and Methods

2.2.1. Plant Material

Twenty-seven rice cultivars were included in this study, and the seeds were obtained from the USDA's rice research center, which is located at Stuttgart, Arkansas. Among these, 13 rice cultivars including four checks, 'Presidio', 'Antonio', 'Della' and 'Jazzman' were used in the agronomic and morphological evaluation. Two cultivars were non-aromatic, and 11 cultivars were aromatic (see Table 2.1). The four check cultivars were developed in the U.S. and are commonly grown in several rice growing states, particularly in Texas and Louisiana.

Entry Nymhor	Name of	Gene Bank Code	Туре	Country of Origin
Entry Number	Cultivars			
1	Amber 33	PI-326029	Aromatic	Iraq
2	Amber 33	GSOR-310278	Aromatic	Iraq
3	Amber	PI-130650	Aromatic	Iraq
4	Amber	GSOR-310793	Aromatic	Iraq
5	Amber Coarse	PI-430978	Aromatic	Iraq
6	Amber Coarse	GSOR-311588	Aromatic	Iraq
7	Amber 43	PI-430980	Aromatic	Iraq
8	Amber 43	GSOR-311672	Aromatic	Iraq
9	Anber 33	-	Aromatic	Iraq
10	Della*‡	CI 9483	Aromatic	USA
11	Jazzman*	PI-658006	Aromatic	USA
12	Antonio*	PI 667755	Non- Aromatic	USA
13	Presidio*	PI636465	Non- Aromatic	USA
14	Della	Clor-9483	Aromatic	USA
15	Basmati T3	PI-159367	Aromatic	India
16	Scented A	PI-184501	Aromatic	Japan
17	Basmati	PI-385456	Aromatic	Pakistan
18	Basmati	PI-385471	Aromatic	Pakistan
19	Basmati Pardar	PI-385809	Aromatic	Pakistan
20	Basmati Medium	PI-385816	Aromatic	Pakistan
21	Basmati	PI-385817	Aromatic	Pakistan
22	Basmati 6313	PI-400680	Aromatic	Pakistan
23	Basmati 37	PI-402762	Aromatic	India
24	Basmati 5853	PI-402764	Aromatic	Pakistan
25	Basmati 5874	PI-402765	Aromatic	Pakistan
26	Basmati	PI-431251	Aromatic	Pakistan
27	Dellmont	PI-546364	Aromatic	USA

Table 2.1. The 27 rice cultivars used in the study and their entry number, gene bank code, type and country of origin.

† PI: plant introduction; GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation
 Oryza
 ‡ *: Check

2.2.2. Field Experiment

2.2.2.1. Field Experiment for Agronomic and Morphological Traits in Beaumont

In this study, the seeds planted in the field comprised 13 cultivars, including nine aromatic types of rice from Iraq and four checks, (2 non-aromatic and 2 aromatic cultivars). The field study was conducted in the month of June in the years 2015, 2016, and 2017, at the Texas A&M AgriLife Research Center in Beaumont. The entries were direct seeded in plots arranged in a randomized complete block design (RCBD) with three replications. Each plot had six rows with 18 centimeters of space between the rows. Each row was six meters long, and the distance between each plot was 30 centimeters. Seventy-five grams of seeds were planted in each plot. The fields were prepared by tilling the soil to make the plots adequate for direct seeding. The Texas production guidelines were followed in the management of the trials. Fertilizer at the rate of (100-50-0) NPK per hectare was applied in a 3-way split, at planting, permanent flood and panicle differentiation, using 36, 32 and 32 kilograms N per hectare. All P was applied before planting. Weeds were controlled by applying herbicides such us Sharpen, Firezone, Command, Halo max, Stam and Ordram.

2.2.2.2. Field Experiment for Studying Molecular Diversity

2.2.2.1. Beaumont

Twenty-seven rice cultivars were planted in the field in Beaumont: nine aromatic rice cultivars from Iraq, six rice cultivars from the United States (four were aromatic and two were non-aromatic), nine aromatic rice cultivars (Basmati) from Pakistan, two aromatic rice cultivars (Basmati) from India, and one aromatic rice cultivar from Japan. The field study was conducted in July 2017 at the Texas A&M AgriLife Research Center in Beaumont. The field experiment was arranged in an augmented design with two replications. All entries were direct seeded in plots with 3 meters rows spaced at 18 centimeters apart.

2.2.2.2.2. Eagle Lake

At Eagle Lake, the same 27 entries and experimental design in Beaumont were used but the field study was conducted in August 2017 at the Texas A&M AgriLife Research Center at Eagle Lake and the entries were transplanted. Seedlings were prepared by planting three seeds in small pots and for each line and cultivars, five plots were planted. After three weeks from the seeding date, seedlings were carefully removed in each pot. At transplanting, each entry had five plants spaced at 20 cm apart in rows spaced at 20 cm. SAS version 9.4 was used for the statistical analysis of the data

2.2.3. Data Collection

Several methods and parameters were used to estimate and measure the agronomic and morphological traits, and these are described below. Most of the traits were obtained using half meter row sample per plot.

2.2.3.1. Days to 50% Heading

Days to 50 % heading was measured as the number of days that passed from the initial planting to when the primary panicles in 50% of the plants headed.

2.2.3.2. Plant Height (cm)

Plant height was measured a week before harvest time in centimeters with a ruler from the ground surface to the tip of the panicles.

2.2.3.3. Number of Tillers (m²)

At the time of harvest, the total number of tillers were counted in a half meter row and calculated in per square meter plot.

2.2.3.4. Percentage of Productive Tillers

Productive tillers are the panicle bearing tillers. All productive tillers and non-productive tillers were counted from a half meter row use in getting the tillers counts. The percentages of the productive tillers were estimated using the formula:

Productive Tillers % = (Number of tillers have panicles / Total number of tillers) x 100.

2.2.3.5. Flag-Leaf Area (cm²)

The flag-leaf area was recorded by measuring the length and the maximum width of the leaf followed by obtaining the area using the following formula: Leaf area = K x length x width, where K = "adjustment factor." K varied with the shape of the leaf, which was affected by factors such as the cultivar and growth stages, 0.75 (IRRI, 1972).

2.2.3.6. Ligule Length (mm)

The average ligule length was calculated at late the vegetative phase when five plants were chosen at random, and the first leaf under the flag leaf of the main tiller was collected to measure the length of the ligule. The ligule length in millimeters was measured with a ruler from the base of the collar to the tip.

2.2.3.7. Panicle Length (cm)

The length of five panicles of each plant was measured from the base of the lowest spikelet to the tip of the latest spikelet on the panicle, excluding the awn at the time of harvest.

2.2.3.8. Number of Grains Per Panicle

The total number of grains per panicle were recorded at harvest and these were obtained from five randomly selected panicles in a half meter row.

2.2.3.9. Sterility Percentage (%)

The sterility percentages of unfilled grains were measured per panicle and recorded after harvest. Five panicles were used, as well as the following formula: Sterility percentage = (Number of unfilled grains / Total number of grains) $\times 100\%$.

2.2.3.10. Fertility Percentage (%)

The fertility percentages of filled grains were measured per panicle and recorded after the harvest. Five panicles were used, as well as the following formula: Fertility percentage = (Number of filled grains / Total number of grains) $\times 100\%$.

2.2.3.11. Thousand Grain Weight (g)

The thousand-grain weight was taken by counting 1000 from the bulked harvested grains and recording their weight.

2.2.3.12. Grain Yield per Hectare (kg ha⁻¹)

The weight of the grains obtained from the half meter row and air-dried to obtain 12 % moisture content was used in estimating the grain yield per hectare. The following formula was used: Yield in kg ha⁻¹= [(Weight of sample) / (Area of sample in square meter)] x 1000.

2.2.3.13. Rice Milling (%)

One hundred grams of dried rough rice per plot were milled using Zaccaria mill. The rice-milling ratio (% total milled rice) was the amount of milled rice obtained after milling rough rice. It was measured with the following formula: Rice-milling percentage = (weight of milled rice sample / weight of rough rice sample) x 100.

2.2.3.14. Seed Length (mm)

Seed length was measured after rice had been milled, and 100 seeds were chosen at random. The STD4800 scanner and WinSEEDLE (2014) (Instruments Canada Inc.) were used to measure the lengths (Fig. 2.1).

2.2.3.15. Seed Width (mm)

Seed width was measured after the rice had been milled. The same 100 milled rice chosen at random for seed length, and the STD4800 scanner and WinSEEDLE (2014) were used to gather seed widths.

2.2.3.16. Chalky Seed Percentage (%)

The chalky seeds were also measured after the rice had been milled, and the same 100 milled rice chosen at random for lengths and widths, and the STD4800 scanner and WinSEEDLE (2014) were used for the measurement.



Figure 2.1. The STD4800 scanner for WinSEEDLE (2014).

2.2.3.17. Aroma (2AP) Analysis Method (GC/TCD with Headspace Autosampler)

2.2.3.17.1. Sample Preparation and Gas Chromatography Condition

The aroma was estimated with GC-MS for 2AP. Milled-rice samples were ground into a powder (less than 0.25 mm in diameter) using the Cyclone lab mill shown in Fig.2.2. Two replications of 1.00 g samples were transferred into a 20 mL headspace glass vial. One µL of 0.5 mg/mL of 2,6-dimethylpyridine (2,6-DMP) was added to the vial as an internal standard before the airtight sealing was performed, which was done with a polytetrafluoroethylene and silicone septum secured by an aluminum cap. Sample vials were placed on the headspace-auto-sampler model HS-20 (Shimadzu, Columbia, MD) and equilibrated at 120 °C for 10 min with high-speed shaking, prior to collecting the volatile components. The pressurizing time, the pressure equilibrium time, and the injection times were 1.00, 0.01, and 2.00 min, respectively. After the pressurizing, a sample of the headspace was collected through a 3-mL sample loop and automatically transferred to the gas chromatography via a heated transfer line for 0.50 min. The oven, sample-line, and transfer-line temperatures were set at 120 °C, 150 °C, and 160 °C, respectively. Gas chromatographic separation was performed on a Shimadzu GC-2010. Another system (Shimadzu, Columbia, MD) was coupled to a flame thermionic detector (FTD) and equipped with LabSolutions software for data collection and evaluation. Separation was performed with a 60 m x 0.32 mm i.d. x 1.0 µm film thickness Rtx-5 capillary column (Restek, USA), with a splitless injection at 250 °C. The temperature of the column was programmed to start at 50 °C at the time of the injection; subsequently, it was set to increase at a rate of 5 °C/min, from 50 °C to 200 °C. Gas chromatography and FTD were performed at the temperature of the detector of 280 °C, and helium was used as a carrier gas, with the flow rate of 3.5 mL/min. The concentration of

2AP was identified by comparing the gas-chromatography retention times with the standard that ran under the same conditions. Peak areas were obtained with the aid of software from LabSolutions (Shimadzu, Columbia, MD).

2.2.3.17.2. Standard Preparation

. A standard of 10 mg of 2AP in a 10% w/w in toluene was purchased from Toronto Research Chemicals (TRC), which is based in Canada. A series of standards with the concentrations of 0, 0.5, 1.0, 1.25, 2.5, and 5.0 mg/g of 2AP in toluene were prepared. A nonaromatic milled-rice sample was ground into a powder with the same method used for the samples. Three replications of 1.00 g of nonaromatic milled rice were weighed and transferred into a 20 mL headspace glass vial for each level of the standard. One μ L of 0.5 mg/mL of 2,6-dimethylpyridine (2,6-DMP) and 1 μ L of the 2AP standard of each concentration level were added into the vial before the airtight sealing, which was done with a polytetrafluoroethylene and silicone septum and secured by an aluminum cap. The headspace auto-sampler and gas chromatography were set up and analyzed in the same manner used for the samples for 2AP analysis (Figure 2.3 and Figure 2.4).



Figure 2.2. Cyclone lab sample mill used for grinding milled rice.



Figure 2.3. The Gas Chromatography-Mass Spectrometry (GC-MS) set-up used for estimating 2-Acetyl-1-pyrroline (2AP).

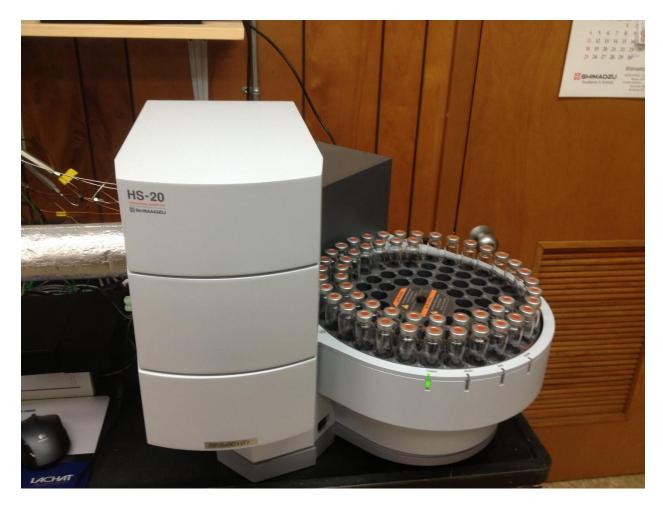


Figure 2.4. Loading samples to the Gas Chromatography-Mass Spectrometry (GC-MS).

2.2.4. Genotypic Analysis

2.2.4.1. Genotyping

Leaves for DNA extraction were obtained from the greenhouse-grown seedlings before transplanting at Eagle Lake. Bulked leaves were obtained in five plants per genotype, frozen using liquid nitrogen and stored frozen under 80 degrees Celsius below zero until DNA extraction.

Genomic DNA from the parents were extracted using a modified CTAB method (Liu et al., 2013), and all extractions were done in the AgriGenomics Laboratory, based in College Station, Texas. The quality of the DNA was checked with agarose gels, and the concentration was tested with a NanoDrop 1000 UV-vis Spectrophotometer (DeNovix DS-II Spectrophotometer). DNA preparation for analysis were done at the AgriGenomics Laboratory.

2.2.4.2. DNA Extraction

Bulked of young leaves from each entry was collected at 21 days after seeding, and these were utilized for DNA extraction using a modified CTAB method (Liu et al., 2013).

2.2.4.3. Genotyping by Sequencing (GBS)

The DNA analysis was performed at the Genomics and Bioinformatics Laboratory at Texas A&M AgriLife, College Station, TX. The laboratory used the following procedure to execute the DNA analysis. One hundred micrograms of DNA per sample on 96 well plates were digested in a final volume of 25 μ l in 1X NEB Cut Smart Buffer and 100 U each of ENZYME 1 and ENZYME 2 (NEB), at 37 °C for 4 hr. Following a 20 min of 80 °C enzyme inactivation, samples were held at 12 °C until ligation. To each 25 μ l digest sample, 3.5 μ l of a 10X ligase buffer (Promega) and 0.5 μ l of T4 DNA ligase (Promega) were added. Adapters containing 1 of 48 unique barcodes and Illumina-compatible P5 sequences that had been coupled to an EcoRI overhang, as well as Illumina-compatible P7 sequences coupled to the MspI overhang, were added as well. The plates

were incubated for 8 hr at 16 °C and heat inactivated at 80 °C for 20 min. Three pools of 45, 45, and 46 samples were mixed and combined with 0.1 volume of 3 M NaAc, pH 5.2, and two volumes of 100% ethanol and placed at -20° C for 1 hr. The three pools were spun at a high speed for 10 min in a bench-top microfuge. The pellets were washed twice in 1 mL of freshly made 70% ethanol and resuspended in 200 µl of EB. Samples were purified with the company Qiagen's PCR Purification columns and eluted in 2X 50 µl EB, for a total of 100 µl. One volume of AMPure XP beads were added to the elutant and DNA purified, as per the manufacturer's suggested protocol, and these were eluted in 35 μ l of EB. Thirty microliters of each pool containing between 1.9 and 2.2 µg of DNA was subjected to a Pippin Prep size selection on a 2% dye-free agarose gel, with the internal size markers aiming for 270-330 bp inserts. Recovered samples were cleaned with 1X AMPure XP beads and quantified on a DeNovix spectrophotometer. One hundred fifty nanograms of each pool was then subjected to a preselection PCR (PreCR) in which a biotinylated forward primer and unique indexed reverse primers were used to amplify and tag the desired DNA fragments. Reactions (200 µl in total) contained 200 nM of dNTPs, biotinylated forward and two P7-index primers per pool, and four units of Phusion Hi-Fidelity Taq (NEB) and were split into 2 x 100 µl volumes for thermocycling. Following an initial denaturation performed at 98 °C for 30 s, samples were subjected to 18 cycles of 98 °C for 10 s, 58 °C for 30 s, and 72 °C for 30 s, and a final elongation was performed for 5 min at 72 °C and held at 4 °C. PCR products were cleaned up in Qiagen's PCR purification columns and 1X AMPure XP beads and quantified as before. Removal of EcoRI-EcoRI and MspI-MspI fragments was achieved with ThermoFisher's Dynabeads M-270 Streptavidin, which are coupled magnetic beads. Briefly, 50 µl of beads per sample were captured and washed twice with the 1X Bead Washing Buffer (1X BWB, 10 mM Tris-HCl [pH 7.5], 1 mM EDTA, and 2 M NaCl). The beads were re-suspended in 100 µl of 2X BWB and mixed with 2000 ng of the PreCR product in 100 μ l of EB. After 20 min at RT, the beads were captured and washed three times in 200 μ l of 1X BWB, twice in 200 μ l of water, and once in 100 μ l of 1X SSC. The beads were re-suspended in 50 μ l of 1X SSC and heated at 98 °C for 5 min and placed on a magnet, and the resulting supernatant was removed as soon as possible. This elution was repeated, and the final supernatants were cleaned up with Qiagen's PCR columns. The eluted ssDNA was DeNovix quantified and diluted to 1 ng/ μ l with EB. A final PCR was performed on 10 ng of the input DNA, and P5 and P7 primers were used in a 75 μ l reaction as described above, but only with 8 cycles. The final PCR products were purified with 1X AMPure XP beads, quantified, and assessed for quality on a Fragment Analyzer (made by Advanced Analytics).

2.2.4.4. RADSeq Data Analysis and SNP Identification

A RADseq analysis was done for 27 samples in the Genomics and Bioinformatics Laboratory at Texas A&M AgriLife. Genomic DNA from each sample was digested with the restriction enzymes PstI and MseI. The restriction-associated DNA (RADs) for all 27 samples were pooled and sequenced in two lanes on Illumina's HiSeq 4000 with a 150 bp pair-end library. The library preparation and sequencing were conducted at AgriLife Research Genomic and Bioinformatics Service, at the Texas A&M University (www.txgen.tamu.edu). The raw sequence reads were de-multiplexed, according to the index reads. First, the sequences were filtered for quality with the program FASTX-Toolkit (http://hannonlab.cshl.edu/fastx-toolkit). The raw sequencing reads were trimmed to remove low-quality bases with scores below 20 on the ends of the reads; next, reads with 30% or more bases showing a low-quality score (Q < 15) were removed. The reference genome for *Oryza indica* (ASM465v1) and *Oryza sativa* (IRGSP-1.0) were downloaded from a plant ensemble (https://plants.ensembl.org). An artificial reference was

constructed by combining both of these two genomes into one single Fasta file. Bowtie 2 [http://bowtie-bio.sourceforge.net/bowtie2/index.shtml] was used to align the quality-controlled reads with the references for the default parameters. The reference-aligned reads were then processed with the ref_map.pl pipeline in Stacks V2.0 (Catchen et al. 2013). First, the uniquely aligned reads were assigned into stacks and subsequently merged to form putative loci. The minimum depth of stack was five reads. To include a locus in the analysis, we required it to be present in at least 50% of the samples. Subsequently, a maximum-likelihood framework was used to call SNPs, and a catalogue was built with all of the existing loci and alleles, against which all individuals were matched. (For more details on the use of the Stacks software package for studies of model and nonmodel organisms, see Catchen et al. 2013.)

The methodology for data analyses for morphological and agronomic traits used the SAS version 9.4. All cultivars were genotyped with genotyping by sequencing (GBS) for doing cluster and principal coordinate analysis (PCoA), and the principal component analyses (PCA) of the phenotypic data of aromatic rice cultivars used R 3.4 software. The data set used in PCA are shown in Table 2.14, Table 2.15 and Table 2.16.

2.3. Results and Discussion

2.3.1 Agronomic and Morphological Traits

2.3.1.1. First Year Result 2015

In 2015, the results showed significant differences among entries in the six measured traits. Table 2.2 and 2.3 provide the agronomic traits of the aromatic-rice cultivars. The Amber 33-PI, Amber 33-GSOR, Amber-PI, and Amber-GSOR cultivars required the most days to reach 50% of their headings: 106.6, 106, 102.6, and 102.3 days, respectively. Antonio, Amber 43-PI, Presidio, and Amber 43-GSOR required the fewest days to reach 50% of their headings: 77, 77.3, 82, and 83.6 days, respectively. It was noted that heading varied among replications.

The entries had significant differences in their heights. The Iraqi aromatic-rice cultivars Amber 43-PI, Amber-PI, Amber 33-GSOR, Amber-GSOR, and Amber 33-PI were the tallest, with heights of 167, 166.3, 165.3, 164.4, and 161.6 cm, respectively. The U.S. rice cultivars Presidio, Jazzman, and Antonio had shorter heights of 92.5, 100.1, and 103 cm, respectively.

In regard to the number of tillers per linear meter, the Amber Coarse-PI, Amber Coarse-GSOR, and Antonio had a higher number of tillers: 311.6, 292, and 227, respectively. The aromatic rice Amber 33-GSOR, Della, and Presidio had fewer tillers per meter squared. Similar to heading, variation among replications was noted for tiller count.

The flag-leaf area of aromatic-rice cultivars Amber 43-PI, Amber Coarse-GSOR, and Della developed the largest flag-leaf areas: 61.1, 57.8, and 56.4 cm², respectively. The non-aromatic-rice cultivars produced much smaller flag leaf areas, such as Presidio (30.7 cm²) and Antonio (33.9 cm²).

The panicle lengths of Amber 33-PI, Amber-GSOR, Amber 33-GSOR and Amber-PI were 28.7 cm, 27.7 cm, 26.1, and 26 cm. respectively and these varied significantly. Presidio, Jazzman, and Antonio had short panicle length as shown in Table 2.2 and 2.3.

Table 2.2. Mean squares of the ANOVA showing the effects of replications and cultivars on days to 50 % heading, plant height, number of tillers, flag leaf area, ligule length and panicle length tested at Beaumont, Texas in 2015.

Source	Days to 50% Heading:	Plant Height (cm)	Number of Tillers	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)
Rep	0.78^{*} †	19.84 ^{ns}	23.69**	0.60 ^{ns}	0.23 ^{ns}	5.68 ^{ns}
Cultivar	365.11**	2327.83^{**}	7295.97**	291.79**	151.71**	40.77**
Error	0.20	16.12	2.18	0.47	1.54	1.87

† ns =Non-significant; * = P<0.05; ** = P<0.01

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; m² = Meter squared; mm = Millimeter

Cultivars	Days to 50 % Heading	Plant Height (cm)	Number of Tillers	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)
Amber 33-PI [†]	106.7	161.6	203.3	53.1	25.0	28.7
Amber -PI	102.7	166.3	213.6	38.6	29.1	26.0
Amber Coarse -PI	92.3	135.2	311.7	50.8	17.7	21.21
Amber 43 -PI	77.3	167.0	214.6	61.1	17.7	22.1
Amber 33 -GSOR	106.0	165.3	152.3	51.6	28.5	26.1
Amber -GSOR	102.3	164.4	202.0	39.9	30.7	27.7
Amber Coarse -GSOR	87.3	133.3	292.0	57.9	16.4	20.2
Amber 43 -GSOR	83.7	144.9	271.3	48.2	15.7	19.5
Jazzman	86.7	100.1	190.0	41.2	15.2	19.0
Della	83.7	128.3	163.3	56.4	11.8	21.3
Presidio	82.0	92.5	186.3	30.7	11.5	18.1
Antonio	77.0	103.0	227.0	33.9	12.0	19.2
Mean	90.6	138.5	219.0	47.0	19.3	22.4
Range	77.0- 106.7	92.5-167.0	152.3-311.7	30.7-61.1	11.5-30.7	18.1-28.7
CV	0.5	2.9	0.7	1.5	6.4	6.1
LSD (0.05)	0.76	6.80	2.50	1.16	2.10	2.32

Table 2.3. Means of agronomic traits of days to 50% heading, plant height, number of tillers, flag leaf area, ligule length and panicle length of rice cultivars tested at Beaumont, Texas in 2015.

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number
§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance
‡ % = Percentage; cm = Centimeter; cm² = Centimeter squared; m² = Meter squared; mm = Millimeter

2.3.1.2. Second Year Result 2016

The results for year 2016 show highly significant variations for the morphological and agronomic traits, as shown in Table 2.4 and Table 2.5. The number of days needed to reach 50% heading differed among the cultivars: Anber 33 required 105 days, and Amber 33-PI and Amber 33-GSOR each required 103.3 days, and these were the longest periods recorded for flowering times. Amber 43-PI and Amber 43-GSOR needed only 73.3 and 75 days, respectively, and these were the shortest periods recorded for the flowering times. Ultimately, the Amber cultivars had a substantial range of flowering times.

In regard to plant heights, significant variations among the rice cultivars were seen. The heights of Amber 43-PI, Amber-PI, Amber-GSOR, Amber 43-GSOR, Amber 33-PI, Amber 33-GSOR, and Amber Coarse were the tallest, with measurements of 168.2, 150.6, 150.2, 150.1, 148.9, 148.8, and 148 cm, respectively. The U.S. rice cultivars Jazzman, Presidio, Antonio, and Della produced shorter plants, with average heights of 87.3, 88.5, 95.7, and 111.3 cm, respectively.

Amber Coarse-GSOR and Amber Coarse-PI had the highest number of tillers: 383.3 and 346.3 tillers per square meter, respectively. Presidio had 192.6 tillers per square meter, and Jazzman had 210 tillers per square meter.

The percentages of the productive tillers showed no significant variations among Antonio and Presidio cultivars, and Jazzman having 100% productive tillers. The Amber 43-GSOR, Amber 33-PI, Amber Coarse-GSOR, Amber-PI, and Della cultivars were within the same range of 99.1% to 96.7%.

The results for the flag-leaf area showed significant differences among the aromatic rice cultivars, and Amber 43-PI (56.7 cm²) and Amber 33-PI (55.6 cm²) had largest flag-leaf areas. The aromatic rice Della (one of the check cultivars) had a flag-leaf area of 45.78 cm², and it showed

similar response from the previous year. Amber 33-GSOR and Amber Coarse-GSOR had average flag-leaf areas of 46.0 and 44.7 cm², respectively. The non-aromatic-rice cultivars Presidio (30.7 cm) and Antonio (33.9 cm²) had the smallest flag-leaf areas.

Amber 33-PI, Amber-GSOR, Amber 33-GSOR, Amber-PI, and Anber 33 had the longest panicle lengths: 27.4, 26.3, 26.3, 24.0, and 24 cm, respectively. However, Della showed similarity with Amber 43 and Amber Coarse cultivars. The three U.S. bred rice, Jazzman, Antonio, and Presidio, had the shortest panicle lengths.

The number of grains per panicle was significantly different among the aromatic and nonaromatic types; Jazzman, Amber 33-PI, Antonio and Amber 33-GSOR had high numbers of grains: 142, 137, 130 and 129, respectively. However Amber 43-GSOR, Amber Coarse-GSOR and Amber Coarse-PI had the lowest numbers of grains, with 62, 72 and 73, respectively. Similarly, the sterility percentages were significantly different among the aromatic and non-aromatic types of rice. Iraqi aromatic rice Amber 43-PI (40.2%), Amber 33-PI (39.4%), Amber Coarse-PI (38.5%), Amber 43-GSOR (36.6%) had the higher percentages of sterility, while Antonio (21.3%) and Amber-PI (19.7%) had lower percentages of sterility. The aromatic types of rice from Iraq showed significant variations in their fertility percentages, as shown in Table 2.6 and Table 2.7. The grains of Amber-PI, Amber-GSOR, Amber Coarse-GSOR, and Amber 33 were filled more than those of Amber 43-GSOR, Amber 43-PI, and Amber Coarse-PI.

Highly significant differences in the weights of the thousand-grain samples were noted. One thousand-grain weight of Amber Coarse-IP weighed 27.1 g, which was considerably heavier than the weights of the Amber-PI sample (19.9 g) and the non-aromatic Presidio sample (23.8 g), as shown in Table 2.6 and Table 2.7.

The grain yields per hectare (kg ha⁻¹) obtained in the non-aromatic rice cultivars from the

U.S. such as Antonio (8825.3 kg ha⁻¹) and Presidio (8447.1 kg ha⁻¹) were significantly higher than those of aromatic rice such as Jazzman (6771.5 kg ha⁻¹) and Della (5413.5 kg ha⁻¹), as well as all Iraqi aromatic rice cultivars, as shown in Table 2.6 and Table 2.7. In regard to milling quality, the percentages of the total milled rice varied among the test entries. Not one of the Iraq aromatic types of rice was comparable to Antonio; however, two were comparable to Presidio, and one was comparable to Jazzman. All of the aromatic types of rice from Iraq, except Amber Coarse-PI with 62.6%, had less than 60% total milled rice, as shown in Table 2.6 and Table 2.7.

The U.S. rice cultivars, both aromatic and non-aromatic, had long seeds. For example, Jazzman, Della, Antonio, and Presidio had seed lengths of 6.6, 6.5, 6.3, and 6.3 mm, respectively, as shown in Table 2.6 and Table 2.7. The Amber cultivars from Iraq were classified as having medium seed lengths, which ranged from 5.9 mm to 5.6 mm.

The seeds of Amber 43-GSOR, Amber Coarse-PI, Amber Coarse-GSOR, and Amber 43-PI were the widest, at 2.7, 2.6, 2.6, and 2.6 mm, respectively. The average seed widths of the aromatic cultivars were the following: Amber 33, 2.2 mm; Amber-GSOR, 2.1 mm; and Amber-PI, 2.1 mm. In addition, no big difference was seen between Jazzman and Presidio, as shown in Table 2.6 and Table 2.7. Chalky seed percentage trait showed significant differences among all Iraqi aromatic rice cultivars. All Amber cultivars recorded high chalky percentage, with range starting with Amber Coarse-GSOR at 4.1% to Anber 33 at 1.7%. In general, the U.S. cultivars had a smaller chalky percentage, such as Jazzman (0.3%), Presidio (0.6%), Della (0.7%), and Antonio (1%), as shown in Table 2.6 and Table 2.7.

Table 2.4 and Table 2.6 show the variation among replications of some morphological and agronomic traits, such as the days needed to reach 50% of the heading, plant heights, percentages of productive tillers, flag-leaf areas, and weights of thousand-grain samples. However, majority of the morphological and agronomic traits were not significantly different among the replications.

Table 2.4. Mean squares of the ANOVA showing the effects of replications and cultivars on days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, panicle length, number of grains per panicle and sterility percentage of rice cultivars tested at Beaumont, Texas in 2016.

Source	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers	Percentage of Productive Tillers (%)	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Grains per Panicle (g)	Sterility Percentage (%)
Rep	1.26^{**} †	80.88^{**}	3.18 ^{ns}	29.00^{*}	3.80^{*}	0.01 ^{ns}	4.29 ^{ns}	508.00 ^{ns}	1.30 ^{ns}
Cultivar	423.60**	4502.77^{**}	9121.73**	21.20^{**}	332.30**	133.57**	36.14**	1944.76**	178.64**
Error	0.09	6.23	20.93	5.44	0.83	0.08	2.01	165.92	13.57

† ns = Non-significant; * = P<0.05; ** = P<0.01

 $\ddagger \%$ = Percentage; cm = Centimeter; cm² = Centimeter squared; m² = Meter square; mm = Millimeter; g = Gram

Table 2.5. Means of agronomic traits of days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, panicle length, number of grains per panicle and sterility percentage of rice cultivars tested at Beaumont, Texas in 2016.

Cultivars	Days to 50% Heading	Plant Height (cm)	Number of Tillers	Percentage of Productive Tillers (%)	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Grains per Panicle	Sterility Percentage (%)
Anber 33	105.0	147.4	277.0	93.3	35.8	20.8	24.0	100.0	23.8
Amber 33-PI	103.3	148.9	269.0	99.0	55.6	24.8	27.4	137.7	39.4
Amber -PI	98.3	150.6	319.7	98.3	35.5	23.6	24.0	112.3	19.7
Amber Coarse -PI	83.3	136.7	346.3	95.9	42.5	10.9	20.7	73.3	38.6
Amber 43 -PI	73.3	168.2	228.0	93.7	56.7	9.3	20.4	97.7	40.3
Amber 33 -GSOR	103.3	148.8	264.0	94.2	46.0	24.2	26.3	129.3	27.4
Amber -GSOR	99.3	150.2	315.6	93.9	28.6	19.8	26.3	110.3	21.6
Amber Coarse -GSOR	83.0	148.1	383.3	99.0	44.7	13.1	20.0	72.3	23.1
Amber 43 -GSOR	75.0	150.1	289.0	99.1	34.2	10.1	19.4	62.3	36.6
Jazzman	79.3	87.3	210.0	100.0	31.3	12.5	18.2	142.0	26.2
Della	79.0	111.4	225.3	96.7	45.8	4.4	19.3	114.0	24.1
Presidio	80.0	88.6	192.6	100.0	23.7	11.2	17.6	107.0	22.2
Antonio	81.0	95.8	273.3	100.0	26.6	11.4	18.1	130.7	21.4
Mean	87.9	133.2	276.4	97.2	39.0	15.1	21.7	106.8	28.0
Dongo	73.3-	87.3-	192.6-	93.3-100.0	23.7-56.7	4.4-	17.6-	62.3-142.0	19.7-40.3
Range	105.0	168.2	383.3			24.7	27.4		19.7-40.5
CV	0.3	1.9	1.7	2.4	2.3	1.9	6.5	12.1	13.1
LSD (0.05)	0.50	2.88	7.71	3.93	1.53	0.49	2.39	21.71	6.20

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number

CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; m² = Meter squared; mm = Millimeter; g = Gram

Table 2.6. Mean squares of the ANOVA showing the effects of replications and cultivars on fertility percentage, thousand grains weight, grain yield, rice milling, seed length, seed width and chalky seed of rice cultivars tested at Beaumont, Texas in 2016.

Source	Fertility Percentage (%)‡	Thousand Grain Weight (g)	Grain Yield (kg ha ⁻¹)	Total Milling Rice (%)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
Rep	1.30 ^{ns} †	0.01^{*}	253585.4 ^{ns}	3.55 ^{ns}	0.0002^{ns}	0.003 ^{ns}	0.05 ^{ns}
Cultivar	178.64**	17.40^{**}	12673031.6**	65.76^{**}	0.32^{**}	0.14^{**}	4.68^{**}
Error	13.57	0.002	151243.6	5.00	0.01	0.002	0.10

† ns = Non-significant; * = P<0.05; ** = P<0.01

% = Percentage; g = Gram; kg ha⁻¹ = Kilogram per hectare; mm = Millimeter

Cultivars	Fertility Percentage (%):	Thousand Grain Weight (g)	Grain Yield (kg ha ⁻¹)	Total Rice Milling (%)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
Anber 33	76.2	21.8	3279.1	53.5	5.8	2.2	1.7
Amber 33-PI [†]	60.6	22.7	3806.4	53.0	5.8	2.2	3.0
Amber -PI	80.3	19.9	3750.1	57.5	5.7	2.1	3.4
Amber Coarse -PI	61.4	27.2	3586.4	62.7	5.9	2.6	2.9
Amber 43 -PI	59.7	21.2	2572.1	53.1	5.6	2.6	1.9
Amber 33 -GSOR	72.6	22.7	4183.7	56.0	5.8	2.3	3.1
Amber -GSOR	78.4	19.8	3501.3	57.4	5.8	2.1	3.4
Amber Coarse -GSOR	76.9	26.4	3190.2	59.4	5.9	2.6	4.1
Amber 43 -GSOR	63.4	26.8	3530.7	54.2	5.7	2.7	2.5
Jazzman	73.8	23.7	6771.5	65.2	6.6	2.1	0.3
Della	75.9	24.8	5413.5	58.1	6.5	2.2	0.7
Presidio	77.8	23.8	8447.1	62.8	6.3	2.1	0.6
Antonio	78.6	23.7	8825.3	66.9	6.3	2.1	1.0
Mean	72.0	23.4	4681.3	58.4	6.0	2.3	2.2
Range	59.7-80.3	19.8-27.2	2572.1- 8825.3	53.0-66.9	5.6-6.6	2.1-2.7	0.3-4.1
CV§	5.1	0.2	8.3	3.8	1.7	1.8	14.4
LSD (0.05)	6.21	0.08	655.36	3.77	0.17	0.07	0.53

Table 2.7. Means of agronomic traits of fertility percentage, thousand grains weight, grain yield, rice milling, seed length, seed width and chalky grain of rice cultivars tested at Beaumont, Texas in 2016.

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number
 § CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; g = Gram; kg ha⁻¹ = Kilogram per hectare; mm = Millimeter

2.3.1.3. Third Year Result 2017

Similar to the two previous years, highly significant variations were noted for the morphological and agronomic traits in 2017, as shown in Table 2.8 and Table 2.9. The results show significant differences in the number of days the cultivars need to reach 50% of their headings. The types of rice from Iraq required the most days to reach 50% headings, and this group included Anber 33, Amber 33-GSOR, Amber-GSOR, Amber 33-PI, and Amber-PI, which needed 125, 124, 123, 122, and 122 days, respectively. Another group of Iraqi rice cultivars needed fewer days to reach 50% headings, and this group included Amber 43-GSOR, Amber Coarse-GSOR, Amber Coarse-PI, and Amber Coarse-PI, which required 111, 111, 108, and 105 days, respectively. The U.S. rice cultivars were the earliest to flower. Antonio required 86 days; Presidio, 90 days; Della, 91 days; and Jazzman, 100 days.

The plant heights varied significantly among the rice cultivars. Table 2.8 and Table 2.9 show that Amber 43-GSOR, Amber 43-PI, and Amber-PI were the tallest, with heights of 153, 152.3, and 150 cm, respectively. The U.S. aromatic-rice cultivars produced shorter plants; for example, the average heights of the Jazzman and Della plants were 97.2 cm and 100 cm, respectively. In addition, Antonio and Presidio produced much shorter plants with an average height of 91 cm and 92.5 cm, respectively.

Table 2.8 and Table 2.9 showed highly significant variation between two types: Amber Coarse-GSOR and 'Amber Coarse-PI compared to other Amber cultivars. They had the highest number of tillers per sq. meter. Amber Coarse-GSOR produced 379 tillers per sq. meter, and Amber Coarse-PI produced 350 tillers per sq. meter. Della, an aromatic-rice cultivar, generated 213 tillers per sq. meter; Presidio, a non-aromatic rice cultivar, generated 231 tillers per sq. meter. These last two produced the fewest tillers.

The percentages of productive tillers showed no significant variation among the U.S. rice cultivars Jazzman, Presidio, Antonio, and Della—which had 99%, 98%, 97%, and 94% productive tillers, respectively. In regard to Amber Coarse-PI, Amber 43-PI, Amber Coarse-GSOR, and Amber 43-GSOR, the % productive tillers were 97%, 96.7%, 96%, and 94%, respectively, as shown in Table 2.8 and Table 2.9.

The flag-leaf areas were significantly different among the aromatic-rice cultivars; for example, Amber 33-PI had the largest flag-leaf area, at 54.7 cm², among all of the Amber cultivars. The aromatic rice Della and Jazzman and the non-aromatic rice Antonio and Presidio had smaller flag-leaf areas, which ranged from 20.8 cm² to 28.1 cm².

The Iraqi aromatic rice Anber 33, Amber 33-PI, Amber-GSOR, Amber-PI, and Amber 33-GSOR had the longest panicle lengths: 29.3, 28.4, 28.2, 27.7, and 26.2 cm, respectively. However, Amber 43-GSOR, Amber Coarse-GSOR, and Amber Coarse-PI showed similarity with the U.S. aromatic rice cultivars Jazzman and Della. Presidio and Antonio as non-aromatic recorded the shortest panicle length (Figure 2.5).

The number of grains per panicle were significantly different among rice cultivars. Amber 33-PI, Amber 33-GSOR, Jazzman, Antonio, Presidio, Amber-PI, Della, and Anber 33 had 124, 123, 121, 114, 109, 108, 105, and 104 had higher number grains per panicle, respectively. Amber Coarse-GSOR and Amber Coarse-PI had 58 and 62, the lowest number of grains per panicle, respectively.

The sterility percentages trait was not significantly different among all of the Amber cultivars, but all had higher sterility percentages than the U.S. rice cultivars, such as Presidio (11.9%), Antonio (19%), Jazzman (22.6%), and Della (28.7%), as shown in Table 2.8 and Table 2.9.

In 2017, all of the U.S. rice cultivars showed highly significant fertility percentages. However, the aromatic rice from Iraq had lower fertility percentages, as shown in Table 2.10 and Table 2.11, ranging from Amber-GSOR at 54.9% to Amber 43-PI at 42.1% filled grains.

The weights of the thousand-grain significantly varied. The weight of the Amber Coarse-IP grains was 25.2 g, and Amber Coarse-GSOR was 24.0 g, which was similar to the Della at 24.3 g. In addition, the Amber 43-GSOR sample weighed 23.2 g, which was similar to the weights of Jazzman (23.6 g), Antonio (23.6 g), and Presidio (22.9 g), as shown in Table 2.10 and Table 2.11.

The grain yields per hectare (kg ha⁻¹) obtained in the non-aromatic rice cultivars from U.S. such as Antonio (8769 kg ha⁻¹) and Presidio (8507.7 kg ha⁻¹) were significantly higher than aromatic rice cultivars such as Jazzman (7451.5 kg ha⁻¹) and Della (6162.8 kg ha⁻¹). However, these aromatic and non-aromatic rice cultivars were significantly high yielder than the Iraqi aromatic rice such as Amber Coarse-GSOR with 4906.1 kg ha⁻¹ and Amber Coarse-PI with 4245.8 kg ha⁻¹. These two Amber rice cultivars were comparable to U.S. bred aromatic, Della, as shown in Table 2.10 and Table 2.11. In regard to milling quality, the percentages of the total milled rice varied among the test entries. Jazzman and Presidio had the highest milling-rice percentages: 72.7% and 70.5%, respectively. The Della (61.3%) percentage was similar to those of several Amber cultivars, such as Anber 33, Amber 33-PI, Amber 33-GSOR, Amber-GSOR, Amber Coarse-PI, Amber-PI, Amber Coarse-GSOR, and Amber 43-GSOR, as shown in Table 2.10 and Table 2.11.

The aromatic and non-aromatic U.S. rice cultivars all had long seeds; for example, Antonio, Jazzman, Della, and Presidio had seed lengths of 6.6, 6.4, 6.2, and 6.1 mm, respectively, as shown in Table 2.10 and Table 2.11. The Iraqi Amber cultivars were classified as having medium-length seeds, which ranged from 6.0 mm to 5.4 mm.

Amber 43-GSOR, Amber Coarse-GSOR, and Amber Coarse-Pi had the widest seed widths: 2.5, 2.5, 2.6, and 2.5 mm, respectively. The average seed widths of Amber-GSOR and Amber-PI were 2.0 mm and 2.0 mm. There were no significant differences among the seed widths of Jazzman, Antonio, Amber 33-PI, Anber 33, and Amber 33-GSOR.

All of the Iraqi aromatic-rice cultivars were very chalky. High percentages were recorded for Amber Coarse-GSOR (3.9%) and Amber Coarse (3.1%). In general, the U.S. cultivars had lower chalky percentages, such as Jazzman (0.3%), Presidio (0.3%), Della (0.5%), and Antonio (0.9%), as shown in Table 2.10 and Table 2.11.

The highest content of 2-Acetyl-1-pyrroline (2AP) were recorded in the aromatic-rice cultivars Amber 33-GSOR and Amber 33-PI, which had concentrations of 0.78 and 0.75, respectively. Della had a 0.67 and Jazzman had 0.47 concentration of 2AP that was not significantly different when compared with other Amber cultivars, such as Anber 33, Amber-GSOR, and Amber-PI. As expected, the U.S. non-aromatic rice cultivars, such as Antonio and Presidio, produced no aroma (2AP) (supplementary Figure 2.10).

Table 2.8 and Table 2.10 show the effect of replications on two morphological and agronomic traits: flag-leaf area and aroma (2AP) that were measured in 2017. The tables show the significant differences among those replications. However, the other morphological and agronomic traits such as the days required to reach 50% headings, plant heights, number of tillers per meter squared, percentages of the productive tillers, ligule lengths, panicle lengths, number of grains per panicle, sterility percentages, fertility percentages, weights of the thousand-grain samples, grain yields, rice milling, seed lengths, the seed widths, and chalky seed percentage were not significantly different among the replications.



Figure 2.5. Comparison of panicle length among 13 rice cultivars. P = Plant introduction, G = Genetic stocks-oryza collection identification number.

Table 2.8. Mean squares of the ANOVA showing the effects of replications and cultivars on days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, panicle length, number of grains per panicle and sterility percentage of rice cultivars tested at Beaumont, Texas in 2017.

Source	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers (m ²)	Percentage of Productive Tillers (%)	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Grains per Panicle	Sterility Percentage (%)
Rep	3.41 ^{ns} †	10.46 ^{ns}	837.00 ^{ns}	34.31 ^{ns}	26.81*	0.06 ^{ns}	10.13 ^{ns}	81.37 ^{ns}	15.30 ^{ns}
Cultivar	587.80^{**}	1846.34**	7035.01**	94.50^{**}	310.30**	113.06**	49.73**	1503.67**	727.11**
Error	1.63	6.05	410.19	19.62	6.33	0.90	4.45	178.16	88.25

† ns = Non-significant; * = P<0.05; ** = P<0.01

 $\ddagger \%$ = Percentage; cm = Centimeter; cm² = Centimeter squared; m² = Meter square; mm = Millimeter; g = Gram

Table 2.9. Means of agronomic traits of days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, panicle length, number of grains per panicle and sterility percentage of rice cultivars tested at Beaumont, Texas in 2017.

Cultivars	Days to 50% ‡	Plant Height	Number of Tillers	Percentage of Productive	Flag Leaf Area (cm ²)	Ligule Length	Panicle Length	Number of Grains per Panicle	Sterility Percentage
<u> </u>	Heading	(cm)		Tillers (%)		(mm)	(cm)		(%)
Anber 33	125.3	144.7	245.7	86.5	43.3	22.2	29.3	104.8	50.1
Amber 33-PI [†]	122.7	146.3	239.7	87.2	54.8	24.1	28.5	124.8	55.1
Amber -PI	122.7	150.1	267.0	81.9	32.9	25.5	27.8	108.0	52.5
Amber Coarse -PI	108.7	136.3	350.0	97.4	38.9	13.7	21.8	62.7	46.9
Amber 43 -PI	105.3	152.3	235.3	96.8	42.8	12.7	23.7	86.9	57.8
Amber 33 -GSOR	124.3	143.1	255.0	89.1	49.2	25.9	26.3	123.9	46.8
Amber -GSOR	123.3	146.7	264.0	87.8	33.9	24.4	28.3	101.1	45.0
Amber Coarse -GSOR	111.7	139.0	379.3	96.2	36.2	15.1	21.0	58.2	51.6
Amber 43 -GSOR	111.7	153.0	298.0	94.4	28.6	12.8	19.4	74.8	55.6
Jazzman	100.7	97.2	235.7	99.2	28.0	13.9	19.6	121.4	22.6
Della	91.3	100.1	213.0	94.5	20.9	8.4	20.1	105.6	28.8
Presidio	90.7	92.5	231.7	98.3	28.2	12.5	19.2	109.1	12.0
Antonio	86.0	91.0	281.7	97.8	22.2	13.2	18.9	114.9	19.1
Mean	109.6	130.2	268.9	92.9	35.4	17.3	23.4	99.7	41.8
Dongo	86.0-125.3	91.0-	213.0-	81.9-99.17	20.9-54.8	8.4-25.9	18.9-	58.2-124.8	12.0-57.8
Range	00.0-125.5	153.0	379.3			0.4-23.9	29.3		12.0-37.8
CV§	1.2	1.9	7.5	4.8	7.1	5.5	9.0	13.4	22.5
LSD (0.05)	2.15	4.15	34.13	7.46	4.24	1.60	3.56	22.49	15.83

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; m² = Meter square; mm = Millimeter; g = Gram

Table 2.10. Mean squares of the ANOVA showing the effects of replications and cultivars on fertility percentage, thousand grains
weight, grain yield, rice milling, seed length, seed width, chalky seed and aroma of rice cultivars tested at Beaumont, Texas in 2017.

Source	Fertility Percentage (%)‡	Thousand Grain Weight (g)	Grain Yield (kg ha ⁻¹)	Total Rice Milling (%)	Seed Length (mm)	Seed Width (mm)	Chalky Grain (%)	Aroma (2AP)
Rep	15.30 ^{ns} †	0.06 ^{ns}	100282.2 ^{ns}	0.51 ^{ns}	0.005 ^{ns}	0.0002^{ns}	1.13 ^{ns}	0.02^{**}
Cultivar	727.11**	28.95^{**}	17028504.7**	78.23**	0.38^{**}	0.13**	3.43**	0.23**
Error	88.25	0.73	322773.0	8.06	0.01	0.003	0.65	0.002

Cultivars	Fertility Percentage (%)‡	Thousand Grain Weight (g)	Grain Yield (kg ha ⁻¹)	Total Rice Milling (%)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)	Aroma (2AP)
Anber 33	49.9	18.6	2800.0	61.5	5.8	2.1	1.4	0.65
Amber 33-PI†	44.9	17.9	2364.5	61.3	5.8	2.1	1.4	0.75
Amber -PI	47.5	16.7	2114.1	58.9	5.6	2.0	1.4	0.60
Amber Coarse -PI	53.1	25.2	4245.8	60.4	6.0	2.5	3.1	0.04
Amber 43 -PI	42.2	19.4	3085.7	56.3	5.4	2.4	1.4	0.00
Amber 33 -GSOR	53.2	18.3	2698.6	61.3	5.6	2.1	1.6	0.78
Amber -GSOR	55.0	17.3	2674.4	60.5	5.6	2.0	2.0	0.61
Amber Coarse -GSOR	48.4	24.0	4906.1	57.3	6.0	2.5	3.9	0.04
Amber 43 -GSOR	44.4	23.2	3799.8	57.1	5.5	2.5	2.5	0.01
Jazzman	77.4	23.6	7451.5	72.8	6.4	2.2	0.3	0.47
Della	71.2	24.3	6162.8	61.4	6.2	2.2	0.5	0.67
Presidio	88.0	22.9	8507.7	70.5	6.1	2.0	0.3	0.00
Antonio	80.9	23.6	8769.0	67.4	6.6	2.1	0.9	0.00
Mean	58.2	21.1	4583.1	62.0	5.9	2.2	1.6	0.35
Range	42.2-88.0	16.7-25.2	2114.1- 8769.0	56.3-72.8	5.4-6.6	2.0-2.5	0.3-3.9	0.00-0.78
CV§	16.2	4.0	12.4	4.6	1.6	2.4	50.97	11.7
LSD (0.05)	15.83	1.44	957.4	4.79	0.16	0.09	1.36	0.09

Table 2.11. Means of agronomic traits of fertility percentage, thousand grains weight, grain yield, rice milling, seed length, seed width, chalky seed and aroma of rice cultivars tested at Beaumont, Texas in 2017.

[†] PI: plant introduction, GSOR: genetic stocks-oryza collection identification number

CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance<math>CV = Coefficient of variation; LSD = Least significant difference at 5% level of significant difference at 5% le

2.3.1.4. Interaction between Years and Cultivars

Analysis of the three-year data showed significant variations and interactions. Results demonstrated no significant differences for all morphological and agronomic traits across replications except four traits such as days to 50 % heading, plant height, flag leaf area and panicle length and all morphological and agronomic traits except grain yield varied significantly (LSD 0.05) across years. Similarly, the cultivars showed highly significant differences in all morphological and agronomic traits in three years. The results of the interactions between cultivars and years, are shown in Table 2.12 and Table 2.13, Five traits; days to 50 % heading, plant height, number of tillers, flag leaf area, and panicle length, had interactions for three years. However, the rest of the traits had interactions for two years only. The interactions between years and cultivars demonstrated that some morphological and agronomic traits (such as days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, sterility percentage, fertility percentage, thousand grains weight, grain yield, rice milling, seed length and seed width) of cultivars varied significantly (LSD, 0.01) with the years it was grown. Panicle length and grain number per panicle, however, were found stable across years.

Table 2.12. Interaction between years, mean squares of the ANOVA showing the effects of replications, years, cultivars and their interaction between years and cultivars on days to 50 % heading, plant height, number of tillers, percentage of productive tillers, flag leaf area, ligule length, panicle length, number of grains per panicle and sterility percentage of rice cultivars tested at Beaumont, Texas in 2015, 2016 and 2017.

Source	Days to	Plant	Number of	Percentage of	Flag Leaf	Ligule	Panicle	Number of	Sterility
	50%	Height	Tillers	Productive	Area	Length	Length	Grains per	Percentage
	Heading‡	(cm)		Tillers (%)	(cm2)	(mm)	(cm)	Panicle	(%)
Year	5102.19**	856.33**	36371.46**	362.50^{*}	1320.60**	196.25**	28.97 ^{ns}	993.98 ^{ns}	3718.73**
Rep (Year)	1.81^{*} †	23.58^*	287.96 ^{ns}	31.64 ^{ns}	10.41^{**}	0.10 ^{ns}	6.70^{*}	294.69 ^{ns}	8.30 ^{ns}
Cultivar	1214.43**	5967.16**	20056.25^{**}	74.27**	709.14^{**}	368.52^{**}	119.11**	3324.81**	637.02**
Year*Cultivar	68.70^{**}	137.95**	1454.85^{**}	41.42^{**}	104.84^{**}	8.97^{**}	2.15 ^{ns}	123.62 ^{ns}	268.73^{**}
Error	0.65	9.95	148.50	12.53	2.60	0.82	2.80	172.04	50.90

† ns = Non-significant; * = P<0.05; ** = P<0.01

 \ddagger % = Percentage; cm = Centimeter; m² = Meter squared; mm = Millimeter; g = Gram

Year was tested with rep(year) as the error term, whereas cultivar and year*cultivar were tested using the residual error.

Table 2.13 Interaction between years, mean squares of the ANOVA showing the effects of replications, years, cultivars and their interaction between years and cultivars on fertility percentage, thousand grains weight, grain yield, rice milling, seed length, seed width and chalky seed of rice cultivars tested at Beaumont, Texas in 2016 and 2017.

Source	Fertility Percentage	Thousand Grain	Grain Yield	Rice Milling	Seed Length	Seed Width	Chalky Grain
	(%)‡	Weight (g)	(kg ha-1)	(%)	(mm)	(mm)	(%)
Year	3718.73**	100.75**	188267.3 ^{ns}	251.44**	0.14^{**}	0.15^{**}	7.69^{*}
Rep (year)	8.30 ^{ns} †	0.04^{ns}	176933.8 ^{ns}	2.03 ^{ns}	0.002^{ns}	0.002^{ns}	0.59 ^{ns}
Cultivar	637.02**	42.70^{**}	28139691.4**	123.96**	0.65^{**}	0.26^{**}	7.29^{**}
Year*Cultivar	268.73**	3.64**	1561844.9**	20.03**	0.04^{**}	0.01^{**}	0.81^{*}
Error	50.91	0.37	237008.3	6.53	0.01	0.002	0.37

† ns = Non-significant; * = P<0.05; ** = P<0.01

[‡] % = Percentage; g = Gram; kg ha⁻¹ = Kilogram per hectare; mm = Millimeter

Year was tested with rep(year) as the error term, whereas cultivar and year*cultivar were tested using the residual error.

2.3.2. Molecular Diversity in Aromatic Rice

Twenty-seven rice cultivars were included in this study. Twenty-one agronomic and morphological traits were collected data from two locations in Beaumont and Eagle Lake in 2017. All cultivars were genotyped with genotyping-by-sequencing (GBS) for doing cluster and principal coordinate analysis (PCoA). Both locations data were used in conducting the principal component analyses (PCA) of the phenotypic data of aromatic rice cultivars as shown in Table 2.14, Table 2.15 and Table 2.16.

2.3.2.1. Cluster of Aromatic Rice Cultivars

To understand the evolutionary status of rice species, the amount of genetic diversity within a species is an important factor to consider. All entries were genotyped with GBS, and marker calls were made in TASSEL 5.0. The marker data were recoded to numeric values based on the allele calls and used for hierarchical clustering that implemented the "Euclidean distance" and "hclust" functions in R 3.4. An average distance was used to compute the distance matrix, and the distance matrix was used for clustering. The 27 accessions were grouped into five distinct clusters (Figure 2.6), as shown in Table 2.14, Table 2.15 and Table 2.16. The first group had four accessions (Basmati PI-385456, Amber 43-GSOR, Amber Coarse-GSOR, and Amber Coarse-PI); the second had two accessions (Amber 43-PI and Basmati-PI-385471); the third had 13 accessions (Amber-PI, Amber 33-PI, Anber 33, Amber 33-GSOR, Amber-GSOR, Basmati 5853-PI, Basmati 6313-PI, Basmati-PI-431251, Basmati 37-PI, Basmati 5874-PI, Basmati Pardar-PI, Basmati Medium-PI, and Basmati-PI-385817); the fourth had two accessions (Basmati T3-PI and Scented A-PI); and the fifth had six accessions (Antonio, Presidio, Dellmont-PI, Della-Clor, Della, and Jazzman). The height in the dendrogram was the average distance calculated based on similarities or dissimilarities among the lines or clusters. The higher the height of the split in the dendrogram, the

more dissimilar the lines or clusters were. Using genetic diversity can improve the target traits of rice for the rice breeder (Salgotra et al., 2015). The height among the five clusters and between each group showed dissimilarities, but the analysis detected the genotypes that were derivatives of genetically similar types (GSOR and its PI source, e.g., Amber 33, GSOR-310278 was derived from PI 326029) and were clustered together. Choudhury et al. (2013) studied rice cultivars from India and, found two clusters within 24 indigenous and improved rice. In addition, Das et al. (2013) found four groups among a set of twenty-six rice cultivars. The Iraqi aromatic accessions were distributed among three clusters: The majority of the cultivars were placed in the third cluster, a few were placed in the first, and only one was placed in the second. All of the aromatic and nonaromatic U.S. rice cultivars were located only in the fifth cluster. The Basmati cultivars were distributed among the third, fourth, first, and second clusters. Roy et al. (2015) based on using SSR marker analysis reported three major groups from a set of 107 Indian aromatic rice cultivars. Islam et. al. (2018) used 12 qualitative phenotypic traits to classify the 113 cultivars into four groups.

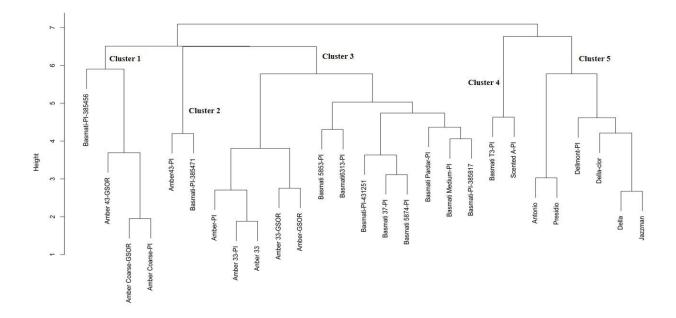


Figure 2.6. Cluster dendrogram showing the genetic relationships among 25 aromatic rice cultivars and 2 non-aromatic rice cultivars.

2.3.2.2. Principal Coordinate Analyses of the Genotypic Data of Aromatic Rice Cultivars

A principal coordinate analysis (PCoA) was used on the genotypic data of 25 aromatic-rice cultivars and 2 nonaromatic rice cultivars. A PCoA is similar to a PCA, which is used to visualize similarities or dissimilarities of data. Using the PCoA to analyze the genetic diversity and population structure of rice germplasm from north-eastern region of India, Choudary et al. (2014) found that the PCoA method obtained accessions that were distributed according to their population structure. For the current study, a PCoA was used on the genotypic data, 24,257 markers in total, to understand the relations among the 27 cultivars (supplementary Table 2.18) and the distributions among and within the cultivars, the rice cultivars and markers data with different genotypic traits. Qualitative and quantitative phenotypic characteristics can impact the genetic diversity of rice cultivars (Sun et al., 2009). Using molecular markers such as SSRs or SNPs are useful to determine the structure of the population (Singh et al., 2013). Islam et. al. (2018) determined correlation between phenotypic and genotypic traits of the tested cultivars which obtained significant statistical relationship between two groups of data. Most of the genotypic variance within the data was explained by the first three principal components (PCo1 = 45%, PCo2= 11%, and PCo3 = 6%). The results obtained for the cultivars were divided into five clusters, which included two clusters of Amber rice cultivars, two clusters of Basmati rice cultivars, and one cluster of U.S. cultivars; all of the clusters were shown as three major groups (Figure 2.7). The first group, represented by the green color, included the Amber aromatic cultivars from Iraq. The second group, represented by the red color, included the Basmati rice cultivars. The third group, represented by the purple color, included the U.S. rice cultivars. The PCoA explained the similarities within a group and differences among the groups.

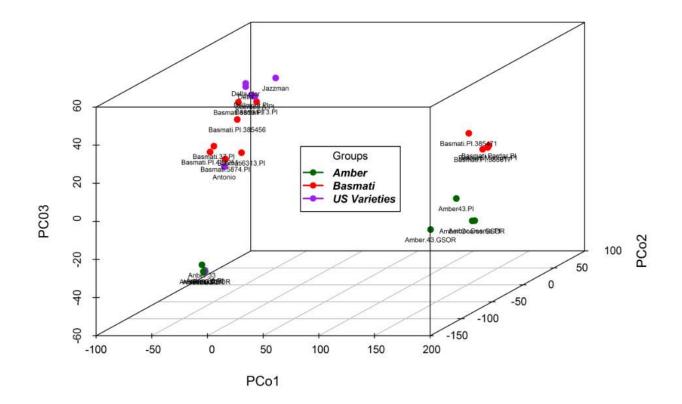


Figure 2.7. Principal co-ordinate analysis of genotypic markers and relationships among 25 aromatic rice cultivars and 2 non-aromatic rice cultivars.

2.3.2.3. The Principal Component Analyses of the Phenotypic Data of Aromatic Rice Cultivars

A PCA visualizes the relations among observations based on multiple variables. The goal of a PCA is to decompose data collected with correlated measurements into a new set of visualizing relations among this data in an easy way. A PCA was used to make the data set less complex and easy to comprehend, and it kept the difference within the data set as far as possible (Ringnér, 2008). The PCA showed that seven independent principal components caused about 75% of the differences (Rabara et al., 2014). The PCA was performed with data of 21 agronomic and morphological traits: the number of days needed to reach 50% of the headings, the plant heights, the number of tillers per plant, the flag-leaf areas, the ligule lengths, the panicle lengths, the number of panicles per plant, the number of branches per panicle, the number of grains per panicle, the number of unfilled grains per panicle, the number of filled grains per panicle, the sterility percentages, the fertility percentages, the weights of the thousand-grain samples, the grain yields per plant, the grain yields per entry, the rice milling total, the seed lengths, the seed widths, the % chalk in grain, and the 2AP content (supplementary Table 2.17) to study the phenotypic diversity of aromatic rice from Iraq, and various sources. A PCA is a dimensionality-reduction multivariate technique that enables the studying and visualizing of the relations among observations based on multiple variables. In this case, the observations were the rice cultivars, and the variables were the different phenotypic traits. Most of the phenotypic variance within the data was explained by the first three principal components (PC1 = 23%, PC2 = 21%, and PC3 = 16%). The first three PCs were plotted in a three-dimensional scatterplot to visualize the grouping and relations among the cultivars. PC1 was grouping or separating the cultivars based on the morphological traits, such as the plant heights, the number of tillers per plant, the number of panicles per plant, the number of

branches per panicle, the number of grains per panicle, and so forth. PC2 was a grouping of the variables based on the yield-, seed-, and grain-related traits such as the weights of the grains, the sterility percentages, the fertility percentages, the weights of the thousand-grain samples, the yields per plant, the number of empty seeds, and so forth. The 27 cultivars were roughly divided into three groups: Amber aromatic rice from Iraq, Basmati, and US cultivars. The groups were color coded and divided into three major groups to aid visualization (Figure 2.8). The first group was represented by the green color, and it includes the Amber class of Iraqi aromatic-rice cultivars, which comprise 9 cultivars (Amber-PI, Amber 33-PI, Anber 33, Amber 33-GSOR, Amber-GSOR, Amber 43-GSOR, Amber 43-PI, Amber Coarse-GSOR, and Amber Coarse-PI). The second group was represented by the red color and included the Basmati rice cultivars, which comprised 11 cultivars (Basmati PI-385456, Basmati PI-385471, Basmati 5853-PI, Basmati 6313-PI, Basmati PI-431251, Basmati 37-PI, Basmati 5874-PI, Basmati Pardar-PI, Basmati Medium-PI, Basmati-PI-385817, and Basmati T3-PI). The third group was represented by the purple color and included six US rice cultivars and 1 cultivar originally from Japan (Antonio, Presidio, Dellmont-PI, Della-Clor, Della, Jazzman, and Scented A-PI). Naz et. al. (2006) reported the relationship between two groups of data is very desirable and had significant value and used for selection because phenotypic traits are dependent on genotypic traits. Islam et. al. (2018) demonstrated the population structure analysis also revealed three populations, P1, P2 and P3, with a majority of cultivars in population one. Which means this grouping agrees with genetic distance based clustering and principal component analysis, PCA1 was 17.67% and PCA2 was 2.03%.

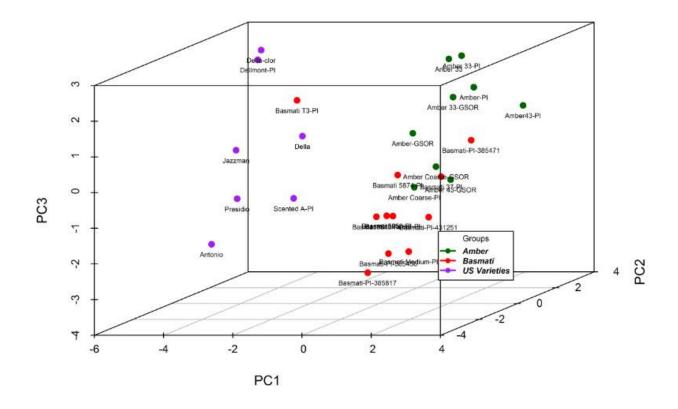


Figure 2.8. Principal component analysis of phenotypic traits, showing the relationships among 25 aromatic rice cultivars and 2 non-aromatic rice cultivars.

2.3.2.4. Correlation Among Traits

Correlation coefficients revealed the relations between two or more phenotypic traits. Several phenotypic traits showed distinct relations among themselves (Figure 2.9). A highly positive correlation between the number of panicles per plant and number of tillers per plant was observed (r = 0.96; P < 0.05), as well as a highly positive correlation between the filled grains and the number of grains per panicle (r = 0.95; P < 0.05). However, a negative correlation between the plant heights and the lengths of the seeds was observed. In addition, a negative correlation between the weights of the thousand-seed samples and the panicle lengths was noted (r = -0.50; P < 0.05). Karim et al. (2014) found the highest positive indirect effect for thousand grain weight by plant height and the highest negative indirect effect for thousand grain weight by number of filled grains per panicle. Also, the result showed the number of panicles per hill found to display significant positive correlation with grain yield per hill only at phenotypic level.

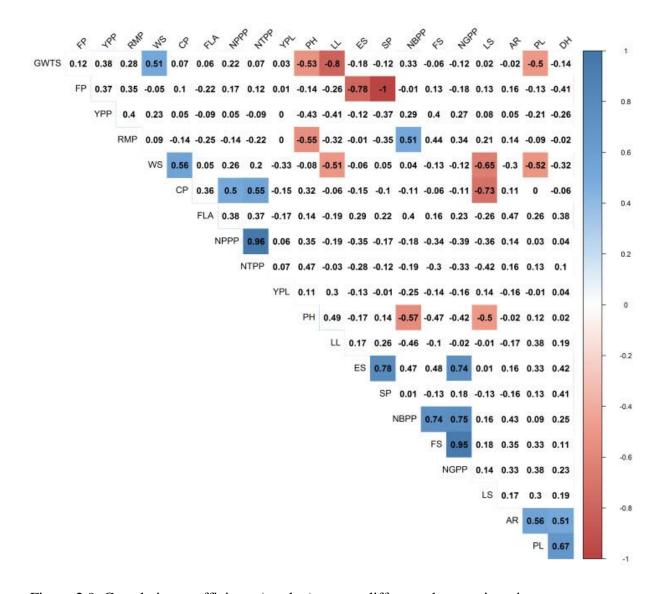


Figure 2.9. Correlation coefficients (r value) among different phenotypic traits. GWTS = Grains weight thousand seeds, FP = Fertility percentage, YPP = Yield per plant, RMP = Rice milling percentage, WS = Width seed, CP = Chalky percentage, FLA = Flag leaf area, NPPP = Number panicle per plant, NTPP = Number of tillers per plant, YPL = Yield per line, PH = Plant height, LL = Ligule length, ES = Empty seeds, SP = Sterility percentage, NBPP = Number branch per panicle, FS = Full seeds(filled grains), NGPP = Number of grains per panicle, LS = Length seed, AR = Aroma, PL = Panicle length and DH = Days to 50 % heading. The color highlighting is the value signify.

Table 2.14. Means of agronomic traits of days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of 27 rice cultivars tested for both locations Beaumont and Eagle Lake, Texas in 2017.

Cultivars	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicle Per plant	Number of Branch per Panicle	Number of Grains per Panicle (g)
Amber33 PI†	99.1	138.3	11.8	43.2	17.3	25.9	10.7	12.1	132.3
Amber PI	102.5	135.7	11.0	35.0	19.7	26.6	9.5	11.7	138.7
Amber Coarse PI	91.0	113.0	13.5	28.7	11.2	19.5	12.5	8.6	58.6
Amber43 PI	91.5	153.9	11.0	41.6	8.9	21.6	10.0	9.3	84.0
Amber33 GSOR	98.0	135.2	13.5	35.2	13.4	23.8	10.5	11.2	101.3
Amber GSOR	99.5	140.6	12.0	33.8	17.9	25.9	9.3	11.8	116.5
Amber Coarse GSOR	89.0	133.5	14.8	30.6	11.8	19.4	14.3	8.7	62.6
Amber43 GSOR	92.5	127.6	11.0	27.1	8.9	19.5	9.8	8.5	65.8
Anber	100.5	142.0	13.0	37.7	17.6	27.1	10.3	14.0	134.7
Jazzman	100.8	93.6	5.8	32.9	8.0	21.9	5.6	17.6	145.5
Della	99.8	117.8	7.0	29.6	7.7	23.1	6.2	17.8	134.0
Antonio	78.5	91.1	5.1	20.6	10.6	20.4	5.0	15.9	148.1
Presidio	80.0	120.2	7.0	27.8	11.7	20.5	6.8	15.5	168.5
Della Clor	99.5	102.4	10.0	40.3	5.3	24.6	9.8	18.7	176.7
Basmati T3	70.5	106.0	9.0	36.0	5.3	20.3	8.8	12.7	133.2
Scented A	82.5	126.5	6.0	19.7	9.3	19.0	5.8	10.5	124.2
Basmati	76.0	131.9	9.0	29.2	9.3	21.8	8.8	8.7	63.5
Basmati	83.0	135.0	8.0	30.8	16.3	17.6	5.8	11.7	105.5
Basmati Pardar	87.5	129.0	9.0	27.8	19.3	24.2	6.3	12.2	157.2
Basmati Medium	87.0	126.4	4.0	25.7	22.3	20.8	3.8	9.5	119.0

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

Table 2.14. Continued

Cultivars	Days to	Plant Usisht	Number of	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Cultivars	50%‡	Height	Tillers per	Area (am^2)	Length	Length	of Panicle	Branch per	Grains per
	Heading	(cm)	Plant	(cm^2)	(mm)	(cm)	Per plant	Panicle	Panicle (g)
Basmati	87.5	127.9	6.0	26.7	18.3	22.0	5.8	11.0	98.5
Basmati 6313	86.5	121.7	11.0	22.9	12.3	25.0	9.5	9.2	94.0
Basmati 37	104.5	113.0	8.0	25.7	19.3	27.7	6.8	9.2	119.7
Basmati 5853	88.0	119.4	7.0	35.5	16.3	21.6	6.8	9.5	107.0
Basmati 5874	101.5	111.5	6.0	27.6	13.3	25.9	5.8	9.5	112.2
Basmati	104.0	126.5	4.0	29.9	16.3	24.8	3.8	8.5	99.5
Dellmont	102.5	92.0	7.0	44.2	4.3	22.6	6.8	21.7	143.7

Table 2.15. Means of agronomic traits of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, grain yield per line, rice milling and seed length of 27 rice cultivars tested for both locations Beaumont and Eagle Lake, Texas in 2017.

Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line¥ (g)	Rice Milling¥ (%)	Seed Length (mm)
Amber33 PI ⁺	32.8	99.5	25.1	75.0	19.2	12.9	232.2	60.5	5.5
Amber PI	45.3	93.2	31.2	68.8	17.7	10.1	219.6	62.6	6.0
Amber Coarse PI	10.9	47.5	19.2	80.8	23.8	14.5	260.3	64.4	6.1
Amber43 PI	28.8	54.9	35.1	64.9	21.4	9.8	193.4	54.7	5.6
Amber33 GSOR	19.7	81.4	19.2	80.8	20.6	13.3	218.6	59.0	5.6
Amber GSOR	15.4	100.8	13.4	86.6	19.0	16.9	187.4	63.5	6.0
Amber Coarse GSOR	13.2	49.2	22.2	77.8	24.8	16.1	318.1	65.2	6.0
Amber43 GSOR	15.0	50.6	22.8	77.2	22.7	12.2	117.4	56.0	5.9
Anber	34.5	100.0	25.9	74.1	20.5	12.5	153.8	61.8	5.8
Jazzman	36.7	108.8	25.4	74.6	23.9	17.0	249.1	72.7	7.0
Della	35.0	98.9	26.5	73.5	22.5	17.4	200.3	65.4	6.7
Antonio	28.0	120.1	18.7	81.3	23.4	19.5	316.4	69.4	6.5
Presidio	30.4	137.8	18.2	81.8	22.7	19.4	350.9	72.4	6.8
Della Clor	46.8	129.7	26.5	73.5	22.6	23.8	123.0	63.7	6.8
Basmati T3	25.0	108.0	18.1	81.9	22.9	22.2	76.7	62.2	5.5
Scented A	19.7	104.3	15.1	84.9	22.6	19.8	136.6	68.3	5.4
Basmati	4.3	59.0	7.8	92.2	20.2	9.3	144.7	63.6	6.7
Basmati	44.7	60.5	38.6	61.4	21.8	8.5	163.5	57.3	5.3
Basmati Pardar	34.0	122.9	22.0	78.0	18.4	7.4	264.1	60.1	6.7
Basmati Medium	35.0	83.7	30.4	69.6	17.5	13.5	112.3	62.6	6.7

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

‡ % = Percentage; g = Gram; mm = Millimeter

¥ Trait measured at one location in Beaumont

Table 2.15. Continued

Cultivars	Number of Unfilled Grains per	Number of Filled Grains per	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight	Grain Yield per Plant	Grain Yield per line¥	Rice Milling¥ (%)	Seed Length (mm)
Basmati	Panicle 15.3	Panicle 82.9	16.0	84.0	(g) 18.1	(g) 18.3	(g) 195.9	63.0	6.7
Basmati 6313	16.7	77.1	18.5	81.5	22.1	15.9	200.8	61.5	7.7
Basmati 37	37.5	82.0	31.9	68.2	19.0	10.1	166.2	61.0	6.7
Basmati 5853	22.5	84.3	21.5	78.5	22.6	18.2	335.6	53.4	6.8
Basmati 5874	33.0	79.0	29.6	70.4	22.0	12.6	116.3	59.8	6.8
Basmati	28.7	70.6	27.8	72.3	20.4	10.1	333.9	60.5	6.8
Dellmont	30.1	113.4	20.5	79.5	24.7	10.3	46.4	65.7	6.8

Lines and Cultivars	Seed Width	Chalky	Aroma¥
Lines and Cultivars	(mm) ‡	Seed (%)	(2AP)
Amber33 PI ⁺	2.2	2.5	0.75
Amber PI	2.1	2.0	0.60
Amber Coarse PI	2.6	1.6	0.04
Amber43 PI	2.6	1.5	0.00
Amber33 GSOR	2.2	2.7	0.78
Amber GSOR	2.1	2.4	0.61
Amber Coarse GSOR	2.5	1.7	0.04
Amber43 GSOR	2.6	1.5	0.01
Anber	2.3	2.0	0.65
Jazzman	2.3	0.5	0.47
Della	2.3	0.6	0.67
Antonio	2.2	0.7	0.00
Presidio	2.2	1.8	0.00
Della Clor	2.2	0.3	0.85
Basmati T3	3.3	3.4	0.02
Scented A	3.0	1.8	0.32
Basmati	2.0	0.4	0.69
Basmati	2.8	1.6	0.07
Basmati Pardar	1.7	0.4	0.02
Basmati Medium	1.9	0.5	0.02

Table 2.16. Means of agronomic traits of seed width, chalky seed and aroma of 27 rice cultivars tested for both locations Beaumont and Eagle Lake, Texas in 2017.

[†] PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

‡ mm = Millimeter; % = Percentage; 2AP = 2-Acetyl-1-pyrroline

¥ Trait measured at one location in Beaumont

Table	2.16.	Continued
1 4010	2.10.	Commuca

Lines and Cultivars	Seed Width (mm)‡	Chalky Seed (%)	Aroma¥ (2AP)
Basmati	1.9	0.4	0.03
Basmati 6313	2.0	1.3	0.51
Basmati 37	2.4	0.6	0.07
Basmati 5853	1.9	0.7	0.37
Basmati 5874	1.9	0.9	0.50
Basmati	2.0	1.1	0.43
Dellmont	2.4	1.5	0.73

2.4. Conclusions

The conclusion to these results are:

- Perhaps aroma was associated with specific agronomic, morphological, or physiological traits. The high positive indirect effect was observed for aroma via panicle length and aroma via days to 50 % heading.
- Phenotypic diversity analysis indicated that aromatic cultivars such as Amber, Amber 33, and Anber 33 exhibit close similarities of their agronomic, botanical, phenological, morphological, and physiological traits. In addition, cultivars such as Amber Coarse and Amber 43 share similarities in their agronomic, botanical, phenological, morphological, and physiological traits.
- Iraqi aromatic-rice cultivars had two groups based on aroma trait. Those in the first group have higher aromas, such as Amber, Amber 33, and Anber33, and those in the second group have lower aromas, such as Amber Coarse and Amber 43.
- The Iraqi aromatic cultivars were distributed among three clusters based on genotyping data: The majority of the cultivars were placed in the third cluster, a few were placed in the first, and only one was placed in the second.
- Using both phenotyping and genotyping data in PCoA, the aromatic cultivars were group mainly based on geographic origin.
- The aroma trait is related to the grain yields. For example, cultivars such as Amber, Amber 33, and Anber 33 produce lower grain yields (kg ha⁻¹) with higher aromas, and Amber Coarse and Amber 43 produce higher grain yields (kg ha⁻¹) with lower aromas. A highly positive correlation between the number of panicles per plant and number of tillers per plant was observed.

2.5. References

- Catchen J, S. Bassham, T. Wilson, M. Currey, C. O'Brien, Q. Yeates, and WA. Cresko. 2013. The population structure and recent colonization history of Oregon threespine stickleback determined using RAD-seq. Mol. Ecol. 22: 2864–2883.
- Chakravarthy, B.K. and R. Naravaneni. 2006. SSR marker based DNA finger printing and diversity study in rice (Oryza Sativa L.). Afr. J. Biotech. 5(9): 684- 688.
- Choudhury D. R., N. Singh, A.K. Singh, S. Kumar, K. Srinivasan, R.K. Tyagi, A. Ahmad, N. K. Singh, and R. Singh. 2014. Analysis of genetic diversity and population structure of rice germplasm from north-eastern region of India and development of a core germplasm set. PLoS ONE 9(11): e113094. <u>https://doi.org/10.1371/journal.pone.0113094</u>
- Choudhury, B., M. L. Khan, and S. Dayanandan. 2013. Genetic structure and diversity of indigenous rice varieties (Oryza sativa) in eastern Himalayan region of northeast India. Springer Plus. 2: 228–237, https://doi.org/10.1186/2193-1801-2-228 PMID: 23741655.
- Das, B., S. Samik, K. P. Swarup, R. Bipasha, G. Mrityunjay, P. Manoj, and K. G. Tapas. 2013. Genetic diversity and population structure of rice landraces from eastern and north eastern states of India. BMC Genet. (14)(71): https://doi.org/10.1186/1471-2156-14-71 PMID: 23945062.
- Dela, Cruz and G. S. Khush. 2000. Effect of temperature during grain development on stability of cooking quality component in rice. Jpn. J. Breed., 39: 299-306.
- Doyle, J.J. and J.E. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19: 11–15.
- Dramé, K. N., I., Sanchez, G., Gregorio, and M. N. Ndjiondjop. 2013. Suitability of a selected set of simple sequence repeats (SSR) markers for multiplexing and rapid molecular characterization of African rice (Oryza glaberrima Steud.). Afr. J. Biotechnol. 10: 6675-6685.
- Food and Agriculture Organization, FAO .2000. Rice Information Volume 2, January.
- Garris, A. J., T. H. Tai, J. Coburn, S. Kresovich, and S. McCouch. 2005. Genetic structure and diversity in Oryza sativa L. Genetics 169: 1631–1638.
- Hossain, M. F., M. S. U. Bhuiya, and M. Ahmed. 2005. Morphological and agronomic attributes of some local and modern aromatic rice varieties. Oryza. 34(3): 201-208.
- International Rice Research Institute. 1972. Annual Report for 1971. Los Baños, Philippines. 238 p.

- Islam, M. Z., M. Khalequzzaman, M. K. Bashar, N. A. Ivy, M. A. K. Mian, B. R. Pittendrigh, M. M. Haque, and M. P. Ali. 2018. Variability assessment of aromatic rice germplasm by pheno-genomic traits and population structure analysis. Sci. Rep. 8: 9911.
- Karim, D., N.A. Siddique, U. Sarkar, M.Z. Hasnat, and J. Sultana. 2014. Phenotypic and genotypic correlation co-efficient of quantitative characters and character association of aromatic rice. J. Biosci.Agric. Res. 01(01): 34-46.
- Liu, S., C. Griffey, M. Hall, A. McKendry, J. Chen, W. Brooks, G. Brown-Guedira, D. Van Sanford, and D.G. Schmale. 2013. Molecular characterization of field resistance to fusarium head blight in two US soft red winter wheat cultivars. Theor. Appl. Genet. 126: 2485-2498. doi:10.1007/s00122-013-2149-y.
- Naik, D., A. Sao, S.K. Sarawagi, and P. Singh 2006. Genetic divergence studies in some indigenous scented rice (Oryza sativa L.) accessions of central India. Asian J. Plant Sci. 5: 197-200.
- Nakano, M., A. Yoshimura and N. Iwata. 1992. Phylogenetic study of cultivated rice and its wild relatives by RFLP. Rice Genet. Newsl. 9: 132-134.
- Naz, N. A. and M. Ahmad. 2006. Genetic and phenotypic correlations for some sexual maturity traits in nili ravi bufalo heifers. Pakistan Vet. J. 26(3): 141–143.
- Mathure, S., A. Shaikh, N. Renuka, K. Wakte, N. Jawali, R. Thengane, and A. Nadaf. 2011. Characterisation of aromatic rice (Oryza sativa 1.) Germplasm and correlation between their agronomic and quality traits. Euphytica.179(2): 237-246.
- Mgonja, E.M., C.H. Park, H. Kang, E.G. Balimponya, S. Opiyo, M. Bellizzi, S.K. Mutiga, F. Rotich, V.D. Ganeshan, R. Mabagala, C. Smeller, J. Correll, B. Zhou, N.J. Talbot, T.K. Mitchell, and G.L. Wang. 2017. Genotyping-by-sequencing-based genetic analysis of African rice cultivars and association mapping of blast resistance genes against Magnaporthe oryzae populations in Africa. Phytopathol. 107(9): 1039–1046.
- Rabara, R.C., M.C. Ferrer, C.L. Diaz, M.C.V. Newingham, and G.O. Romero. 2014. Phenotypic diversity of farmers' traditional rice varieties in the Philippines. Agron. 4(2): 217-241.
- Rabbani, M., Z. Pervaiz and M. Masood. 2008. Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (Oryza sativa L.) using RAPD markers. Electron. J Biotechnol. 11(3).
 http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v11n3-3/11

Ringnér, M. 2008. What is principal component analysis? Nat. Biotechnol. 26: 303–304.

- Roy, S., A. Banerjee, B. Mawkhlieng, A.K. Misra, A. Pattanayak, G.D. Harish, S. K. Singh, S. V. Ngachan, and K. C. Bansal. 2015. Genetic diversity and population structure in aromatic and quality rice (Oryza sativa L.) landraces from north-eastern India. PLoS ONE. 10(6): e0141405, <u>https://doi.org/10.1371/journal.pone.0129607</u>.
- Saha, P., M. Islam, M. Islam, and M. Salam. 2015. Analysis of yield components and aroma of small grain aromatic rice (Oryza sativa L.) in Bangladesh. The Agriculturists. 13(2): 17-24.
- Salgotra, R. K., B. B. Gupta, J. A. Bhat, and S. Sharma. 2015. Genetic diversity and population structure of basmati rice (Oryza sativa L.) germplasm collected from northwestern Himalayas using trait linked SSR markers. PLoS ONE, 10(7), e0131858, https://doi. org/10.1371/journal.pone.0131858.
- Sekhar, B.P.S. and G.M. Reddy. 1982. Amino acid profiles in some scented rice varieties. Theor. Appl. Genet. 62: 35-37.
- Singh, R.K., U.S. Singh, and G.S. Khush. 2000. Aromatic Rices. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp: 1-4.
- Singh, N., D.R. Choudhury, A.K. Singh, S. Kumar, K. Srinivasan, R. K. Tyagi, N. K. Singh, and R. Singh. 2013. Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. PLoS ONE. 8(12): e84136, https://doi.org/10.1371/journal.pone.0084136.
- Sun, X. P. and Q.W. Yang. 2009. Comparative study on genetic diversity of wild rice (Oryza rufpogon Grif.) in China and three countries in southeast Asia. Acta Agron. Sinica. 35(4): 679–684.
- Tahir, M., D. Wandan, and A. Zada. 2002. Genetic variability of different plant yield characters in rice. Sarhad J. Agric. 18(2): 22-27.
- Teixeira da Silva, J.A. 2005. Molecular markers for phylogeny, breeding and ecology in agriculture. In: Thangadurai. D., Pullaiah, T., Tripathy. L. (Eds) Genetic Resources and Biotechnology, Regency Publications, New Delhi, India (Vol III): 221-256.
- Tu Anh, T.T., T.D. Khanh, T.D. Dat, and T.D. Xuan. 2018. Identification of phenotypic variation and genetic diversity in rice (Oryza sativa L.) mutants. Agriculture. 8(2): 30.

United States Department of Agriculture (USDA).2016. https://ipad.fas.usda.gov/highlights/2016/12/Iraq/Index.htm

Wang, Z.Y. and S.D. Tanksley. 1989. Restriction fragment length polymorphism in Oryza sativa L. Genome. 32: 1113-1118.

- Younan, H.Q., A.K. Al-kazaz, and B.K. Sulaiman. 2011. Investigation of genetic diversity and relationships among a set of rice varieties in Iraq using random amplified polymorphic DNA (RAPD) analysis. Jordan J. Bio Sci. 4: 249-256.
- Zong, G., A. Wang, L. Wang, G. Liang, M. Gu, T. Sang, and H. Han. 2012. A pyramid breeding of eight grain-yield relate quantitative trait loci based on marker-assistant and phenotype selection in rice (Oryza sativa L.). J. Genet. Genomics. 39(7): 335-350.

Entry	Traits	PC1	PC2	PC3	PC4	
Number						
1	PH†	0.35	0.13	0.00	-0.09	
2	NTPP	0.28	-0.06	0.30	-0.21	
3	NPPP	0.26	-0.13	0.30	-0.20	
4	СР	0.18	-0.12	0.34	-0.01	
5	LL	0.14	0.30	-0.24	-0.09	
6	WS	0.09	-0.24	0.27	0.36	
7	SP	0.04	0.34	0.09	0.39	
8	FLA	0.03	0.13	0.42	-0.08	
9	YPL	0.00	-0.02	-0.19	-0.10	
10	DH	-0.03	0.32	0.18	-0.15	
11	PL	-0.04	0.32	0.10	-0.36	
12	FP	-0.04	-0.34	-0.09	-0.39	
13	AR	-0.09	0.13	0.27	-0.37	
14	GWTS	-0.10	-0.30	0.17	0.17	
15	ES	-0.21	0.33	0.16	0.26	
16	YPP	-0.21	-0.26	0.07	-0.04	
17	LS	-0.25	0.06	-0.27	-0.21	
18	RMP	-0.29	-0.19	0.00	-0.07	
19	NBPP	-0.36	0.00	0.27	0.00	
20	NGPP	-0.37	0.13	0.15	0.01	
21	FS	-0.38	0.02	0.12	-0.10	

Table 2.17. Principal component analysis (PCA) of phenotypic traits.

[†] PH = Plant height, NTPP = Number of tillers per plant, NPPP = Number panicle per plant, CP = Chalky percentage, LL = Ligule length, WS = Width seed, SP = Sterility percentage, FLA = Flag leaf area, YPL = Yield per line, DH = Days to 50 % heading, PL = Panicle length, FP = Fertility percentage, AR = Aroma, GWTS = Grains weight thousand seeds, ES = Empty seeds, YPP = Yield per plant, LS = Length seed, RMP = Rice milling percentage, NBPP = Number branch per panicle, NGPP = Number of grains per panicle and FS = Full seeds.

Entry	Cultivars	PCo1	PCo2	PCo3
Number				
1	Basmati.Pardar.PI ⁺	160.03	13.07	31.62
2	Basmati.PI.385817	152.54	14.01	31.90
3	Amber.Coarse.PI	152.47	1.29	-47.03
4	Basmati.Medium.PI	149.68	13.18	37.14
5	Amber.Coarse.GSOR	148.57	0.36	-50.17
6	Basmati.PI.385471	132.68	4.72	41.61
7	Amber43.PI	118.67	30.58	-35.41
8	Amber.43.GSOR	97.92	26.07	-62.44
9	Basmati6313.PI	-8.30	-86.59	15.66
10	Basmati.5874.PI	-11.63	-103.31	14.30
11	Basmati.37.PI	-19.10	-104.14	25.06
12	Basmati.PI.431251	-28.76	-103.83	21.90
13	Basmati.PI.385456	-42.19	-21.64	23.48
14	Jazzman	-42.50	38.48	41.43
15	Scented.A.PI	-63.27	44.59	23.38
16	Basmati.T3.PI	-63.50	44.98	21.44
17	Basmati.5853.PI	-69.73	32.47	18.52
18	Dellmont.PI	-71.46	51.49	27.41
19	Della.clor	-72.49	49.62	32.19
20	Anber.33	-74.74	-11.51	-34.64
21	Della	-74.94	50.33	29.23
22	Amber.GSOR	-75.70	-6.75	-40.88
23	Amber.PI	-77.76	-6.66	-41.47
24	Amber.33.GSOR	-77.96	1.02	-39.55
25	Presidio	-79.12	-0.21	-40.38
26	Amber.33.PI	-79.39	0.75	-40.58
27	Antonio	-80.00	27.64	-3.70

Table 2.18. Principal co-ordinate analysis (PCoA) of phenotypic traits.

† PI: plant introduction; GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

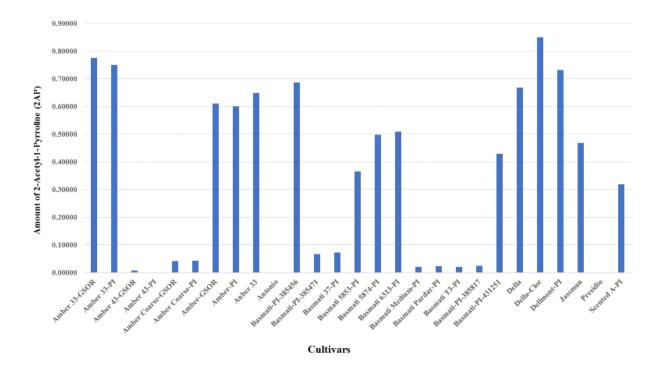


Figure 2.10. The amount of aroma as 2-Acetyl-1-Pyrroline (2AP) among 27 cultivars.

CHAPTER III

DETERMINING VARIABILITY IN AGRONOMIC AND MORPHOLOGICAL TRAITS, AND MAPPING QUANTITATIVE TRAIT LOCI AND GENES ASSOCIATED WITH AROMA IN A RICE RECOMBINANT INBRED POPULATION

3.1. Introduction

Aromatic rice (Oryza sativa) cultivars are preferred in most countries in Asia and the Middle East because this type of rice has a unique characteristic associated with aroma. Aromatic rice cultivars such as Jasmine and Basmati are extremely popular around the world; farmers have cultivated the Basmati rice cultivar for hundreds of years (Siddig et al., 2012). Demand for aromatic rice is increasing worldwide, especially in the Middle East, Europe, and the United States (Mahajan et al., 2018). Previous studies aimed to characterize the physiological and morphological traits of rice cultivars. Accordingly, studying agronomic and morphological characterization is fundamental to providing plant-breeding programs with information about Iraqi aromatic rice cultivars. Most existing studies have focused on investigating properties of Iraqi rice within and among local cultivars, but it is important to study the differences between Iraqi and United States aromatic rice cultivars as well. Rice breeders want to better understand the relationships among aromatic rice cultivars, including Amber rice, and variability in their agronomic, morphological, and genetic characteristics, as well as the distribution of Amber rice parentage in United States and world rice cultivars. Diversity within the rice germplasm is essential for agricultural development and thus for increasing food production and promoting economic growth. Genetic diversity is required for all crops, particularly rice, to improve yield, aroma, and tolerance to biotic and abiotic stresses. The amount of genetic diversity within rice is important for understanding the evolutionary status of this crop and the relationships among the indica, japonica, and aromatic rice groups. The first mapping of grain aroma in rice occurred in 1992 (Ahn et al., 1992); 12 years later, scientists identified the gene controlling 2-Acetyl-1-pyrroline (2AP) responsible for grain aroma (Vanavichit et al., 2004, 2005). The accumulation of 2AP in the aromatic rice phenotype could be regulated by genetic effect only or by a combination of genetic effect and environmental conditions. Genetic differences for a quantitative trait might be controlled by one gene or by the collective effects of many genes, or quantitative trait loci (QTLs). It is challenging to identify QTLs using only traditional phenotypic evaluation and without linked DNA markers; using molecular markers helps identify QTLs and is essential in mapping the genome of plants and thus improving the crops through breeding programs (Asins, 2002). The two most commonly used methods for QTL mapping are association mapping and linkage analysis. Use of QTL mapping is a common strategy for discovering genes associated with many important quality traits in rice. Many mapping studies have been conducted in the past decade to identify QTLs for aroma (Ahn et al., 1992; Tian et al., 2005; Amarawathi et al., 2008; Ahamadi et al., 2008). One of the main objectives of the present study is to construct a GBS-based linkage map of rice. Although there are many traditional and improved cultivars of rice available in Iraq, no study has produced a complete characterization or systematic analysis on their genetic base and diversity (Younan et al., 2011). This study aims to improve understanding of diversity among Iraqi aromatic rice cultivars. In addition, genetic control of these traits in Iraqi aromatic rice is not fully understood. This study aims to understand the differences pertaining to aroma among Amber cultivars at the molecular level.

3.2. Materials and Methods

3.2.1. Plant Material

This study involved 147 rice lines and cultivars, including 120 rice lines of F₇ seed population developed as recombinant inbred lines (RILs), nine aromatic rice cultivars from Iraq, six rice cultivars from the U.S. (four aromatic and two non-aromatic), nine aromatic rice cultivars (Basmati) from Pakistan, two aromatic rice cultivars (Basmati) from India, and one aromatic rice cultivar from Japan. Table 3.1 provides a brief description of these rice lines and cultivars.

Entry	Lines and	Gene Bank	Туре	Country of Origin
Number	Cultivars	Code	•••	
1	F6-1 to F6-120‡	-	RIL	USA
2	Amber 33*§	PI†-326029	Aroma	Iraq
3	Amber 33	GSOR-310278	Aroma	Iraq
4	Amber	PI-130650	Aroma	Iraq
5	Amber	GSOR-310793	Aroma	Iraq
6	Amber Coarse	PI-430978	Aroma	Iraq
7	Amber Coarse	GSOR-311588	Aroma	Iraq
8	Amber 43	PI-430980	Aroma	Iraq
9	Amber 43	GSOR-311672	Aroma	Iraq
10	Anber 33	-	Aroma	Iraq
11	Della*	CI 9483	Aroma	USA
12	Jazzman*	PI-658006	Aroma	USA
13	Antonio*	PI 667755	Non- Aroma	USA
14	Presidio	PI636465	Non- Aroma	USA
15	Della	Clor-9483	Aroma	USA
16	Basmati T3	PI-159367	Aroma	India
17	Scented A	PI-184501	Aroma	Japan
18	Basmati	PI-385456	Aroma	Pakistan
19	Basmati	PI-385471	Aroma	Pakistan
20	Basmati Pardar	PI-385809	Aroma	Pakistan
21	Basmati Medium	PI-385816	Aroma	Pakistan
22	Basmati	PI-385817	Aroma	Pakistan
23	Basmati 6313	PI-400680	Aroma	Pakistan
24	Basmati 37	PI-402762	Aroma	India
25	Basmati 5853	PI-402764	Aroma	Pakistan
26	Basmati 5874	PI-402765	Aroma	Pakistan
27	Basmati	PI-431251	Aroma	Pakistan
28	Dellmont	PI-546364	Aroma	USA

Table 3.1. The rice Recombinant Inbred Lines (RILs) population and cultivars used in this study and their entry number, gene bank code, type and country of origin.

† PI: plant introduction; GSOR: genetic stocks-oryza collection identification number; CIor: Cereal investigation oryza

 $\ddagger F6$ = Filial six generation; RILs = Recombinant inbred lines

§ *: Check

3.2.2. Development of the Recombinant Inbred Population (RIL)

3.2.2.1. Crossing

The crossing that resulted in this RIL population was conducted at experimental greenhouses at the Texas A&M AgriLife Research Center in Beaumont, Texas and the main campus at College Station, Texas. Planting began during the first week of June 2014 in Beaumont and during the first week of July 2014 in College Station, Texas (only for crossing). The parental seeds were planted in four batches each, with a time interval of one week to allow for availability of pollen grain to use during crossing. Iraqi Amber lines, namely Amber 33 (PI 326029), Amber 33 (GSOR 310278), Amber (PI 130650), Amber (GSOR 310793), Amber Coarse (PI 430978), Amber Coarse (GSOR 311588), Amber 43 (PI 430980), and Amber 43 (GSOR 311672) were used as the female parents. Texas A&M AgriLife cultivar 'Antonio' was used as the male parent (Tabien et al., 2015) (see Table 3.2).

Table 3.2. Crossing scheme between eight Iraqi aromatic rice cultivars and 'Antonio' rice cultivar.

Female parent (\bigcirc_+)	Х	Male parent (
Cultivar (\bigcirc_{+}) †	Code	Cultivar (\mathcal{O})
Amber 33	‡PI-326029	Antonio
Amber 33	GSOR-310	278
Amber	PI-130650	
Amber	GSOR-310	793
Amber Coarse	PI-430978	
Amber Coarse	GSOR-311	588
Amber 43	PI-430980	
Amber 43	GSOR-311	672

† ♀ = Female parent; \bigcirc = Male parent

‡ PI: plant introduction; GSOR: genetic stocks-oryza collection identification number

3.2.2.2. Emasculation and Pollination

A small homemade vacuum pump used suction force to extract the immature anthers from the spikelets of selected female parents during emasculation. The device contained a pump, incoming air, and a plastic tube and filters for filtering pollen grains. The plastic tube was connected on one side to the vacuum pump and on the other to a glass pipette with a narrow nozzle. The tube was used for sucking anthers from the clipped spikelets. One or two panicles from each plant was selected as a female parent. The spikelets chosen were based on their potential for emasculation, with 20 to 30 chosen in the middle of a panicle one to two days after the plant flowered. Each selected spikelet was cut from the top to create space to vacuum anthers out of the spikelet; each spikelet had six anthers. The glass pipette was inserted into the spikelet to suck out anthers without damaging the stigma. After all spikelets in a rice plant panicle were emasculated, these were covered by a glassine cross-bag and labeled. This prepared the plant for pollination, and the plant was then moved back to the greenhouse. The following day, the pollen from the selected male parents was transferred to the emasculated spikelets of female parents. Generally, the time that male flowers budded was 10:00 a.m. approximately. The male's panicles were gently moved to the emasculated plant's panicles, with the emasculated panicle touching two or three male panicles to release more pollen grains. After pollination was complete, the pollinated panicle was covered by same bag. Additionally, the label included the name of the male specimen, Antonio, and the date of pollination, and the panicle was kept covered by the cross-bag. After four to five weeks, the cross seed was harvested (see Figure 3.1).



Figure 3.1. Selected plants after pollination. An illustration of part of the crossing scheme used to produce the recombinant inbred line population. Panicles were covered with glycine bags following shedding of anthers from the male parent, Antonio into Amber 33, the female parent.

3.2.2.3. Greenhouse Experiment-Development of Recombinant Inbred Lines

The experiment was conducted at an experimental greenhouse at the Texas A&M AgriLife Research Center in Beaumont, Texas. The planting date was the first week of January 2015. The F₁ seeds from above crosses were planted in Jiffy pots, with the seedlings transferred to small pots in a large plastic tub (with a capacity of 25 small pots) two weeks after planting. After placing the pots in the large plastic tub, all pots were filled with clay soil sourced from the field. One seedling was planted in each small pot and labeled. This study used the single seed descent method after each plant matured and was ready for harvesting F₂ seeds for one cross only {Amber33 (PI 326029) x Antonio}. The F2 derived line were grown two times per year in a greenhouse, and the population was advanced to the F₇ generation using the single seed descent method. The population from aromatic rice type Amber33 (PI 326029) crossed with Antonio was advanced as shown in Figures 3.2 and 3.3, and this study focused on developing the RIL population from this cross.

```
♀ Amber 33 (PI 326029) P1 X P2 (Antonio) ♂
                              \mathbf{1}
                               F1
                               ↓ (self)
                               F2
                                \mathbf{1}
                                     (self) and (single seed descent method)
                            F3 (F2:3)
                                ↓ (self) and (single seed descent method )
                            F4 (F2:4)

    (self) and (single seed descent method)

                            F5 (F2:5)
                                ↓ (self) and (single seed descent method )
                           F6 (F2:6)
                                ↓ (self) and (single seed descent method )
                           F7 (F2:7)
```

Figure 3.2. Flow chart showing the crossing scheme used in the development of a recombinant inbred population that was phenotyped and genotyped in this study.



Figure 3.3. Setup for the generation of the recombinant inbred lines mapping population in the greenhouse.

3.2.3. Field Experiment

3.2.3.1. Beaumont

The seeds planted in the field in Beaumont, Texas included 120 RILs at F_6 , 2 parents, and 25 cultivars. The field study was conducted in July 2017 at the Texas A&M AgriLife Research Center in Beaumont, using an augmented design with two replications. All entries were direct seeded using Hege planter in 3 meters row spaced at 18 centimeters apart. Plants were maintained following the Texas Production Guidelines.

3.2.3.2. Eagle Lake

The field study was conducted in August 2017 at the Texas A&M AgriLife Research Center in Eagle Lake. The same set of entries, experimental design and replication in Beaumont was used at Eagle Lake but all were transplanted. Seedling were produced in the greenhouse and transplanted at 21 days after seeding. Distance of transplanting was set at 20 cm x 20 cm. Each entry was represented by five plants grown in two rows. The SAS version 9.4 was used for the statistical analysis of the data.

3.2.4. Data Collection

3.2.4.1. Days to 50 % Heading

Days to 50 % heading was measured as the number of days that passed from the initial planting to when the primary panicles in 50% of the plants headed.

3.2.4.2. Plant Height (cm)

Plant Height was measured a week before harvest time in centimeters with a ruler from the ground surface to the tip of the panicles.

3.2.4.3. Number of Tillers Per Plant

The total numbers of tillers were recorded from a half meter row in Beaumont and in hills basis at Eagle Lake at the time of harvest.

3.2.4.4. Flag Leaf Area (cm²)

The flag-leaf area was recorded by measuring the length and the maximum width of the flag leaf followed by obtaining the area using the following formula: Leaf area = K x length x width, where K = "adjustment factor." K varied with the shape of the leaf, which was affected by factors such as the cultivar and growth stages, 0.75 (IRRI, 1972).

3.2.4.5. Ligule Length (mm)

The average ligule length was calculated at late vegetative phase from five randomly chosen plants. The first leaf under the flag leaf of the main tiller was collected to measure the length of the ligule. The ligule length in millimeters was measured with a ruler from the base of the collar to the tip.

3.2.4.6. Panicle Length (cm)

The length of five panicles of each plant was measured from the base of the lowest spikelet to the tip of the latest spikelet on the panicle, excluding the awn at the time of harvest.

3.2.4.7. Number of Panicles Per Plant

The total number of panicles was recorded at the time of harvest in half meter row in Beaumont and in each plant at Eagle Lake.

3.2.4.8. Number of Branches Per Panicle

Number of branches was measured by counting the number of primary branches on the panicle axis length of five panicles.

3.2.4.9. Number of Grains Per Panicle

After harvest, the total number of grains per panicle were recorded in five panicles.

3.2.4.10. Number of Unfilled Grains Per Panicle

The total number of unfilled grains per panicle were recorded after harvest from five panicles.

3.2.4.11. Number of Filled Grains Per Panicle

The total number of filled grains per panicle were recorded after harvest from five panicles.

3.2.4.12. Sterility Percentage (%)

The sterility percentages of unfilled grains were measured per panicle and recorded after harvest. Five panicles were used, as well as the following formula: Sterility percentage = (Number of unfilled grains / Total number of grains) $\times 100\%$.

3.2.4.13. Fertility Percentage (%)

The fertility percentages of the filled grains were measured per panicle and recorded after the harvest. Five panicles were used, as well as the following formula: Fertility percentage = $(Number of filled grains / Total number of grains) \times 100\%$.

3.2.4.14. Thousand Grain Weight (gm)

The thousand-grain weight was taken by counting 1000 from the bulked harvested grains and recording their weight.

3.2.4.15. Grain Yield Per Plant (gm)

The weight of grains per plant dried at 12% from half meter row was recorded after harvest and milling of each plant in grams.

3.2.4.16. Grain Yield Per Line (gm)

The weight of grains from half meter row dried at 12% was recorded after harvest of each line in Beaumont only.

3.2.4.17. Rice Milling (%)

The rice-milling ratio was the amount of milled rice to rough rice, which was measured from the harvest Beaumont with the following formula: Rice-milling percentage = (Milled rice sample / Rough rice sample) x 100%.

3.2.4.18. Seed Length (mm)

Seed length was measured after rice had been milled, and 100 seeds were chosen at random. The STD4800 scanner and WinSEEDLE (2014) (Instruments Canada Inc.) were used to measure the lengths.

3.2.4.19. Seed Width (mm)

Seed width was measured after rice had been milled, and 100 seeds were chosen at random. The seed width was measured with the STD4800 scanner and WinSEEDLE (2014).

3.2.4.20. Chalky Seed Percentage (%)

The chalky seeds were measured after the rice had been milled, and 100 seeds were chosen at random. The STD4800 scanner and WinSEEDLE (2014) were used for the measurement.

3.2.4.21. Aroma (2AP)

The test materials were phenotyped for aroma using Gas Chromatography-Mass Spectrometry. All RILs, parents, and checks from 147 milling samples with two replications were screened for aroma in Dr. Manoch Kongchum's laboratory at H. Rouse Caffey Research Station-LSU AgCenter in Crowley, Louisiana. The aroma in rice is normally determined by estimating the aroma, separating the compounds and then identifying the compounds by gas chromatography mass spectrometry (GC-MS) analysis (Bullard and Holguin, 1977).

3.2.4.21.1. Sample Preparation and Gas Chromatography Conditions

The aroma was estimated with GC-MS for 2AP. Milled-rice samples were ground into a powder (less than 0.25 mm in diameter). Two replications of 1.00 g samples were transferred into a 20 mL headspace glass vial. One µL of 0.5 mg/mL of 2,6-dimethylpyridine (2,6-DMP) was added to the vial as an internal standard before the airtight sealing was performed, which was done with a polytetrafluoroethylene and silicone septum secured by an aluminum cap. Sample vials were placed on the headspace-auto-sampler model HS-20 (Shimadzu, Columbia, MD) and equilibrated at 120 °C for 10 min with high-speed shaking, prior to collecting the volatile components. The pressurizing time, the pressure equilibrium time, and the injection times were 1.00, 0.01, and 2.00 min, respectively. After the pressurizing, a sample of the headspace was collected through a 3-mL sample loop and automatically transferred to the gas chromatography via a heated transfer line for 0.50 min. The oven, sample-line, and transfer-line temperatures were set at 120 °C, 150 °C, and 160 °C, respectively. Gas chromatographic separation was performed on a Shimadzu GC-2010 Another system (Shimadzu, Columbia, MD) and was coupled to a flame thermionic detector (FTD) and equipped with LabSolutions software for data collection and evaluation. Separation was performed with a 60 m x 0.32 mm i.d. x 1.0 µm film thickness Rtx-5 capillary column (Restek, USA), with a splitless injection at 250 °C. The temperature of the column was programmed to start at 50 °C at the time of the injection; subsequently, it was set to increase at a rate of 5 °C/min, from 50 °C to 200 °C. Gas chromatography and FTD were performed at the temperature of the detector of 280 °C, and helium was used as a carrier gas, with the flow rate of 3.5 mL/min. The concentration of 2AP was identified by comparing the gaschromatography retention times with the standard that ran under the same conditions. Peak areas were obtained with the aid of software from LabSolutions (Shimadzu, Columbia, MD).

3.2.4.21.2. Standard Preparation

A standard of 10 mg of 2AP in a 10% w/w in toluene was purchased from Toronto Research Chemicals (TRC), which is based in Canada. A series of standards with the concentrations of 0, 0.5, 1.0, 1.25, 2.5, and 5.0 mg/g of 2AP in toluene were prepared. A nonaromatic milled-rice sample was ground into a powder with the same method used for the samples. Three replications of 1.00 g of nonaromatic milled rice were weighed and transferred into a 20 mL headspace glass vial for each level of the standard. One μ L of 0.5 mg/mL of 2,6-dimethylpyridine (2,6-DMP) and 1 μ L of the 2AP standard of each concentration level were added into the vial before the airtight sealing, which was done with a polytetrafluoroethylene and silicone septum and secured by an aluminum cap. The headspace auto-sampler and gas chromatography were set up and analyzed in the same manner used for the samples.

3.2.5. Genetic Analysis

3.2.5.1. Genotyping

Genomic DNA of the parents and the mapping population (RILs) were extracted using the modified CTAB method (Doyle and Doyle, 1987). DNA extraction for 147 samples was done in the AgriGenomics Laboratory in College station. The quality of DNA was checked using agarose gels and the concentration was tested using a Nanodrop 1000 UV-vis Spectrophotometer (DeNovix DS-II Spectrophotometer). DNA preparations for all test materials were done at the AgriGenomics Laboratory.

3.2.5.2. DNA Extraction

Samples were collected from young leaves and a modified CTAB method (Liu et. al., 2013) was used for DNA extraction.

3.2.5.3. Genotyping by Sequencing (GBS)

The DNA analysis was performed at the Genomics and Bioinformatics Laboratory at Texas A&M AgriLife. The lab used the following procedure to execute the DNA analysis. One hundred micrograms of DNA per sample on 96 well plates were digested in a final volume of 25 μ l in 1X NEB Cut Smart Buffer and 100 U each of ENZYME 1 and ENZYME 2 (NEB), at 37 °C for 4 hr. Following a 20 min of 80 °C enzyme inactivation, samples were held at 12 °C until ligation. To each 25 µl digest sample, 3.5 µl of a 10X ligase buffer (Promega) and 0.5 µl of T4 DNA ligase (Promega) were added. Adapters containing 1 of 48 unique barcodes and Illumina-compatible P5 sequences that had been coupled to an EcoRI overhang, as well as Illumina-compatible P7 sequences coupled to the MspI overhang, were added as well. The plates were incubated for 8 hr at 16 °C and heat inactivated at 80 °C for 20 min. Three pools of 45, 45, and 46 samples were mixed and combined with 0.1 volume of 3 M NaAc, pH 5.2, and two volumes of 100% ethanol and placed at -20° C for 1 hr. Next, the three pools were spun at a high speed for 10 min in a benchtop microfuge. The pellets were washed twice in 1 mL of freshly made 70% ethanol and resuspended in 200 µl of EB. Samples were purified with the company Qiagen's PCR Purification columns and eluted in 2X 50 µl EB, for a total of 100 µl. One volume of AMPure XP beads were added to the elutant and DNA purified, as per the manufacturer's suggested protocol, and they were eluted in 35 µl of EB. Thirty microliters of each pool containing between 1.9 and 2.2 µg of DNA was subjected to a Pippin Prep size selection on a 2% dye-free agarose gel, with the internal size markers aiming for 270–330 bp inserts. Recovered samples were cleaned with 1X AMPure

XP beads and quantified on a DeNovix spectrophotometer. One hundred fifty nanograms of each pool was then subjected to a preselection PCR (PreCR) in which a biotinylated forward primer and unique indexed reverse primers were used to amplify and tag the desired DNA fragments. Reactions (200 µl in total) contained 200 nM of dNTPs, biotinylated forward and two P7-index primers per pool, and four units of Phusion Hi-Fidelity Taq (NEB) and were split into 2 x 100 µl volumes for thermocycling. Following an initial denaturation performed at 98 °C for 30 s, samples were subjected to 18 cycles of 98 °C for 10 s, 58 °C for 30 s, and 72 °C for 30 s, and a final elongation was performed for 5 min at 72 °C and held at 4 °C. PCR products were cleaned up in Qiagen's PCR purification columns and 1X AMPure XP beads and quantified as before. Removal of EcoRI-EcoRI and MspI-MspI fragments was achieved with ThermoFisher's Dynabeads M-270 Streptavidin, which are coupled magnetic beads. Briefly, 50 µl of beads per sample were captured and washed twice with the 1X Bead Washing Buffer (1X BWB, 10 mM Tris-HCl [pH 7.5], 1 mM EDTA, and 2 M NaCl). The beads were re-suspended in 100 µl of 2X BWB and mixed with 2000 ng of the PreCR product in 100 µl of EB. After 20 min at RT, the beads were captured and washed three times in 200 µl of 1X BWB, twice in 200 µl of water, and once in 100 µl of 1X SSC. The beads were re-suspended in 50 µl of 1X SSC and heated at 98 °C for 5 min and placed on a magnet, and the resulting supernatant was removed as soon as possible. This elution was repeated, and the final supernatants were cleaned up with Qiagen's PCR columns. The eluted ssDNA was DeNovix quantified and diluted to 1 ng/µl with EB. A final PCR was performed on 10 ng of the input DNA, and P5 and P7 primers were used in a 75 μ l reaction as described above, but only with 8 cycles. The final PCR products were purified with 1X AMPure XP beads, quantified, and assessed for quality on a Fragment Analyzer (made by Advanced Analytics).

3.2.5.4. RADSeq Data Analysis and SNP Identification

A RADSeq analysis was done for 147 samples in the Genomics and Bioinformatics Laboratory at Texas A&M AgriLife. Genomic DNA from each sample was digested with the restriction enzymes PstI and MseI. The restriction-associated DNA (RADs) for all 27 samples were pooled and sequenced in two lanes on Illumina's HiSeq 4000 with a 150 bp pair-end library. The library preparation and sequencing were conducted at AgriLife Research Genomic and Bioinformatics Service, at the Texas A&M University (www.txgen.tamu.edu). The raw sequence reads were demultiplexed, according to the index reads. First, the sequences were filtered for quality with the program FASTX-Toolkit (http://hannonlab.cshl.edu/fastx-toolkit). The raw sequencing reads were trimmed to remove low-quality bases with scores below 20 on the ends of the reads; next, reads with 30% or more bases showing a low-quality score (Q < 15) were removed. The reference genome for Oryza indica (ASM465v1) and Oryza sativa (IRGSP-1.0) were downloaded from a plant ensemble (https://plants.ensembl.org). An artificial reference was constructed by combining both of these two genomes into one single Fasta file. Bowtie 2 [http://bowtie-bio.sourceforge.net/bowtie2/index.shtml] was used to align the quality-controlled reads with the references for the default parameters. The reference-aligned reads were then processed with the ref_map.pl pipeline in Stacks V2.0 (Catchen et al. 2013). First, the uniquely aligned reads were assigned into stacks and subsequently merged to form putative loci. The minimum depth of stack was five reads. To include a locus in the analysis, it should be present in at least 50% of the samples. Subsequently, a maximum-likelihood framework was used to call SNPs, and a catalogue was built with all of the existing loci and alleles, against which all individuals were matched. (For more details on the use of the Stacks software package for studies of model and non-model organisms, see Catchen et al. 2013.)

3.2.5.5. Linkage Mapping and QTL Analysis

Linkage mapping is a tool in molecular breeding used for genetic mapping of QTL cosegregating with traits of interest in bi-parental populations and develop molecular markers which can be used in marker assisted breeding. A linkage map is a tool in linkage mapping that facilitates the localization of co-segregating markers to specific positions of the genome based on recombination frequencies. JoinMap 4.0 (Kyazma ®, Wageningen, Netherlands) was used to prepare a linkage map. Markers with more than 5% missing data and severe segregation distortion were removed from the analysis and remaining markers were used to create a linkage map in JoinMap 4.0. A LOD score of (LOD > 3) was used to create linkage groups within chromosomes using the 'Kosambi' mapping function. The linkage map was used to conduct the mapping using interval mapping and the multiple QTL mapping (MQM) algorithm in MapQTL 6.0 (Kyazma ®, Wageningen, Netherlands). A combined adjusted means from the two locations (Beaumont and Eagle Lake) for 21 traits (see Table 3.15, Table 3.16 and Table 3.17) was used along with marker data from RADSeq A permutation test of 10,000 iterations was done to calculate the significance threshold of LOD score. The results from the MQM algorithm were later visualized in MapChart (Wageningen University and Research, Netherlands) and figures were created.

3.3. Results and Discussion

3.3.1. Agronomic and Morphological Traits

3.3.1.1 Beaumont

In Beaumont, significant differences existed in terms of agronomic and morphological traits (Tables 3.3 to 3.8). Three lines (F6-43, F6-90, and F6-77) had the longest number of days to 50% heading (111, 102, and 102 days, respectively); parents Amber 33-PI and Antonio took 101 and 77.5 days to reach 50% heading, respectively. Twelve lines (F6-6, F6-24, F6-115, F6-93, F6-37, F6-9, F6-112, F6-33, F6-30, F6-25, F6-116, and F6-10) ranged from 73 to 76 days to 50% heading, earlier than Antonio.

The plant height trait demonstrated significant variation among the lines and cultivars. Tall height is characteristic of Amber's cultivars. Amber 33-PI was the tallest parent, with a height of 142.9 cm; Antonio was 93.7 cm. F6-72, F6-7, F6-89, F6-108, F6-79, F6-83, F6-113, F6-75, F6-73, F6-115, F6-12, and F6-5 were 179.7 cm, 170.1 cm, 162.7 cm, 161.8 cm, 157.7 cm, 152.2 cm, 149 cm, 148.8 cm, 146.8 cm, 145.2 cm, 143.5 cm, and 143.3 cm, respectively. Some of the rice lines were shorter than Antonio, including F6-17, F6-85, F6-84, F6-67, F6-1, and F6-31 (92 cm, 91 cm, 89.8 cm, 89.3 cm, 88 cm, and 86 cm, respectively).

F6-99, F6-13, F6-47, F6-104, F6-65, F6-6, F6-71, F6-76, and F6-85 had a higher number of tillers per plant than both parents, ranging from 14 to 16; F6-10, F6-116, F6-31, F6-88, F6-107, F6-108, F6-112, F6-22, F6-3, F6-30, F6-9, and F6-90 had the lowest number of tillers per plant, ranging from 4 to 5.

Flag leaf area exhibited significant differences among lines and rice cultivars. Only three lines had a greater flag leaf area than Amber 33-PI (the female parent); F6-93 was 47.5 cm², F6-56 was 44 cm², and F6-92 was 43.2 cm². Some lines had a smaller flag leaf area than Antonio

(such as F6-37 and F6-32). This shows both positive and negative transgressive segregation for this trait. The remaining lines had flag leaf area values ranging between the two parents.

Differences existed in ligule length among lines and rice cultivars. Some cultivars, such as Basmati cultivars, and lines, such as F6-47 and F6-90, had longer ligules compared to the U.S. rice cultivars such as 'Jazzman' and 'Della'. In addition, some rice lines such as F6-72 and F6-36 had the shortest ligule length.

Aromatic rice cultivars and some lines demonstrated the longest panicle length, ranging from 26.3 cm to 32.1 cm and longer than Amber 33-PI (the female parent). However, Amber Coarse and Amber 43 cultivars and a few lines (such as F6-10, F6-68, and F6-67) were significantly similar to Antonio (the male parent) and had a short panicle length.

The number of panicles per plant was significantly different among the lines and cultivars. Amber Coarse, Amber 43, and Della had 14.5, 12, and 11.5 panicles per plant, respectively. In addition, lines F6-99, F6-71, F6-85, F6-47, F5-55, F6-84, F6-1, and F6-2 had a higher number of panicles per plant than both parents (Amber 33-PI and Antonio), with 15, 13.5, 13, 12, 12, 11.5, 11.3, and 11.3 panicles per plant, respectively.

The number of branches per panicle demonstrated highly significant differences among the lines and cultivars. Some cultivars such as 'Della-Clor', 'Delmont', Della, 'Presidio', Jazzman, and 'Basmati T3' had a higher number of branches per panicle (19, 18, 16.6, 16.5, 16, and 15, respectively) than both parents. In addition, lines F6-94, F6-64, F6-74, F6-87, F5-98, F6-43, F6-27, F6-60, F6-71, F6-105, F6-28, and F6-4 had a higher number of branches per panicle, ranging from 14.7 to 21.

There were significant differences in the number of grains per panicle among the lines and cultivars. Some cultivars such as Presidio, Della-Clor, 'Basmati Pardar', Basmati T3, and Jazzman

(162.5, 155.5, 153.5, 144.5, and 142.8 grains per panicle, respectively) had a higher number of grains per panicle than Antonio (the male parent, with 140.31 grains per panicle). Additionally, some lines had a greater number of grains per panicle than both parents, such as F6-27, F6-94, F6-7, F6-98, F5-87, F6-1, F6-18, F6-89, F6-74, F6-54, F6-105, F6-64, F5-60, F6-43, F6-116, and F6-24.

The number of unfilled grains per panicle trait varied significantly among lines and cultivars. Some cultivars such as 'Basmati- PI-385471', Basmati T3, Basmati Pardar, Della-Clor, 'Scented A', and Basmati 37, as well as several lines, had a greater number of unfilled grains per panicle than Amber 33-PI (the female parent, which had 29.8 unfilled grains per panicle). Conversely, some cultivars such as Basmati- PI-385817, Amber Coarse, Basmati- PI-431251, 'Amber-GSOR', 'Amber 43-GSOR', and Basmati- PI-385456, as well as several rice lines (such as F6-86, F6-70, F6-80, F6-8, F5-104, F6-112, F6-51, F6-101, F6-26, and F6-95), had a lower number of unfilled grains per panicle than Antonio (the male parent, with 15.4 unfilled grains per panicle).

The number of filled grains per panicle demonstrated significant variance among lines and cultivars. The Presidio cultivar had 136.5 filled grains per panicle, and lines F6-7, F6-27, F6-98, F5-1, F6-94, F6-116, F6-51, F6-64, F6-48, and F6-87 had 163, 152, 147, 145, 139, 137, 136, 135, 128, and 124.8 filled grains per panicle, respectively – higher than the number for Antonio, the male parent, and Jazzman, the check (at 124.4 and 120.9, respectively).

Sterility percentage demonstrated significant differences among lines and cultivars. Basmati- PI-385456 had a percentage of sterility of 11.1% and Amber-GSOR of 10.2%, numerically lower than Antonio's 11.2%. Additionally, several lines – such as F6-86, F6-48, F6-8, F6-112, F6-26, and F6-51 at 11.0%, 11.0%, 10.9%, 10.8%, 9.8%, and 7.9%, respectively – had a lower percentage of sterility than Antonio.

Two cultivars, Basmati-PI-385456 and Amber-GSOR, as well as the lines F6-86, F6-48, F6-8, F6-112, F6-26, and F6-51, demonstrated a higher proportion of and differences in fertility percentage than Antonio (Tables 3.5 and 3.6). However, many lines and several cultivars (such as Basmati 5853, Basmati 37, Basmati T3, Amber 43-PI, and Basmati- PI-38547) demonstrated a smaller proportion than Amber 33-PI (the female parent, at 73.7%).

Significant variation existed for thousand-grain weight. The thousand-grain weight for Amber Coarse-IP, Amber Coarse-GSOR, Dellmont, Scented A, Amber 43-GSOR, and Jazzman was 25.1 g, 25.0 g, 24.5 g, 24.3 g, 24.1 g, and 24.0 g, respectively – significantly heavier than Antonio at 23.6 g. Additionally, some lines such as F6-84, F6-15, F6-77, F6-62, F6-26, F6-85, F6-83, F6-110, F6-30, F6-56, F6-42, F6-43, F6-115, F6-17, F6-90, F6-112, F6-101, F6-12, F6-65, F6-21, F6-50, and F6-60 ranged between 23.7 g and 27.5 g, heavier than Antonio.

There were no significant differences in grain yield per plant (g) among cultivars and lines compared to the four checks Della, Jazzman, Amber 33-PI, and Antonio.

The trait grain yield per line demonstrated significant differences among cultivars and lines. Della-Clor, Basmati T3, Basmati 5853, Basmati-PI-385817, and Scented A were 28.1 g, 25.5 g, 23.7 g, 23.3 g, and 22.9 g, respectively; these were heavier than parents Antonio and Amber 33-PI and checks Della and Jazzman. Some lines also had a heavier grain weight than the four checks, such as F6-85, F6-98, F6-27, F6-47, F6-86, F6-76, F6-99, F6-115, F6-84, and F6-71.

The percentage of milled rice varied among the checks, lines, and cultivars.

Jazzman (at 72.7%) and Presidio (at 72.4%) had a higher percentage of rice milling than Antonio (69.4%). F6-94, F6-97, F6-16, and F6-64 (at 71.6%, 71.1%, 69.9%, and 69.8%, respectively) demonstrated a higher percentage of rice milling than both parents (Antonio at 69.4% and Amber

33-PI at 60.5%). None of the Basmati or Iraq aromatic rice cultivars – such as Basmati-PI-431251, Basmati Pardar, Basmati 5874, Basmati-PI-385471, Basmati 5853, Amber 33-GSOR, Amber 43-GSOR, and Amber 43-PI at 60.5%, 60.1%, 59.8%, 57.3%, 53.4%, 59.0%, 56.0%, and 54.7% total milled rice, respectively – were comparable to Antonio and Amber 33-PI.

The U.S. rice cultivars (aromatic and non-aromatic) Jazzman, Della-Clor, Della, Presidio, and Dellmont had 6.9 mm, 6.8 mm, 6.7 mm, 6.7 mm, and 6.7 mm were classified as long seed, respectively. Additionally, Basmati rice cultivars Basmati 6313, Basmati 5874, Basmati 5853, Basmati-PI-431251, Basmati 37, Basmati-PI-385456, Basmati-PI-385817, Basmati Medium, and Basmati Pardar had a seed length of 7.6 mm, 6.8 mm, 6.8 mm, 6.8 mm, 6.7 mm, 6.7 mm, 6.6 mm, 6.6 mm, and 6.3 mm, respectively. Some lines had longer seeds than Antonio. Several cultivars and lines – such as Basmati T3, Scented A, Basmati-PI-385471, F6-64, F6-1, F6-18, and F6-51 at 5.5 mm, 5.3 mm, 5.2 mm, 5.4 mm, 5.3 mm, 5.3 mm, and 5.2 mm, respectively – were shorter than Amber 33-PI (the female parent, classified as having medium-length seeds with a 5.5 mm seed length).

For seed width, the lines ranged from F6-63 (2.0 mm) to F6-98 (2.7 mm). The Iraqi aromatic rice cultivars ranged from Amber-GSOR (2.0 mm) to Amber 43-GSOR (2.7 mm), and the U.S. rice cultivars ranged from Della-Clor (2.1 mm) to Dellmont (2.4 mm). The Basmati cultivars ranged from Basmati Pardar (1.8 mm) to Basmati T3 (3.3 mm) – the greatest range among the cultivars and rice lines.

For chalky seed percentage, two rice cultivars (Basmati T3 at 3.1% and Amber 33-GSOR at 2.5%) had a numerically higher percentage than Amber 33-PI (the female parent, at 2.3%) and Antonio (the male parent, at 0.6%) as checks. F6-25 was recorded as having the highest percentage of chalky seeds among rice lines, 7.7%, compared to F6-51, with 0.2% chalky seeds.

In Beaumont, as presented in Tables 3.7 and 3.8, the highest aroma as 2AP recorded in 11 lines: F6-3, F6-69, F6-5, F6-12, F6-100, F6-11, F6-67, F6-80, F6-49, F6-106, and F6-70, with 1.31, 1.23, 0.94, 0.90, 0.84, 0.83, 0.80, 0.78, 0.78, 0.75, and 0.75 2AP concentration, respectively. Amber 33-PI, the female parent, had 0.75 2AP concentration. Ten lines – F6-112, F6-113, F6-13, F6-16, F6-20, F6-23, F6-59, F6-60, F6-14, and F6-15 – had zero concentration of 2AP, similar to Antonio (the male parent) and Presidio as the U.S. non-aromatic rice cultivars, in Appendix 3.

Table 3.3. Mean squares of the ANOVA showing the effects of cultivars on days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at Beaumont, Texas in 2017.

	Days to	Plant	Number of	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Source	50%‡	Height (cm)	Tillers per	Area (cm ²)	Length	Length	of Panicle	Branch per	Grains per
	Heading		Plant		(mm)	(cm)	Per plant	Panicle	Panicle (g)
Line	93.71**	374.00**	9.98^{**}	70.25**	27.89^{**}	7.20^{**}	6.60^{**}	6.51**	1155.31**
Error	4.54	18.12	2.85	11.88	1.91	0.79	1.78	1.52	248.05

† ns = Non-significant; * = P<0.05; ** = P<0.01

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

Table 3.4. Means of agronomic traits of days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at Beaumont, Texas in 2017.

Lines and Cultivars	Days to 50%‡	Plant Height	Number of Tillers	Flag Leaf Area	Ligule Length	Panicle Length	Number of Panicle	Number of Branch per	Number of Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	Panicle	Panicle (g)
F6-1¥	101.0	88.3	12.0	35.1	15.9	24.4	11.4	14.8	194.0
F6-2	101.0	139.3	13.0	27.7	14.4	26.1	11.4	13.3	148.0
F6-3	97.0	132.3	4.0	21.1	8.4	24.2	3.9	13.5	116.5
F6-4	93.0	109.1	8.0	26.9	9.9	25.3	4.4	16.0	162.5
F6-5	80.0	143.3	6.0	29.0	8.9	22.5	5.9	10.5	97.5
F6-6	76.0	117.0	14.0	33.1	9.9	24.1	7.4	13.3	119.8
F6-7	81.0	170.2	9.0	36.5	11.9	29.2	8.9	14.5	214.5
F6-8	92.0	107.2	10.0	18.9	9.9	23.1	4.9	10.5	108.3
F6-9	74.0	111.7	4.0	25.6	11.9	24.2	4.4	11.8	113.5
F6-10	73.0	107.2	5.0	14.6	7.9	20.3	5.4	10.5	99.3
F6-11	88.0	106.8	6.0	15.7	7.4	24.0	5.4	11.8	103.0
F6-12	98.0	143.5	9.5	20.1	9.9	29.0	5.4	10.8	121.0
F6-13	97.0	99.5	15.0	34.9	7.9	21.7	6.9	13.3	127.3
F6-14	79.0	103.7	10.0	32.3	24.4	24.3	7.4	15.5	150.8
F6-15	99.0	101.5	8.0	30.8	19.9	26.1	7.4	14.8	117.3
F6-16	100.0	98.3	10.0	40.9	11.9	24.7	7.4	15.0	166.3
F6-17	96.0	92.7	12.0	22.4	3.9	24.0	6.4	14.3	146.0
F6-18	99.0	105.3	8.0	26.0	20.4	26.4	4.4	15.8	193.8
F6-19	87.0	94.7	6.0	27.8	9.9	22.7	5.9	12.0	131.0
F6-20	87.0	126.0	6.0	32.7	7.9	24.8	6.4	11.0	89.3

[†] PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

F6 = Filial six generation

Table 3.4. Continued

Lines and Culture	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Lines and Cultivars	50%‡ Heading	Height (cm)	of Tillers per Plant	Area (cm ²)	Length (mm)	Length (cm)	of Panicle Per plant	Branch per Panicle	Grains per Panicle (g)
EC 01	Ŭ		1	, ,	· /	· · · ·			
F6-21	79.0	120.7	13.0	19.3	7.9	22.6	7.9	10.5	101.8
F6-22	84.0	113.2	4.0	24.7	19.9	24.6	5.4	12.8	109.8
F6-23	100.0	100.8	8.0	23.9	12.4	25.1	8.4	12.3	126.8
F6-24	76.0	135.3	6.0	24.5	4.9	24.5	5.4	14.0	167.0
F6-25	73.0	115.5	6.0	18.8	9.9	22.3	5.4	15.8	145.0
F6-26	78.0	139.3	8.0	15.8	9.9	24.9	7.9	10.8	110.0
F6-27	86.0	115.5	10.0	19.6	11.4	28.6	8.9	16.8	231.8
F6-28	100.0	111.2	6.0	27.4	10.9	27.3	7.9	16.0	131.5
F6-29	93.0	118.3	6.0	40.1	15.4	27.3	6.4	14.8	144.8
F6-30	73.0	102.7	4.0	33.9	11.9	22.4	4.4	11.8	101.3
F6-31	84.0	86.3	5.0	19.9	4.4	23.0	5.4	10.5	87.0
F6-32	95.0	130.5	4.0	14.3	3.9	22.8	4.4	11.0	101.0
F6-33	73.0	129.5	11.0	41.9	24.9	26.9	10.4	10.0	136.8
F6-34	89.0	138.0	11.0	27.5	5.9	26.4	7.9	11.5	125.3
F6-35	91.0	96.0	8.0	20.2	16.9	27.3	7.4	12.8	116.3
F6-36	99.0	137.7	8.0	25.7	3.9	26.6	8.4	10.3	80.0
F6-37	75.0	117.3	8.0	13.1	9.9	21.7	6.9	11.0	93.3
F6-38	79.0	129.2	6.0	36.2	17.9	25.3	5.9	11.8	109.0
F6-39	101.0	125.2	8.0	29.2	13.9	25.5	7.9	11.3	103.8
F6-40	95.0	129.5	7.0	20.0	15.9	22.9	5.9	11.3	111.8
F6-41	98.0	129.8	6.0	30.0	10.9	23.5	4.9	12.5	135.0
F6-42	94.0	111.3	5.5	24.4	11.9	27.5	4.9	12.5	126.5
F6-43	111.0	135.7	6.0	34.9	9.9	27.6	5.9	17.0	170.3
F6-44	92.0	103.5	6.0	18.9	4.9	22.0	6.4	14.3	116.5
F6-45	92.0	108.8	8.0	25.8	7.9	22.0	7.4	11.5	110.8
F6-46	98.0	136.2	6.0	26.9	9.9	21.4	5.4	10.8	120.3
F6-47	101.0	125.0	15.0	31.8	27.9	29.1	12.0	13.5	161.5

Table 3.4. Continued

Lines and Cultivars	Days to 50%‡	Plant	Number of Tillers	Flag Leaf Area	Ligule	Panicle	Number of Panicle	Number of Brench per	Number of
Lines and Cultivars	Heading	Height (cm)	per Plant	(cm^2)	Length (mm)	Length (cm)	Per plant	Branch per Panicle	Grains per Panicle (g)
F6-48	78.0	115.7	6.0	21.7	11.9	25.4	6.0	11.3	143.0
F6-49	100.0	123.8	8.0	26.0	12.4	21.4	8.0	10.8	97.5
F6-50	96.0	126.3	10.0	23.4	11.4	25.9	7.5	11.5	108.5
F6-51	89.0	125.2	6.0	30.8	16.4	23.6	5.5	11.3	146.8
F6-52	101.0	118.0	10.0	28.3	15.4	23.4	8.5	12.0	115.3
F6-53	90.0	132.0	8.0	25.0	3.9	25.6	6.5	14.3	160.3
F6-54	89.0	117.0	9.5	18.7	18.4	25.3	9.0	13.3	180.0
F6-55	85.0	113.0	13.0	19.0	8.4	21.6	12.0	13.0	144.8
F6-56	101.0	120.3	14.0	44.0	12.4	27.8	10.0	12.0	116.3
F6-57	96.0	130.3	8.0	18.2	15.9	24.2	5.0	12.5	100.0
F6-58	78.0	119.5	10.5	24.4	10.9	25.9	9.0	13.3	115.3
F6-59	96.0	118.0	10.0	26.4	12.4	24.3	9.0	13.0	102.3
F6-60	101.0	140.5	8.0	36.2	6.4	28.0	5.0	16.8	171.8
F6-61	101.0	116.2	8.0	39.2	12.4	27.3	4.5	12.0	136.5
F6-62	101.0	124.3	10.0	30.2	19.9	30.2	6.5	11.3	127.5
F6-63	89.0	137.7	6.0	30.2	15.4	26.6	3.5	11.5	95.3
F6-64	91.0	97.0	6.0	31.6	15.9	22.1	5.0	20.5	173.3
F6-65	88.0	115.7	6.0	20.1	10.4	22.9	5.0	12.5	106.0
F6-66	85.0	100.8	6.0	25.7	8.4	26.7	4.5	13.8	165.0
F6-67	98.0	89.3	10.0	20.4	8.4	19.3	7.0	15.0	104.8
F6-68	92.0	124.3	10.0	17.1	7.4	20.0	6.5	12.5	80.3
F6-69	100.0	114.2	12.0	18.2	10.4	26.3	10.0	10.8	112.0
F6-70	92.0	125.7	12.0	24.8	15.9	24.0	10.0	13.5	100.3
F6-71	89.0	95.5	14.0	15.3	6.4	24.7	13.5	16.8	151.3
F6-72	100.0	179.7	10.0	33.4	3.4	32.1	8.5	14.5	142.0
F6-73	98.0	146.8	10.0	21.2	10.4	24.3	8.0	12.3	122.8
F6-74	101.0	119.3	8.0	27.8	10.4	24.3	8.0	19.5	190.8

Table 3.4. Continued

	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Lines and Cultivars	50%‡	Height	of Tillers	Area	Length	Length	of Panicle	Branch per	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	Panicle	Panicle (g)
F6-75	97.0	148.8	6.0	28.9	5.4	26.3	6.0	13.8	129.0
F6-76	100.0	138.7	14.0	37.0	15.4	27.4	8.5	11.5	124.5
F6-77	102.0	124.8	10.0	17.4	10.4	23.8	7.5	13.0	104.5
F6-78	100.0	142.5	12.0	27.3	20.9	27.3	11.0	11.8	115.0
F6-79	88.0	157.7	10.0	15.0	20.4	23.1	9.0	15.8	112.3
F6-80	88.0	129.3	6.0	23.1	5.4	28.4	6.0	12.0	99.3
F6-81	101.0	126.8	6.0	41.6	11.4	26.3	5.5	15.3	115.3
F6-82	98.0	130.2	10.0	21.8	16.4	21.6	9.5	15.3	165.3
F6-83	101.0	152.2	12.0	40.3	10.4	28.4	8.0	13.3	139.8
F6-84	93.0	89.8	12.0	17.1	7.4	23.2	11.5	12.3	99.0
F6-85	96.0	91.7	14.0	36.0	12.4	25.9	13.0	12.5	132.0
F6-86	99.0	124.8	8.0	28.6	10.4	23.6	8.0	11.5	115.3
F6-87	94.0	128.3	8.0	40.4	14.4	24.3	8.0	18.3	201.0
F6-88	98.0	135.7	5.0	23.0	13.4	26.4	6.0	12.8	121.3
F6-89	85.0	162.7	10.0	34.4	12.4	27.4	9.5	15.8	193.8
F6-90	102.0	112.3	4.0	19.0	25.9	23.4	4.0	11.8	115.5
F6-91	101.0	128.7	8.0	40.2	18.4	29.3	6.0	12.0	136.3
F6-92	90.0	122.5	7.0	43.3	24.4	27.0	6.5	15.0	156.5
F6-93	75.0	131.0	8.0	47.5	7.6	26.7	7.1	13.8	116.8
F6-94	99.0	99.2	6.0	32.0	13.6	21.2	5.6	21.0	216.3
F6-95	93.0	120.0	6.0	22.5	6.6	23.7	5.1	11.0	58.3
F6-96	93.0	142.0	8.0	31.3	9.6	26.3	5.6	15.3	127.8
F6-97	92.0	93.8	10.0	15.5	7.6	23.5	9.6	13.5	111.0
F6-98	99.0	134.5	11.0	41.0	8.1	25.5	10.6	18.3	201.8
F6-99	97.0	136.5	16.0	30.8	9.6	27.0	15.1	14.0	143.3
F6-100	90.0	128.3	12.0	28.4	13.1	27.1	7.6	9.8	109.0
F6-101	90.0	122.3	8.0	27.2	9.6	21.1	6.1	11.8	78.8

Table 3.4. Continued

	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Lines and Cultivars	50%‡	Height	of Tillers	Area	Length	Length	of Panicle	Branch per	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	Panicle	Panicle (g)
F6-102	96.0	100.7	8.0	23.6	10.6	24.0	7.1	12.3	104.3
F6-103	90.0	139.7	12.0	34.4	21.1	25.3	9.1	12.8	155.0
F6-104	79.0	128.3	14.0	18.4	8.6	25.0	9.1	11.5	78.8
F6-105	83.0	111.8	12.0	25.3	22.1	27.6	9.6	16.0	175.5
F6-106	95.0	116.7	7.0	17.8	11.6	22.1	6.6	13.5	107.0
F6-107	79.0	114.2	4.0	30.2	9.6	27.4	2.6	11.3	166.8
F6-108	83.0	161.8	4.0	36.0	5.6	26.0	3.1	14.5	142.5
F6-109	85.0	102.3	10.0	24.4	7.6	22.5	7.6	12.8	99.3
F6-110	92.0	116.8	8.0	29.0	11.6	25.6	6.1	15.8	143.5
F6-111	100.0	114.3	8.0	29.8	4.6	26.7	8.6	12.8	110.0
F6-112	74.0	121.7	4.0	27.9	10.1	25.9	4.6	14.3	118.3
F6-113	92.0	149.0	10.0	20.8	5.6	24.5	6.6	13.3	105.3
F6-114	91.0	119.0	10.0	21.0	10.1	26.2	7.6	12.3	83.0
F6-115	76.0	145.2	8.0	31.3	23.1	25.7	9.1	10.3	119.8
F6-116	73.0	115.3	5.0	35.5	17.6	26.3	4.1	12.0	167.8
F6-117	78.0	102.2	8.0	28.0	12.6	23.4	7.1	14.0	126.8
F6-118	100.0	116.0	10.5	31.2	8.6	20.8	6.6	9.3	78.0
F6-119	84.0	106.3	6.0	24.8	8.6	25.7	5.1	15.3	154.0
F6-120	99.0	133.3	10.0	29.4	9.6	22.4	9.6	14.3	110.3
Amber33 PI†	101.0	142.9	13.0	43.2	13.7	26.3	11.2	10.5	114.8
Amber PI	100.0	143.3	12.0	38.4	15.6	27.2	9.1	11.5	120.0
Amber Coarse PI	91.0	125.7	15.0	34.4	9.6	20.1	13.1	9.3	57.8
Amber43 PI	89.0	163.5	14.0	36.9	6.6	21.5	12.1	9.3	70.5
Amber33 GSOR	101.0	140.8	14.0	37.8	3.6	27.3	9.1	12.5	124.3
Amber GSOR	100.0	146.8	14.0	33.3	14.1	26.5	8.6	10.3	113.0
Amber Coarse GSOR	94.0	141.0	16.0	36.5	10.1	18.8	14.6	8.5	53.3
Amber43 GSOR	91.0	136.2	10.0	31.3	7.6	18.6	9.6	8.0	46.3

Table 3.4. Continued

Lines and Cultivers	Days to	Plant Usisht	Number	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Lines and Cultivars	50%‡ Heading	Height	of Tillers	Area (cm ²)	Length (mm)	Length	of Panicle	Branch per Panicle	Grains per
Amban	Heading	(cm)	per Plant	, ,	(mm)	(cm)	Per plant		Panicle (g)
Anber	101.0	144.8	14.0	37.4	14.6	28.3	8.6	13.5	128.0
Jazzman	96.3	98.0	6.4	30.3	6.0	21.4	6.0	16.0	142.8
Della	96.8	125.0	8.3	26.9	8.2	23.8	6.7	16.7	132.6
Antonio	77.5	93.7	5.3	23.0	9.5	20.4	5.0	14.5	140.3
Presidio	81.0	118.8	6.0	28.4	11.6	21.7	5.6	16.5	162.5
Della Clor	100.0	114.7	12.0	37.4	5.6	24.9	11.6	19.0	155.5
Basmati T3	68.0	117.0	10.0	52.3	5.6	21.0	9.6	15.0	144.5
Scented A	82.0	143.0	8.0	19.3	9.6	21.7	7.6	12.5	134.5
Basmati	75.0	151.7	8.0	41.0	9.6	23.5	7.6	9.0	43.0
Basmati	78.0	149.0	10.0	39.7	16.6	21.0	5.6	14.0	121.0
Basmati Pardar	80.0	147.0	10.0	38.8	19.6	27.7	4.6	14.0	153.5
Basmati Medium	76.0	142.7	4.0	30.2	22.6	23.7	3.6	11.5	120.0
Basmati	75.0	149.7	6.0	36.5	18.6	24.3	5.6	11.5	87.0
Basmati 6313	81.0	120.3	12.0	26.6	12.6	28.0	9.6	10.0	80.0
Basmati 37	102.0	126.0	10.0	34.3	19.6	30.1	7.6	10.0	114.5
Basmati 5853	79.0	140.7	8.0	45.5	16.6	22.9	7.6	10.5	102.0
Basmati 5874	98.0	126.0	6.0	28.1	13.6	28.9	5.6	9.5	93.5
Basmati	104.0	139.0	4.0	31.1	16.6	26.4	3.6	8.5	85.0
Dellmont	99.0	96.0	6.0	41.5	4.6	22.1	5.6	18.0	128.5
Mean	91.0	123.0	8.7	28.8	11.7	24.6	7.3	13.1	126.1
Range	68.0- 111.0	86.3- 179.7	4.0-16.0	13.1-52.3	3.4- 27.9	18.6- 32.1	2.6-15.1	8.0-21.0	43.0-231.8
CV%§	2.3	3.5	19.3	11.9	11.7	3.6	18.2	9.4	12.5

Table 3.5. Mean squares of the ANOVA showing the effects of lines on number of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, grain yield per line, rice milling, and seed length of rice lines and cultivars tested at Beaumont, Texas in 2017.

Source	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
Line	707.67**	846.26**	24.05**	282.96**	7.63**	29.22 ^{ns}	5733.74**	19.57**	0.26^{**}
Error	51.05	177.23	282.96	24.05	1.46	13.95	1517.48	3.44	0.02

† ns = Non-significant; * = P<0.05; ** = P<0.01

* % = Percentage; g = Gram; mm = Millimeter

Table 3.6. Means of agronomic traits of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, grain yield per line, rice milling, and seed length of rice lines and cultivars tested at Beaumont, Texas in 2017.

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
¥F6-1	49.0	145.5	24.8	75.2	15.8	17.5	79.6	66.6	5.3
F6-2	74.2	74.2	49.7	50.3	19.0	13.9	122.5	66.0	6.6
F6-3	26.7	90.2	22.5	77.5	21.8	8.7	220.1	54.2	6.2
F6-4	51.5	111.5	31.3	68.8	20.5	11.3	186.1	59.9	6.3
F6-5	37.5	60.5	38.0	62.0	20.2	9.2	128.1	61.6	6.0
F6-6	39.2	81.0	32.3	67.7	16.6	8.2	237.3	62.6	5.7
F6-7	51.2	163.7	23.5	76.6	19.6	23.4	216.8	69.4	5.8
F6-8	12.2	96.5	10.9	89.1	17.7	7.5	310.7	66.4	5.6
F6-9	45.0	69.0	39.2	60.8	20.8	9.2	108.4	62.2	6.4
F6-10	40.7	59.0	40.6	59.4	21.5	6.5	151.4	63.4	6.8
F6-11	51.2	52.2	49.3	50.7	20.0	6.7	174.4	56.8	5.8
F6-12	47.0	74.5	38.4	61.6	23.8	9.5	169.3	64.4	6.9
F6-13	100.5	27.2	78.5	21.5	22.1	7.4	44.1	66.7	6.6
F6-14	32.0	119.2	20.8	79.2	21.8	16.6	195.4	60.8	6.5
F6-15	48.7	69.0	41.1	58.9	27.0	15.6	180.8	66.1	6.7
F6-16	61.0	105.7	36.3	63.8	19.7	13.8	83.9	69.9	5.9
F6-17	92.7	53.7	63.1	36.9	24.2	10.8	135.8	66.7	6.1
F6-18	90.7	103.5	46.4	53.6	19.7	10.7	104.9	67.0	5.3
F6-19	64.2	67.2	48.6	51.4	22.8	9.3	172.1	63.7	5.9
F6-20	31.5	58.2	34.9	65.1	23.0	11.6	76.7	65.1	7.3

[†] PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

* % = Percentage; g = Gram; mm = Millimeter

F6 = Filial six generation

Table 3.6. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
F6-21	21.0	81.2	20.2	79.8	23.7	8.8	315.9	60.5	6.9
F6-22	35.5	74.7	31.9	68.1	15.9	7.5	200.7	68.1	6.3
F6-23	19.2	108.0	14.8	85.3	21.9	16.7	233.5	61.9	6.4
F6-24	93.5	74.0	55.6	44.5	21.8	9.8	194.9	60.4	6.3
F6-25	42.7	102.7	29.1	71.0	20.8	13.0	260.7	66.1	6.8
F6-26	11.2	99.2	9.8	90.2	25.9	16.6	209.4	65.3	7.2
F6-27	79.2	153.0	33.8	66.2	21.6	26.6	382.1	64.8	6.7
F6-28	25.7	106.2	19.1	80.9	18.8	15.9	120.2	64.7	6.3
F6-29	65.2	80.0	44.6	55.4	22.0	12.5	275.1	66.8	6.7
F6-30	28.5	73.2	27.7	72.3	24.9	13.9	312.0	56.8	6.4
F6-31	26.0	61.5	29.5	70.6	22.6	7.9	120.5	60.5	6.2
F6-32	27.7	73.7	27.0	73.0	22.7	7.0	180.7	56.8	6.2
F6-33	35.5	101.7	25.5	74.5	23.4	17.8	241.2	54.1	6.3
F6-34	65.0	60.7	51.5	48.5	19.9	8.5	244.3	62.3	6.4
F6-35	42.2	74.5	35.9	64.1	20.8	9.0	193.1	65.2	6.3
F6-36	35.7	44.7	44.3	55.8	19.7	9.4	160.0	60.7	6.9
F6-37	37.0	56.7	39.2	60.8	22.8	8.4	149.3	57.7	6.6
F6-38	38.7	70.7	35.1	64.9	22.8	9.8	141.2	61.3	6.7
F6-39	43.0	61.2	41.0	59.0	22.3	11.1	236.9	65.1	7.1
F6-40	24.0	88.2	21.0	79.0	20.3	10.1	163.3	66.1	6.2
F6-41	21.5	114.0	15.5	84.5	20.0	13.2	327.2	65.7	6.0
F6-42	23.7	103.2	18.3	81.7	24.7	12.3	240.2	69.1	5.8
F6-43	122.7	48.0	71.7	28.3	24.6	8.6	101.5	68.9	7.2
F6-44	54.2	62.7	46.1	53.9	19.3	12.6	121.8	69.3	6.7
F6-45	79.5	31.7	71.4	28.7	21.9	7.3	112.1	58.2	6.6
F6-46	28.0	92.7	22.9	77.2	17.3	9.1	334.6	64.6	6.0

Table 3.6. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
F6-47	72.5	90.3	44.7	55.4	22.4	26.0	267.5	65.9	6.1
F6-48	16.0	128.3	11.0	89.1	20.4	15.0	238.8	66.6	6.1
F6-49	28.5	70.3	29.0	71.0	21.3	12.4	99.7	63.5	6.5
F6-50	29.8	80.0	27.2	72.8	23.7	14.5	205.5	60.5	7.2
F6-51	12.0	136.0	7.9	92.1	17.1	12.9	203.4	63.9	5.2
F6-52	26.8	89.8	23.0	77.0	20.4	11.9	153.6	65.1	6.0
F6-53	46.8	114.8	28.9	71.1	22.4	11.8	264.4	63.2	6.7
F6-54	64.3	117.0	35.5	64.5	20.4	16.7	172.3	69.3	6.5
F6-55	126.3	19.8	87.0	13.0	22.9	11.1	101.4	63.5	6.5
F6-56	27.3	90.3	23.2	76.8	24.8	14.7	271.0	59.7	6.5
F6-57	35.8	65.5	35.5	64.5	20.0	5.6	84.7	67.1	6.5
F6-58	17.8	98.8	15.2	84.8	21.4	15.8	246.4	58.4	7.1
F6-59	33.0	70.5	32.0	68.0	21.6	12.2	233.7	52.3	6.2
F6-60	74.5	98.5	43.1	56.9	23.7	12.2	164.4	60.8	7.3
F6-61	80.8	57.0	58.9	41.1	22.5	8.1	175.5	62.3	6.2
F6-62	53.0	75.8	41.3	58.7	26.6	13.2	202.6	59.8	6.5
F6-63	33.5	63.0	34.9	65.1	21.0	6.7	201.6	61.5	7.4
F6-64	39.5	135.0	22.6	77.4	19.2	12.2	155.9	69.8	5.4
F6-65	38.0	69.3	35.6	64.4	23.8	9.8	117.0	63.6	6.9
F6-66	75.0	91.3	45.2	54.8	18.9	8.9	264.9	64.7	6.2
F6-67	29.8	76.3	28.2	71.8	18.8	10.0	134.7	65.8	5.9
F6-68	55.5	26.0	68.9	31.1	19.2	6.3	126.8	64.2	6.3
F6-69	55.5	57.8	49.3	50.7	22.4	11.7	248.3	66.5	7.0
F6-70	12.3	89.3	12.0	88.0	19.4	17.2	269.2	65.0	6.8
F6-71	66.0	86.5	43.4	56.6	21.1	20.4	142.0	66.0	6.5
F6-72	50.5	92.8	35.3	64.7	21.7	19.6	145.0	60.9	7.2

Table 3.6. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
F6-73	43.5	80.5	35.2	64.8	22.6	11.4	275.5	65.7	7.0
F6-74	106.8	85.3	55.7	44.3	16.8	16.7	112.4	65.2	6.1
F6-75	19.3	111.0	14.7	85.3	23.3	13.7	223.1	60.3	6.7
F6-76	24.0	101.8	19.0	81.0	20.0	13.3	165.1	59.1	6.3
F6-77	78.5	27.3	74.9	25.1	27.0	8.9	116.7	66.7	6.5
F6-78	32.3	84.0	27.8	72.2	18.3	12.4	166.1	62.2	6.2
F6-79	19.3	94.3	16.9	83.1	17.6	13.7	133.8	68.9	5.9
F6-80	12.3	88.3	12.1	87.9	23.0	11.4	167.5	67.3	7.0
F6-81	33.8	82.8	29.1	71.0	20.5	13.5	224.4	63.4	6.8
F6-82	79.8	86.8	48.0	52.0	19.8	19.5	230.6	65.8	6.4
F6-83	46.0	95.0	32.7	67.3	25.5	14.4	88.6	63.8	6.3
F6-84	43.3	57.0	43.5	56.6	27.6	21.3	189.5	56.3	7.3
F6-85	37.0	96.3	27.8	72.2	25.8	33.5	101.6	59.3	7.1
F6-86	13.0	103.5	11.0	89.0	19.7	23.8	228.4	65.0	6.3
F6-87	77.5	124.8	38.3	61.7	18.2	17.0	254.8	66.5	5.9
F6-88	66.5	56.0	54.6	45.4	17.3	6.9	194.6	61.8	6.9
F6-89	106.5	88.5	54.7	45.3	14.5	11.4	70.0	60.5	6.0
F6-90	21.8	95.0	18.6	81.4	24.2	18.0	191.0	63.1	6.8
F6-91	43.3	94.3	31.5	68.5	21.4	12.8	261.6	58.6	6.5
F6-92	92.3	65.5	58.7	41.3	16.0	12.2	172.1	53.9	6.6
F6-93	53.5	61.5	46.5	53.5	19.5	8.3	160.4	55.1	6.3
F6-94	75.0	139.5	35.4	64.6	14.6	16.8	123.7	71.6	5.9
F6-95	8.5	48.0	15.3	84.7	23.5	7.8	244.0	64.4	6.8
F6-96	30.3	95.8	24.4	75.7	18.8	10.4	136.5	63.2	6.4
F6-97	20.5	88.8	19.1	80.9	23.1	17.5	344.3	71.1	6.2
F6-98	53.0	147.0	26.9	73.1	21.4	30.3	97.9	65.8	6.7

Table 3.6. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line (g)	Rice Milling (%)	Seed Length (mm)
F6-99	24.3	117.3	17.6	82.4	17.6	22.8	236.7	63.3	6.2
F6-100	43.5	63.8	40.6	59.4	16.5	10.9	325.9	61.6	6.2
F6-101	11.3	65.8	15.0	85.0	23.9	10.2	176.4	60.1	6.8
F6-102	24.3	78.3	23.9	76.1	22.2	11.1	130.8	64.9	6.7
F6-103	40.5	112.8	26.8	73.2	20.3	19.3	279.2	66.0	6.2
F6-104	12.0	65.0	15.9	84.1	21.8	10.0	169.4	60.5	6.9
F6-105	69.0	104.8	40.0	60.0	19.9	16.3	108.0	58.1	6.4
F6-106	18.3	87.0	17.7	82.3	21.1	11.0	264.0	64.5	6.4
F6-107	127.0	38.0	76.8	23.2	13.7	5.2	205.8	63.2	6.1
F6-108	27.5	113.3	20.0	80.0	21.0	11.4	164.9	58.2	6.8
F6-109	20.5	77.0	21.3	78.7	21.4	11.9	206.6	63.6	6.4
F6-110	38.3	103.5	27.3	72.7	25.4	18.1	267.7	69.2	6.8
F6-111	24.5	83.8	23.0	77.1	17.5	11.4	157.4	67.5	6.1
F6-112	12.0	104.5	10.8	89.2	24.1	14.7	315.1	65.3	7.2
F6-113	36.3	67.3	35.1	64.9	21.1	9.5	165.3	63.5	6.7
F6-114	16.0	65.3	20.0	80.1	20.3	9.2	169.0	67.6	6.4
F6-115	25.8	92.3	22.2	77.8	24.6	22.7	211.5	60.4	6.3
F6-116	29.0	137.0	18.0	82.0	19.3	17.7	374.6	67.6	6.2
F6-117	27.8	97.3	22.6	77.4	22.9	12.1	324.4	54.2	6.3
F6-118	38.3	38.0	49.7	50.3	22.0	6.4	153.7	62.7	6.2
F6-119	81.0	71.3	53.3	46.7	18.5	11.4	196.8	61.6	6.4
F6-120	17.0	91.5	16.1	83.9	23.0	16.8	124.3	66.5	7.1
Amber33 PI†	29.8	84.6	26.3	73.7	19.1	13.1	232.2	60.5	5.5
Amber PI	17.5	100.8	15.3	84.7	17.4	15.4	219.6	62.6	6.0
Amber Coarse PI	14.3	41.8	25.4	74.7	25.1	15.1	260.3	64.4	6.0
Amber43 PI	23.5	45.3	34.0	66.0	19.5	8.2	193.4	54.7	5.6

Table 5.0. Continued			0, 11,	D (11)	TT1 1	<u> </u>	<u> </u>	D'	C 1
	Number of	Number of	Sterility	Fertility	Thousand	Grain	Grain	Rice	Seed
Lines and Cultivars	Unfilled	Filled	Percentage	Percentage	Grain	Yield per	Yield	Milling	Length
	Grains per	Grains per	(%)‡	(%)	Weight	Plant (g)	per line	(%)	(mm)
<u> </u>	Panicle	Panicle	22.6	77.4	(g)	10.6	(g)	50.0	
Amber33 GSOR	27.3	95.3	22.6	77.4	20.9	19.6	218.6	59.0	5.7
Amber GSOR	10.8	100.5	10.2	89.8	18.2	14.0	187.4	63.5	5.9
Amber Coarse GSOR	13.8	37.8	26.5	73.5	25.0	13.6	318.1	65.2	6.0
Amber43 GSOR	9.3	35.3	20.7	79.3	24.1	11.0	117.4	56.0	5.9
Anber	26.3	100.0	21.2	78.8	20.5	12.8	153.8	61.8	5.9
Jazzman	21.5	120.9	15.1	85.0	24.0	17.5	249.1	72.7	6.9
Della	27.0	105.1	20.3	79.7	22.1	17.5	200.3	65.4	6.8
Antonio	15.4	124.4	11.2	88.8	23.6	19.7	316.4	69.4	6.5
Presidio	24.3	136.5	15.6	84.4	22.9	18.2	350.9	72.4	6.7
Della Clor	37.5	116.3	24.8	75.2	22.9	28.1	123.0	63.7	6.8
Basmati T3	40.0	102.8	28.4	71.6	22.4	25.5	76.7	62.2	5.5
Scented A	33.0	99.8	25.2	74.8	24.3	22.9	136.6	68.3	5.3
Basmati	4.5	36.8	11.1	88.9	20.1	7.7	144.7	63.6	6.7
Basmati	80.0	39.3	66.8	33.2	21.4	6.5	163.5	57.3	5.2
Basmati Pardar	38.5	113.3	25.8	74.2	18.2	9.4	264.1	60.1	6.6
Basmati Medium	22.5	95.8	19.4	80.6	16.7	14.9	112.3	62.6	6.6
Basmati	14.5	70.8	17.3	82.7	18.0	23.3	195.9	63.0	6.6
Basmati 6313	17.0	61.3	21.9	78.1	22.5	16.9	200.8	61.5	7.6
Basmati 37	31.5	81.3	28.2	71.8	20.6	12.4	166.2	61.0	6.7
Basmati 5853	21.0	79.3	21.3	78.7	21.2	23.7	335.6	53.4	6.8
Basmati 5874	25.0	66.8	27.4	72.6	22.2	14.3	116.3	59.8	6.8
Basmati	11.5	71.8	14.2	85.8	20.9	11.5	333.9	60.5	6.8
Dellmont	15.5	111.3	12.7	87.3	24.5	11.6	46.4	65.7	6.7
Mean	39.7	86.4	30.7	69.3	21.3	13.7	197.4	63.5	6.4
							44.1-	52.3-	
Range	4.5-127.0	19.8-163.7	7.9-87.0	13.0-92.1	13.7-27.5	5.2-33.5	382.1	72.7	5.2-7.6
CV%§	18.0	15.4	16.0	7.1	5.7	27.2	19.7	2.9	2.2

Table 3.6. Continued

Table 3.7. Mean squares of the ANOVA showing the effects of lines on seed width, chalky seed and aroma of rice lines and cultivars tested at Beaumont, Texas in 2017.

Source	Seed Width (mm) ‡	Chalky Seed (%)	Aroma (2AP)
Line	0.04^{**}	0.79^{**}	0.11^{**}
Error	0.002	0.11	0.0000002

† ns = Non-significant; * = P<0.05; ** = P<0.01
‡ mm = Millimeter; % = Percentage; 2AP = 2-Acetyl-1-pyrroline</pre>

	Seed Width	Chalky	Aroma
Lines and Cultivars	(mm) ‡	Seed (%)	(2AP)
F6-1¥	2.6	1.4	$\frac{(2AF)}{0.00}$
F6-2	2.2	1.2	0.05
F6-3	2.4	1.3	1.31
F6-4	2.2	0.8	0.01
F6-5	2.4	1.7	0.94
F6-6	2.2	1.6	0.01
F6-7	2.3	0.9	0.00
F6-8	2.4	2.1	0.00
F6-9	2.1	0.6	0.00
F6-10	2.2	0.8	0.68
F6-11	2.1	1.5	0.83
F6-12	2.2	1.3	0.90
F6-13	2.4	1.7	0.00
F6-14	2.2	0.4	0.00
F6-15	2.3	0.4	0.00
F6-16	2.3	0.9	0.00
F6-17	2.4	1.4	0.00
F6-18	2.2	1.1	0.65
F6-19	2.4	1.4	0.00
F6-20	2.4	1.7	0.00
F6-21	2.4	0.8	0.06
F6-22	2.1	0.5	0.51

Table 3.8. Means of agronomic traits of seed width, chalky seed and aroma of rice lines and cultivars tested at Beaumont, Texas in 2017.

[†] PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

‡ mm = Millimeter; % = Percentage; 2AP = 2-Acetyl-1-pyrroline

 $\dot{\mathbf{Y}}$ F6 = Filial six generation

Table 3.8. Continued

Lines and Cultivars	Seed Width (mm) ‡	Chalky Seed (%)	Aroma (2AP)
F6-23	2.5	1.5	0.00
F6-24	2.3	0.9	0.46
F6-25	2.0	7.7	0.10
F6-26	2.3	1.1	0.40
F6-27	2.1	0.5	0.01
F6-28	2.1	1.8	0.04
F6-29	2.3	0.5	0.03
F6-30	2.4	1.4	0.01
F6-31	2.5	1.5	0.55
F6-32	2.2	1.7	0.01
F6-33	2.3	0.6	0.43
F6-34	2.2	1.3	0.71
F6-35	2.3	0.5	0.01
F6-36	2.2	2.4	0.65
F6-37	2.2	0.7	0.30
F6-38	2.2	1.8	0.02
F6-39	2.4	0.9	0.02
F6-40	2.2	0.8	0.31
F6-41	2.3	0.3	0.03
F6-42	2.6	1.4	0.18
F6-43	2.3	2.0	0.01
F6-44	2.1	1.7	0.23
F6-45	2.1	1.8	0.18
F6-46	2.2	0.4	0.03
F6-47	2.3	2.2	0.04
F6-48	2.3	0.6	0.28
F6-49	2.4	0.9	0.78
F6-50	2.1	1.0	0.68

Table 3.8. Continued

Lines and Cultivars	Seed Width (mm) ‡	Chalky Seed (%)	Aroma (2AP)
F6-51	2.2	0.2	0.02
F6-52	2.2	0.2	0.66
F6-53	2.1	0.7	0.00
F6-54	2.2	2.1	0.01
F6-55	2.1	0.6	0.01
F6-56	2.3	2.8	0.03
F6-57	2.1	1.7	0.21
F6-58	2.1	1.7	0.03
F6-59	2.1	0.4	0.00
F6-60	2.1	1.8	0.00
F6-61	2.4	2.1	0.55
F6-62	2.4	2.8	0.22
F6-63	2.0	0.4	0.72
F6-64	2.3	0.5	0.02
F6-65	2.3	1.5	0.03
F6-66	2.2	1.1	0.41
F6-67	2.7	1.9	0.80
F6-68	2.3	0.9	0.10
F6-69	2.4	1.5	1.23
F6-70	2.1	0.8	0.75
F6-71	2.3	1.3	0.02
F6-72	2.0	1.4	0.69
F6-73	2.3	1.0	0.71
F6-74	2.2	0.8	0.68
F6-75	2.3	0.5	0.04
F6-76	2.2	2.1	0.67
F6-77	2.4	1.3	0.64
F6-78	2.2	2.2	0.63

Table 3.8. Continued

Lines and Cultivars	Seed Width (mm) ‡	Chalky Seed (%)	Aroma (2AP)
F6-79	2.2	1.1	0.49
F6-80	2.1	2.6	0.78
F6-81	2.3	0.9	0.04
F6-82	2.2	1.2	0.08
F6-83	2.6	2.0	0.66
F6-84	2.2	1.6	0.01
F6-85	2.3	1.2	0.00
F6-86	2.2	2.4	0.44
F6-87	2.4	1.3	0.66
F6-88	2.3	3.3	0.21
F6-89	2.1	2.4	0.01
F6-90	2.4	2.0	0.58
F6-91	2.3	2.4	0.68
F6-92	2.3	0.8	0.03
F6-93	2.2	0.6	0.00
F6-94	2.2	1.2	0.02
F6-95	2.3	1.4	0.03
F6-96	2.1	0.6	0.03
F6-97	2.4	0.6	0.01
F6-98	2.8	1.3	0.01
F6-99	2.1	1.1	0.69
F6-100	2.1	1.3	0.84
F6-101	2.4	1.4	0.21
F6-102	2.1	0.5	0.00
F6-103	2.1	1.9	0.01
F6-104	2.1	1.8	0.19
F6-105	2.3	1.5	0.03
F6-106	2.4	0.8	0.75

Table 3.8. Continued

Lines and Cultivars	Seed Width (mm) ‡	Chalky Seed (%)	Aroma (2AP)
F6-107	2.2	1.5	0.55
F6-108	2.0	0.4	0.02
F6-109	2.4	1.3	0.53
F6-110	2.3	0.4	0.02
F6-111	2.1	2.1	0.00
F6-112	2.1	0.5	0.00
F6-113	2.1	1.1	0.00
F6-114	2.2	0.8	0.14
F6-115	2.2	0.6	0.61
F6-116	2.2	0.8	0.26
F6-117	2.4	2.2	0.02
F6-118	2.4	2.5	0.02
F6-119	2.2	1.1	0.36
F6-120	2.2	0.6	0.02
Amber33 PI [†]	2.2	2.3	0.75
Amber PI	2.1	2.0	0.60
Amber Coarse PI	2.6	1.6	0.04
Amber43 PI	2.5	0.9	0.00
Amber33 GSOR	2.2	2.5	0.78
Amber GSOR	2.0	2.1	0.61
Amber Coarse GSOR	2.6	1.7	0.04
Amber43 GSOR	2.7	1.4	0.01
Anber	2.2	2.0	0.65
Jazzman	2.3	0.6	0.47
Della	2.3	0.5	0.67
Antonio	2.2	0.6	0.00
Presidio	2.2	1.6	0.00
Della Clor	2.1	0.2	0.85

Table 3.8. Continued

Lines and Cultivars	Seed Width	Chalky	Aroma	
Lines and Cultivals	(mm) ‡	Seed (%)	(2AP)	
Basmati T3	3.3	3.1	0.02	
Scented A	3.0	1.9	0.32	
Basmati	1.9	0.4	0.69	
Basmati	2.8	1.5	0.07	
Basmati Pardar	1.8	0.4	0.02	
Basmati Medium	1.9	0.4	0.02	
Basmati	1.8	0.5	0.03	
Basmati 6313	2.0	1.3	0.51	
Basmati 37	2.4	0.6	0.07	
Basmati 5853	1.9	1.1	0.37	
Basmati 5874	1.9	0.9	0.50	
Basmati	2.0	1.0	0.43	
Dellmont	2.5	1.5	0.73	
Mean	2.3	1.3	0.30	
Range	1.8-3.3	0.2-7.7	0.00-1.31	
CV%§	2.0	25.5	0.1	

3.3.1.2. Eagle Lake

In Eagle Lake, significant variation was noted in some morphological and agronomic traits (see Tables 3.9 to 3.12). Many lines, including F6-98, F6-57, F6-56, F6-120, F6-60, F6-62, and F6-79 had longer days to 50% heading (at 110, 108, 106, 105.8, 105.8, 105.8, and 105.8 days, respectively) than Jazzman (the check, at 105.2 days) and parents Amber 33-PI (101 days) and Antonio (79.5 days), the latter of which was not significantly different than Presidio. Nine lines were recorded as having fewer days to 50% heading than Antonio, ranging from 72 to 78 days: F6-66, F6-14, F6-38, F6-44, F6-72, F6-30, F6-9, F6-93, and F6-117.

Plant height presented significant variation among the rice lines and cultivars. The F6-108, F6-7, F6-5, F6-115, F6-72, F6-63, F6-89, F6-90, F6-113, F6-80, and F6-38 lines had a height of 152.5 cm, 149 cm, 145 cm, 139.5 cm, 138.1 cm, 137.9 cm, 136.9 cm, 136.4 cm, 135.8 cm, 134.8 cm, and 134.0 cm, respectively. Additionally, Amber 43-PI, Anber 33, and Amber-GSOR rice cultivars had a height of 144.8 cm, 139.5 cm, and 134.8 cm, respectively – greater than the tallest parent (Amber 33-PI, at 133.8 cm). Dellmont, a US aromatic cultivar, was 88.5 cm and had the shortest plant height among all rice cultivars. At 78.5 cm, F6-98 had the shortest plant height among all rice lines.

The number of tillers per plant varied significantly among rice lines. Seven rice lines had a higher number of tillers per plant than both parents: F6-47, F6-50, F6-52, and F6-91 had 11.9 tillers, F6-111 had 11.8 tillers, and F6-1 and F6-45 had 11.2 tillers. Amber Coarse-GSOR, Amber 33-GSOR, Amber 43-GSOR, Amber Coarse-PI, and Anber 33 presented significant variance and a higher number of tillers per plant compared to the checks Jazzman, Della, Antonio, and Amber 33-PI. The U.S. rice cultivar Dellmont, the Iraqi rice cultivar Amber 43-PI, and two lines F6-83 and F6-23 had the higher flag leaf area among rice cultivars, lines, and checks, at 46.8 cm², 46.1 cm², 46.9 cm², and 44.5 cm², respectively. The F6-71 line was 3.4 cm², the smallest flag leaf area, and significant variation existed for flag leaf area among rice lines and cultivars.

Ligule length differed among rice lines and cultivars, The F6-47, F6-33, F6-90, F6-92, F6-115, F6-57, F6-105, and F6-14 rice lines had a ligule length of 26.5 mm, 25.5 mm, 24.5 mm, 23 mm, 22.5 mm, 22.5 mm, 21.5 mm, and 21.5 mm, respectively – longer than both parents. The Amber-PI, Amber 33-GSOR, Basmati Medium, and Amber-GSOR cultivars (at 23.8 cm, 23.1 cm, 22 cm, and 21.6 cm, respectively) demonstrated no significant differences in ligule length compared to Amber 33-PI (as check, 20.85 cm). The F6-72 and F6-53 rice lines, at 2 mm and 2.5 mm, respectively, had the shortest ligule length among rice lines and cultivars.

Panicle length varied significantly across lines and cultivars. Eighteen rice lines had the longest panicle among lines and cultivars, ranging from 26.0 cm to 33.5 cm. The Amber-PI and Anber 33 cultivars were recorded as having a longer panicle than the four checks (Jazzman, Della, Amber 33-PI, and Antonio). The Basmati-PI-385471 and Scented A cultivars and the F6-16 and F6-82 rice lines were recorded as having the shortest panicle, at 14.0 cm, 16.3 cm, 14.9 cm, and 16.0 cm, respectively.

The number of panicles per plant demonstrated significant differences among the lines and cultivars, with some cultivars such as Amber Coarse-GSOR, Amber 33-GSOR, Amber Coarse-PI, and Anber 33 ranging from 12 to 14 panicles per plant. In addition, lines F6-47, F6-50, F6-52, and F6-91 had a higher number of panicles per plant than both parents, ranging from 11 to 12; Amber 33-PI and Antonio had 10.3 and 5 panicles per plant, respectively. Additionally, the Scented A cultivar had 4 panicles per plant, the F6-41 line had 3, and the F6-104 line had 3.4, the lowest

numbers of panicles per plant in Eagle Lake.

The number of branches per panicle presented highly significant differences among the lines and cultivars. Delmont, Jazzman, Della, and Della-Clor had the highest number of branches per panicle among cultivars (25.5, 19.1, 18.9, and 18.6 branches per panicle, respectively). In addition, the F6-59 rice line had higher number of branches per panicle at 26.1; the F6-104 rice line had the lowest, at 9.1.

In Eagle Lake, no significant variation was noted in some morphological and agronomic traits, such as the number of grains per panicle, the number of unfilled grains per panicle, the sterility percentage, the fertility percentage, and the grain yield per plant (see Tables 3.9 to 3.12).

Thousand-grain weight exhibited highly significant variation. The thousand-grain weight of Dellmont, Amber Coarse-GSOR, Basmati 5853, Jazzman, Basmati T3, and Amber 43-PI were 24.8 g, 24.6 g, 24.0 g, 23.8 g, 23.5 g, and 23.3 g, respectively – significantly heavier than Antonio at 23.1 g. Additionally, the F6-73 rice line was 25.0 g, heavier than the other lines and both parents in thousand-grain weight. F6-111 had the lowest grain weight, 16.1 g, among rice lines.

For seed length, the U.S. rice cultivars Jazzman, Della-Clor, Dellmont, Presidio, Della, and Antonio had 7.1 mm, 6.9 mm, 6.8 mm, 6.8 mm, 6.7 mm, and 6.5 mm, respectively, were classified as long seed. Additionally, Basmati rice cultivars such as Basmati 6313, Basmati 5853, Basmati 5874, Basmati-PI-385817, Basmati-PI-431251, Basmati Medium, Basmati-PI-385817, Basmati 37, and Basmati Pardar had long seeds (at 7.7 mm, 6.9 mm, 6.9 mm, 6.8 mm, 6.8 mm, 6.7 mm, 6.7 mm, 6.7 mm, and 6.7 mm seed length, respectively). In addition, some lines showed longer seeds than Antonio, with seed length ranging from 6.6 mm to 7.3 mm. Iraqi Amber cultivars were classified as medium seed. Several cultivars and lines – such as Amber 33-GSOR, Scented A,

Basmati T3, Basmati-PI-385471, F6-64, F6-1, and F6-51, at 5.6 mm, 5.5 mm, 5.5 mm, 5.3 mm, 5.3 mm, 5.2 mm, and 5.0 mm, respectively had shorter seed than Amber 33-PI, the female parent classified as having medium seeds with a 5.6 mm seed length.

Seed width exhibited significant range among rice lines, from F6-67 (2.7 mm) to F6-57 (1.9 mm). The Iraqi aromatic rice cultivars ranged from Amber 43-PI (2.6 mm) to Amber-PI (2.1 mm). The U.S. rice cultivars ranged from Dellmont (2.3 mm) to Della-Clor (2.2 mm). The Basmati cultivars ranged from Basmati T3 (3.2 mm) to Basmati Pardar (1.7 mm), the largest range among both cultivars and rice lines.

Chalky seed percentage had highly significant differences among the lines and cultivars: F6-112, F6-89, F6-56, F6-88, F6-117, F6-108, F6-53, F6-62, F6-103, F6-86, and F6-118 had 4.5%, 4.1%, 3.8%, 3.6%, 3.5%, 3.3%, 3.2%, 3.1%, 2.9%, 2.8%, and 2.8% chalky seeds, respectively; these were greater than both parents, with Amber 33-PI at 2.7% and Antonio at 0.9%. Basmati T3 and Amber 33-GSOR were recorded as having a chalky seed percentage of 3.6% and 2.8%, respectively – higher than checks Jazzman, Della, Amber 33-PI, and Antonio. By comparison, the Della-Clor cultivar, recorded at 0.3%, had lower chalky seed percentage, and F6-64, recorded at 0.2%, had the lowest chalky seed percentage.

Table 3.9. Mean squares of the ANOVA showing the effects of cultivars on days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at Eagle Lake, Texas in 2017.

Source	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicle Per plant	Number of Branch per Panicle	Number of Grains per Panicle (g)
Line	81.90**	331.00**	6.33**	83.10*	32.33**	8.86*	5.82**	17.032*	1065.14 ^{ns}
Error	3.94	14.05	1.435	31.10	0.77	3.19	1.65	6.38	513.72

† ns = Non-significant; * = P<0.05; ** = P<0.01

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

Table 3.10. Means of agronomic traits of days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at Eagle Lake, Texas in 2017.

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-1¥	96.4	88.5	11.2	25.9	15.0	19.5	10.0	22.9	152.4
F6-2	95.4	117.5	8.2	21.6	13.5	23.2	8.0	18.9	142.4
F6-3	98.4	117.5	4.2	17.7	8.5	23.9	4.0	13.9	150.9
F6-4	97.4	116.0	6.2	39.6	9.0	27.4	6.0	20.9	181.4
F6-5	86.4	145.0	8.2	15.2	8.0	19.1	8.0	9.4	92.4
F6-6	85.4	95.0	10.2	18.7	9.0	22.7	8.0	12.9	114.9
F6-7	92.4	149.0	7.2	38.8	11.0	26.8	7.0	15.9	103.9
F6-8	93.4	92.5	8.2	13.3	9.0	21.9	8.0	16.9	134.9
F6-9	75.4	107.0	4.2	13.6	11.0	23.7	4.0	16.4	152.4
F6-10	85.4	100.5	9.2	10.3	8.5	17.6	8.0	10.4	84.9
F6-11	84.4	87.0	6.2	18.9	6.5	23.2	6.0	10.4	95.9
F6-12	95.4	131.0	6.2	27.6	7.5	27.1	6.0	9.9	120.4
F6-13	86.4	79.0	10.2	19.9	7.0	19.6	10.0	13.4	118.9
F6-14	77.4	90.5	4.2	26.2	21.5	17.4	4.0	10.4	59.9
F6-15	101.4	82.5	8.2	18.7	17.5	14.9	8.0	12.4	73.4
F6-16	93.4	91.5	6.2	13.3	11.0	19.7	6.0	14.4	113.4
F6-17	93.4	79.5	8.2	20.1	3.0	22.7	8.0	10.9	132.4
F6-18	91.4	105.0	4.2	27.4	19.5	24.1	4.0	17.9	95.4
F6-19	91.4	84.5	8.2	22.4	9.0	20.8	7.0	11.9	107.9
F6-20	95.4	106.0	4.2	24.9	7.0	20.8	4.0	14.9	114.9

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

F6 = Filial six generation

Table 3.10. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-21	83.4	129.0	7.2	20.6	7.0	25.0	7.0	15.4	150.4
F6-22	95.4	94.3	9.2	15.0	19.0	24.3	9.0	13.9	140.4
F6-23	99.4	85.5	10.2	44.5	11.5	27.0	10.0	18.1	187.9
F6-24	85.4	120.5	4.2	17.1	4.0	21.1	4.0	16.4	130.9
F6-25	86.4	100.5	4.2	11.7	9.0	20.6	4.0	14.4	69.4
F6-26	81.4	117.5	10.2	11.5	9.0	23.4	9.0	10.9	88.9
F6-27	87.4	117.0	4.2	22.6	10.5	23.0	4.0	14.4	111.4
F6-28	96.4	100.0	6.2	18.6	10.0	26.0	6.0	15.9	140.9
F6-29	97.4	113.0	6.2	39.2	16.5	27.8	6.0	20.9	185.9
F6-30	75.4	82.5	8.2	18.1	11.0	22.3	8.0	15.9	109.9
F6-31	96.4	85.0	6.2	29.3	5.5	21.1	6.0	10.9	97.9
F6-32	95.4	105.0	8.2	14.1	3.0	19.1	8.0	10.9	101.4
F6-33	80.4	133.3	6.2	22.0	25.5	24.3	4.0	11.9	74.4
F6-34	82.4	132.0	8.2	17.8	5.0	24.1	8.0	10.4	90.4
F6-35	91.4	79.0	8.2	17.8	16.0	29.3	8.0	13.9	181.9
F6-36	94.4	120.0	9.2	41.9	3.0	25.2	9.0	15.9	118.9
F6-37	80.4	82.5	8.2	8.7	9.0	22.1	8.0	15.9	89.9
F6-38	77.4	134.0	6.2	28.4	18.5	20.3	6.0	16.4	90.9
F6-39	93.4	128.5	9.2	16.7	13.0	20.5	9.0	17.4	129.4
F6-40	97.4	117.0	4.2	25.3	15.0	23.2	4.0	18.9	151.9
F6-41	96.4	112.5	4.2	30.2	10.0	26.3	3.0	12.9	136.9
F6-42	92.4	109.0	6.2	21.3	11.0	20.3	6.0	13.4	133.4
F6-43	102.4	117.0	6.2	38.4	9.0	24.6	6.0	20.9	181.4
F6-44	77.4	93.0	6.2	16.3	4.0	19.5	6.0	17.9	72.9
F6-45	82.4	92.0	11.2	13.5	7.0	22.2	10.0	17.4	106.4
F6-46	94.4	123.3	6.2	21.6	9.0	22.3	6.0	12.4	103.9
F6-47	97.8	106.2	12.0	28.3	26.5	24.9	12.0	21.1	131.5

Table 3.10. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-48	80.8	108.4	4.0	15.1	8.5	24.3	4.0	14.6	165.0
F6-49	92.8	124.9	4.0	24.1	11.0	23.6	4.0	17.6	150.0
F6-50	95.8	115.4	12.0	25.5	10.0	25.5	12.0	13.6	113.5
F6-51	90.8	119.9	6.0	26.5	15.0	23.9	6.0	17.6	198.0
F6-52	95.8	98.2	12.0	15.9	14.0	21.0	12.0	16.1	126.5
F6-53	95.8	128.4	6.0	29.7	2.5	25.7	6.0	22.1	135.5
F6-54	79.8	103.9	8.0	11.4	17.0	20.3	8.0	14.1	103.5
F6-55	90.8	99.4	10.0	14.2	7.0	20.2	10.0	15.6	115.0
F6-56	106.8	95.4	8.0	16.1	11.0	22.7	8.0	19.1	135.0
F6-57	108.8	101.4	4.0	22.6	22.5	22.2	4.0	11.6	150.0
F6-58	96.8	108.4	6.0	9.7	9.5	26.8	6.0	19.6	143.0
F6-59	98.8	94.4	10.0	15.9	11.0	25.6	9.0	26.1	166.0
F6-60	105.8	114.9	5.0	33.9	5.0	26.8	5.0	11.1	167.5
F6-61	104.8	93.9	6.0	23.5	11.0	26.6	6.0	14.1	161.0
F6-62	105.8	106.4	8.0	27.6	18.5	25.4	8.0	19.6	116.5
F6-63	82.8	137.9	4.0	26.0	14.0	23.1	4.0	13.6	155.0
F6-64	86.8	97.7	4.0	22.6	14.5	25.4	4.0	15.1	189.5
F6-65	97.8	105.2	4.0	17.3	9.0	22.2	4.0	15.3	148.5
F6-66	78.8	110.9	8.0	19.2	7.0	22.3	8.0	13.6	133.0
F6-67	99.8	87.4	8.0	14.4	7.0	23.1	8.0	12.6	111.5
F6-68	98.8	109.9	4.0	8.5	6.0	21.1	4.0	15.1	125.0
F6-69	100.8	87.4	8.0	18.3	9.0	26.6	8.0	15.6	171.0
F6-70	94.8	116.4	6.0	10.0	14.5	25.5	6.0	15.6	125.0
F6-71	83.8	84.4	4.0	3.4	5.0	25.1	4.0	18.6	134.5
F6-72	76.8	138.2	4.0	29.2	2.0	27.9	4.0	19.1	101.0
F6-73	95.8	123.9	4.0	17.4	9.0	23.4	4.0	11.1	174.0
F6-74	99.8	119.4	6.0	30.3	9.0	20.7	6.0	19.6	181.0

Table 3.10. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-75	92.8	131.9	6.0	23.4	6.0	23.5	6.0	13.6	154.0
F6-76	96.8	116.9	8.0	26.8	14.0	24.3	8.0	15.1	130.5
F6-77	98.8	90.9	4.0	18.9	9.0	24.3	4.0	18.1	141.0
F6-78	93.8	113.7	10.0	21.5	19.5	22.9	10.0	17.6	154.0
F6-79	105.8	107.4	6.0	30.2	19.0	19.6	6.0	17.3	165.0
F6-80	95.8	134.9	4.0	21.5	4.0	24.7	4.0	15.1	115.0
F6-81	99.8	114.9	8.0	23.2	10.0	25.1	8.0	19.6	113.5
F6-82	84.8	98.4	8.0	8.8	15.0	16.0	8.0	12.6	116.0
F6-83	94.8	130.9	6.0	46.9	9.0	33.5	6.0	18.1	142.5
F6-84	94.8	80.9	8.0	17.0	6.0	24.7	8.0	14.1	151.0
F6-85	92.8	82.4	8.0	34.3	11.0	25.5	8.0	12.1	132.5
F6-86	103.8	102.9	6.0	25.9	9.0	22.4	5.0	15.1	141.0
F6-87	95.8	107.4	7.0	23.1	13.0	22.6	7.0	20.1	113.5
F6-88	101.8	109.9	8.0	11.8	12.0	23.3	8.0	11.6	151.0
F6-89	94.8	136.9	6.0	23.4	11.0	23.2	6.0	11.1	145.5
F6-90	92.8	136.4	8.0	20.0	24.5	17.8	8.0	9.6	114.0
F6-91	97.8	107.4	12.0	32.8	17.0	25.5	11.0	13.1	139.5
F6-92	91.8	118.9	4.0	20.9	23.0	26.5	4.0	14.6	173.0
F6-93	74.8	117.6	4.8	24.2	7.0	20.4	5.0	13.6	81.6
F6-94	99.8	90.6	6.8	29.1	13.0	24.7	6.0	25.1	136.6
F6-95	98.8	113.1	3.8	21.3	6.0	22.4	4.0	9.6	94.6
F6-96	84.8	124.1	7.8	22.9	9.0	26.2	8.0	13.1	148.1
F6-97	89.8	84.6	5.8	27.0	7.0	18.7	6.0	10.6	92.1
F6-98	110.8	78.6	3.8	28.6	7.5	22.5	4.0	14.6	113.1
F6-99	90.8	126.6	5.8	30.0	9.0	24.7	6.0	13.6	163.1
F6-100	87.8	122.8	9.8	22.9	12.5	21.6	10.0	11.1	115.6
F6-101	85.8	111.1	3.8	35.7	9.0	24.2	4.0	12.6	81.6

Table 3.10. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-102	92.8	97.6	5.8	17.7	10.0	23.3	6.0	12.1	130.1
F6-103	84.8	117.8	3.8	20.3	20.5	21.5	4.0	11.1	158.1
F6-104	91.8	121.8	3.8	13.4	8.0	23.5	3.5	9.1	90.1
F6-105	82.8	111.6	3.8	14.6	21.5	24.7	4.0	13.1	107.1
F6-106	100.8	131.6	5.8	31.1	11.0	22.6	6.0	15.1	211.1
F6-107	100.8	98.1	5.8	29.7	9.0	23.7	6.0	15.6	171.1
F6-108	86.8	152.6	5.8	24.8	5.0	28.8	6.0	15.1	193.6
F6-109	96.8	81.1	3.8	22.5	7.0	23.2	4.0	13.1	152.1
F6-110	99.8	122.3	3.8	36.2	11.0	26.0	4.0	18.6	196.6
F6-111	97.8	114.6	11.8	24.9	4.0	25.7	10.0	19.6	208.1
F6-112	88.8	122.6	5.8	23.5	9.5	22.4	6.0	15.6	154.1
F6-113	90.8	135.8	7.8	20.7	5.0	23.2	8.0	16.1	132.6
F6-114	96.8	96.6	6.8	12.5	9.5	23.2	7.0	17.6	132.6
F6-115	81.8	139.6	7.8	26.7	22.5	22.0	8.0	9.6	108.6
F6-116	83.8	107.3	9.8	23.3	17.0	19.4	10.0	10.6	79.1
F6-117	72.8	86.1	5.8	21.5	12.0	20.5	6.0	12.6	106.1
F6-118	99.8	94.1	9.8	18.2	8.0	21.9	10.0	11.6	117.1
F6-119	83.8	95.6	5.8	23.3	8.0	16.4	6.0	19.6	140.6
F6-120	105.8	102.6	7.8	18.3	9.0	21.6	8.0	19.1	135.6
Amber33 PI†	97.2	133.8	10.5	43.2	20.9	25.4	10.3	13.6	150.9
Amber PI	104.8	128.6	9.8	31.5	23.8	25.8	10.0	12.1	161.6
Amber Coarse PI	90.8	100.8	11.8	22.9	12.8	18.8	12.0	8.1	63.6
Amber43 PI	93.8	144.8	7.8	46.1	11.2	21.7	8.0	9.6	101.6
Amber33 GSOR	94.8	130.1	12.8	32.4	23.1	20.3	12.0	10.1	82.6
Amber GSOR	98.8	134.8	9.8	34.2	21.6	25.2	10.0	13.6	124.1
Amber Coarse GSOR	83.8	126.6	13.3	24.6	13.4	19.9	14.0	9.1	76.1
Amber43 GSOR	93.8	119.6	11.8	22.7	10.2	20.3	10.0	9.1	89.6

Table 3.10. Continued

Lines and Cultivars	Days to 50%‡	Plant Height	Number of Tillers	Flag Leaf Area	Ligule Length	Panicle Length	Number of Panicles	Number of Branches	Number of Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
Anber	99.8	139.6	11.8	37.9	20.6	25.8	12.0	14.6	145.6
Jazzman	105.2	89.3	5.2	35.4	9.9	22.4	5.3	19.1	149.3
Della	102.7	110.8	5.7	32.3	7.3	22.4	5.8	18.9	136.4
Antonio	79.5	88.6	4.8	18.3	11.7	20.5	5.0	17.4	157.0
Presidio	78.8	122.1	7.8	27.0	11.7	19.1	8.0	14.6	178.6
Della Clor	98.8	90.6	7.8	43.1	5.0	24.1	8.0	18.6	202.1
Basmati T3	72.8	95.6	7.8	19.5	5.0	19.5	8.0	10.6	126.1
Scented A	82.8	110.6	3.8	19.9	9.0	16.3	4.0	8.6	118.1
Basmati	76.8	112.6	9.8	17.3	9.0	20.0	10.0	8.6	88.1
Basmati	87.8	121.6	5.8	21.8	16.0	14.0	6.0	9.6	94.1
Basmati Pardar	94.8	111.6	7.8	16.7	19.0	20.5	8.0	10.6	165.1
Basmati Medium	97.8	110.6	3.8	21.0	22.0	17.9	4.0	7.6	122.1
Basmati	99.8	106.6	5.8	16.7	18.0	19.7	6.0	10.6	114.1
Basmati 6313	91.8	123.6	9.8	19.1	12.0	22.0	9.5	8.6	112.1
Basmati 37	106.8	100.6	5.8	17.0	19.0	25.2	6.0	8.6	129.1
Basmati 5853	96.8	98.6	5.8	25.4	16.0	20.3	6.0	8.6	116.1
Basmati 5874	104.8	97.6	5.8	27.0	13.0	22.7	6.0	9.6	135.1
Basmati	103.8	114.6	3.8	28.5	16.0	23.2	4.0	8.6	118.1
Dellmont	105.8	88.6	7.8	46.8	4.0	22.9	8.0	25.6	163.1
Mean	92.9	109.2	7.0	23.8	11.6	22.8	6.8	14.6	131.5
Range	72.8- 110.8	78.6- 152.6	3.8-13.3	3.4-46.9	2.0- 26.5	14.0- 33.5	3.0-14.0	7.6-26.1	59.9-211.1
CV%§	2.1	3.4	17.2	23.4	7.6	7.8	18.8	17.3	17.2

Table 3.11. Mean squares of the ANOVA showing the effects of lines on number of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, seed length seed width and chalky seed percentage of rice lines and cultivars tested at Eagle Lake, Texas in 2017.

Source	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
Line	329.23 ^{ns}	663.80 ^{ns}	120.39 ^{ns}	120.39 ^{ns}	4.11**	23.22 ^{ns}	0.24^{*}	0.04^{**}	0.79**
Error	146.25	554.29	81.63	81.63	0.23	18.02	0.08	0.004	0.12

† ns = Non-significant; * = P<0.05; ** = P<0.01

* % = Percentage; g = Gram; mm = Millimeter

Table 3.12. Means of agronomic traits of number of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, seed length, seed width and chalky seed percentage of rice lines and cultivars tested at Eagle Lake, Texas in 2017.

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
F6-1¥	77.9	77.2	47.7	52.3	17.3	9.0	5.3	2.5	1.7
F6-2	30.1	115.0	19.6	80.4	17.8	12.9	6.6	2.1	1.5
F6-3	60.6	93.0	37.4	62.6	19.7	9.6	6.2	2.4	1.7
F6-4	71.4	112.7	37.1	62.9	18.9	9.8	6.3	2.2	1.2
F6-5	41.7	53.4	40.3	59.7	16.6	10.3	6.1	2.3	1.8
F6-6	46.4	71.3	36.8	63.2	17.0	12.0	5.6	2.2	1.9
F6-7	16.0	90.6	13.9	86.1	21.0	12.9	5.8	2.5	1.0
F6-8	8.6	129.0	5.9	94.1	18.0	14.2	5.7	2.4	2.1
F6-9	28.5	126.6	17.5	82.6	19.6	17.3	6.4	2.1	0.8
F6-10	20.6	67.0	21.5	78.5	20.8	15.2	6.8	2.2	1.1
F6-11	51.9	46.7	48.5	51.5	21.6	9.7	5.8	2.1	1.9
F6-12	31.0	92.1	23.6	76.4	20.0	11.6	6.9	2.2	1.3
F6-13	62.4	59.2	48.0	52.0	23.8	8.6	6.5	2.4	1.8
F6-14	18.1	44.5	25.5	74.5	20.5	8.0	6.6	2.2	0.4
F6-15	30.9	45.3	36.5	63.5	21.6	8.0	6.7	2.2	0.3
F6-16	37.9	78.2	30.4	69.6	20.0	11.3	5.9	2.3	1.3
F6-17	53.6	81.5	37.4	62.6	20.3	16.4	6.2	2.4	2.4
F6-18	33.5	64.6	31.5	68.5	19.6	11.4	5.9	2.2	1.7
F6-19	50.2	60.4	42.2	57.8	22.0	10.5	6.1	2.4	1.9
F6-20	43.4	74.2	34.5	65.5	22.5	7.4	6.9	2.3	1.7

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

***** % = Percentage; g = Gram; mm = Millimeter

F6 = Filial six generation

Table 3.12. Continued

	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%)‡	(%)	Weight	per Plant	(mm)	(mm)	(%)
	Panicle	Panicle	< / ·	~ /	(g)	(g)			
F6-21	56.1	97.1	34.7	65.3	23.1	14.0	7.0	2.4	0.8
F6-22	45.0	98.1	29.7	70.3	16.3	11.5	6.4	2.3	0.6
F6-23	80.5	110.2	40.4	59.6	20.9	20.5	6.6	2.5	1.7
F6-24	56.4	77.2	39.7	60.3	20.3	9.7	6.5	2.3	0.3
F6-25	14.6	57.6	18.1	81.9	21.1	9.0	6.7	2.0	1.5
F6-26	21.4	70.2	21.4	78.6	22.2	12.6	7.0	2.4	1.7
F6-27	39.4	74.7	32.2	67.8	20.6	12.9	6.8	2.1	0.6
F6-28	30.0	113.7	19.7	80.3	18.9	17.2	6.3	2.1	1.5
F6-29	61.0	127.7	31.0	69.1	21.0	13.1	6.8	2.4	1.8
F6-30	31.2	81.4	25.8	74.2	21.3	11.8	6.7	2.4	1.3
F6-31	21.6	79.1	19.8	80.2	21.5	9.1	6.1	2.5	1.6
F6-32	15.7	88.4	14.0	86.1	23.3	8.6	6.1	2.2	2.1
F6-33	7.5	69.6	8.8	91.2	22.1	16.7	6.5	2.3	1.6
F6-34	23.0	70.1	22.7	77.4	19.3	8.4	6.4	2.2	1.8
F6-35	36.1	148.6	18.7	81.3	19.8	14.6	6.4	2.2	0.6
F6-36	39.6	82.0	30.5	69.5	18.4	18.7	7.1	2.1	1.4
F6-37	18.8	73.9	18.6	81.4	22.8	8.1	6.6	2.2	2.4
F6-38	27.9	65.8	27.3	72.7	24.5	13.5	6.9	2.2	2.1
F6-39	33.7	98.4	24.0	76.0	23.9	13.9	7.0	2.3	1.3
F6-40	60.2	94.4	36.9	63.1	20.1	10.7	6.2	2.2	0.6
F6-41	18.8	120.8	12.7	87.3	20.7	6.7	6.2	2.2	0.3
F6-42	26.4	109.7	18.3	81.7	19.9	11.9	6.4	2.5	2.4
F6-43	46.1	138.1	23.9	76.1	20.4	10.2	7.3	2.3	2.2
F6-44	30.1	45.5	35.9	64.1	19.6	9.6	6.7	2.1	2.0
F6-45	56.0	53.2	47.6	52.4	21.5	10.6	6.6	2.1	1.8
F6-46	29.6	77.0	25.7	74.3	19.3	12.7	6.0	2.3	0.5

Table 3.12. Continued

Lines and Cultivars	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%)‡	(%)	Weight	per Plant	(mm)	(mm)	(%)
	Panicle	Panicle			(g)	(g)			
F6-47	68.8	60.1	53.1	46.9	18.5	10.0	5.9	2.3	2.0
F6-48	35.7	126.6	21.9	78.1	20.1	9.3	6.0	2.3	0.6
F6-49	38.0	109.3	25.7	74.3	20.8	10.0	6.3	2.4	1.3
F6-50	45.0	65.9	40.3	59.7	21.2	8.9	7.2	2.0	1.6
F6-51	18.5	176.8	9.4	90.6	19.2	10.8	5.0	2.2	1.5
F6-52	17.7	106.1	14.2	85.8	18.6	13.9	5.7	2.2	1.0
F6-53	39.3	93.5	29.5	70.5	20.3	10.8	6.6	2.1	3.2
F6-54	35.6	65.2	35.1	64.9	20.6	10.2	6.3	2.2	1.9
F6-55	48.0	64.3	42.5	57.5	21.5	5.2	6.3	2.1	0.6
F6-56	51.4	80.9	38.7	61.3	18.8	4.7	6.5	2.3	3.8
F6-57	39.5	107.8	26.7	73.3	21.3	3.6	6.2	2.0	2.0
F6-58	57.0	83.3	40.4	59.6	22.9	6.8	6.8	2.1	1.5
F6-59	61.2	102.1	37.3	62.7	19.6	6.5	5.9	2.1	0.4
F6-60	71.0	93.8	42.9	57.1	22.9	3.8	7.0	2.2	2.1
F6-61	67.7	90.7	42.6	57.5	21.9	4.8	6.0	2.4	2.4
F6-62	35.0	78.8	30.5	69.5	19.6	6.1	6.4	2.4	3.2
F6-63	56.9	95.4	37.2	62.8	24.8	11.1	7.1	2.4	0.8
F6-64	35.5	151.3	18.9	81.1	19.3	5.7	5.3	2.3	0.2
F6-65	49.8	96.0	34.0	66.0	18.1	6.3	6.7	2.3	1.6
F6-66	48.6	81.8	37.1	62.9	23.1	20.4	6.4	2.2	1.3
F6-67	43.1	65.7	39.4	60.6	22.4	8.4	5.6	2.7	1.6
F6-68	43.4	78.9	35.3	64.7	21.8	7.5	6.0	2.3	0.5
F6-69	58.3	110.0	34.5	65.5	24.0	8.3	6.8	2.4	1.9
F6-70	24.8	97.5	20.2	79.8	21.9	9.1	6.6	2.1	1.2
F6-71	59.7	72.1	45.1	54.9	21.3	6.2	6.3	2.2	1.7
F6-72	38.4	59.9	38.8	61.2	23.1	8.4	7.0	2.0	1.5

Table 3.12. Continued

	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%) ‡	(%)	Weight	per Plant	(mm)	(mm)	(%)
	Panicle	Panicle	(70)*	(70)	(g)	(g)	(IIIII)	(IIIII)	(70)
F6-73	90.9	80.4	52.9	47.1	25.0	31.4	7.0	2.3	1.2
F6-74	65.5	112.8	36.6	63.4	20.9	8.8	5.9	2.3	1.0
F6-75	35.2	116.2	23.1	76.9	22.8	10.2	6.5	2.1	0.7
F6-76	25.1	102.7	19.6	80.4	21.3	9.8	6.1	2.2	2.4
F6-77	49.6	88.7	35.7	64.3	19.5	7.7	6.3	2.4	1.7
F6-78	21.5	129.8	14.2	85.9	22.1	15.4	6.1	2.2	2.4
F6-79	62.9	99.4	38.6	61.4	19.3	4.0	5.7	2.2	1.2
F6-80	42.4	69.9	37.5	62.5	22.4	8.3	6.8	2.1	2.6
F6-81	32.2	78.6	28.9	71.2	19.9	6.8	6.7	2.2	1.0
F6-82	44.4	68.9	39.0	61.1	21.1	18.4	6.2	2.2	1.3
F6-83	39.8	100.0	28.3	71.7	21.2	14.0	6.1	2.6	2.4
F6-84	59.9	88.4	40.2	59.8	21.3	12.4	6.9	2.2	1.5
F6-85	71.8	58.0	55.0	45.0	22.1	8.0	7.0	2.3	1.3
F6-86	26.4	111.9	19.0	81.0	21.5	9.6	6.1	2.0	2.8
F6-87	42.2	68.6	37.8	62.2	21.7	18.2	6.2	2.5	1.3
F6-88	59.9	88.4	40.2	59.8	23.1	7.7	6.7	2.3	3.6
F6-89	55.0	87.8	38.3	61.7	20.8	4.1	5.9	2.1	4.1
F6-90	21.1	90.2	18.8	81.2	22.0	28.1	6.2	2.3	2.0
F6-91	26.8	110.0	19.5	80.5	22.0	15.7	6.4	2.2	2.4
F6-92	50.3	120.0	29.4	70.6	24.8	7.6	6.4	2.3	0.3
F6-93	32.4	49.2	44.7	55.3	22.5	3.9	6.4	2.2	0.6
F6-94	39.6	97.0	31.1	68.9	17.4	8.7	6.0	2.2	1.4
F6-95	18.2	76.4	21.3	78.7	21.4	11.0	6.4	2.3	1.8
F6-96	12.8	135.2	9.2	90.8	24.3	14.4	6.5	2.1	0.9
F6-97	17.2	74.9	20.7	79.3	24.8	5.6	6.3	2.4	0.5
F6-98	32.1	81.0	30.9	69.1	20.9	6.1	6.5	2.5	1.3

Table 3.12. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
F6-99	18.2	144.9	11.8	88.2	19.8	10.8	6.3	2.1	0.8
F6-100	29.0	86.6	27.2	72.8	21.5	12.6	6.4	2.1	1.7
F6-101	15.1	66.4	20.9	79.1	19.8	10.9	6.9	2.3	1.2
F6-102	32.9	97.2	27.2	72.8	19.7	8.2	6.8	2.1	1.5
F6-103	52.5	105.5	35.3	64.8	21.8	7.6	6.2	2.2	2.9
F6-104	19.5	70.6	24.0	76.0	21.0	9.4	6.4	2.1	2.2
F6-105	36.3	70.8	37.0	63.0	23.8	6.3	6.5	2.3	1.7
F6-106	78.0	133.0	38.6	61.4	24.6	7.3	6.5	2.4	1.3
F6-107	47.2	123.9	29.1	70.9	23.3	8.1	6.2	2.2	1.9
F6-108	53.1	140.5	28.8	71.2	21.6	11.6	6.9	2.1	3.3
F6-109	54.4	97.7	38.0	62.0	20.9	4.4	6.5	2.4	1.2
F6-110	53.0	143.5	28.3	71.7	20.5	11.5	6.9	2.3	0.8
F6-111	68.4	139.7	34.4	65.6	16.1	8.2	6.2	2.1	2.3
F6-112	50.3	103.7	34.7	65.3	21.7	6.5	7.2	2.1	4.5
F6-113	52.0	80.5	42.1	57.9	22.2	8.7	7.3	2.0	1.6
F6-114	26.8	105.7	21.7	78.3	21.7	10.5	6.5	2.1	1.3
F6-115	37.7	70.8	37.9	62.1	22.6	9.2	6.4	2.2	1.0
F6-116	14.2	64.9	20.3	79.7	23.0	11.0	6.3	2.2	0.7
F6-117	19.8	86.3	20.4	79.6	23.6	6.5	6.4	2.5	3.5
F6-118	31.5	85.6	29.2	70.8	22.7	6.1	6.3	2.4	2.8
F6-119	53.4	87.2	40.6	59.4	21.4	10.3	6.4	2.2	1.0
F6-120	53.5	82.0	42.3	57.7	22.1	10.4	7.2	2.2	0.6
Amber33 PI†	35.8	115.1	24.0	76.0	19.4	12.5	5.6	2.2	2.7
Amber PI	73.1	88.4	48.0	52.1	18.1	4.2	6.1	2.1	2.0
Amber Coarse PI	7.5	56.1	13.8	86.3	22.4	13.3	6.2	2.5	1.6
Amber43 PI	34.2	67.4	36.9	63.1	23.3	10.9	5.7	2.6	2.0

Table 3.12. Continued

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
Amber33 GSOR	12.2	70.4	16.6	83.4	20.3	6.4	5.6	2.2	2.8
Amber GSOR	20.1	104.0	17.4	82.6	19.9	19.2	6.1	2.1	2.6
Amber Coarse GSOR	12.5	63.5	18.7	81.3	24.7	18.1	6.1	2.5	1.7
Amber43 GSOR	20.7	68.9	25.7	74.3	21.4	12.8	6.0	2.6	1.5
Anber	42.8	102.8	31.3	68.7	20.6	11.7	5.7	2.3	2.1
Jazzman	51.8	97.5	35.9	64.1	23.8	16.3	7.1	2.3	0.4
Della	43.0	93.4	33.0	67.1	23.0	17.2	6.7	2.3	0.6
Antonio	40.6	116.4	26.4	73.6	23.2	19.1	6.5	2.2	0.9
Presidio	36.6	142.0	21.6	78.4	22.6	20.1	6.8	2.2	2.1
Della Clor	56.0	146.1	29.0	71.0	22.3	18.9	6.9	2.2	0.3
Basmati T3	10.0	116.1	8.6	91.5	23.5	18.3	5.5	3.2	3.6
Scented A	6.3	111.7	5.8	94.2	20.8	16.1	5.5	3.0	1.7
Basmati	4.1	84.0	5.1	94.9	20.3	10.4	6.7	2.0	0.4
Basmati	9.4	84.6	11.1	88.9	22.1	9.9	5.3	2.7	1.7
Basmati Pardar	29.6	135.5	19.0	81.0	18.7	4.8	6.7	1.7	0.5
Basmati Medium	47.5	74.5	42.1	58.0	18.3	11.5	6.7	1.9	0.5
Basmati	16.2	97.9	15.4	84.6	18.3	12.7	6.8	1.9	0.4
Basmati 6313	16.4	95.7	15.9	84.1	21.7	14.3	7.7	2.1	1.4
Basmati 37	43.5	85.6	36.3	63.8	17.5	7.1	6.7	2.4	0.6
Basmati 5853	24.0	92.1	22.4	77.6	24.1	12.3	6.9	1.9	0.4
Basmati 5874	41.0	94.0	32.6	67.4	21.8	10.4	6.9	1.9	0.8
Basmati	45.8	72.3	42.0	58.0	19.9	8.2	6.8	2.0	1.2
Dellmont	44.6	118.4	29.0	71.0	24.9	8.4	6.8	2.3	1.5
Mean	39.2	92.3	29.5	70.5	21.2	11.3	6.4	2.2	1.5
Range	4.1-90.9	44.5-176.8	5.1-55.0	45.0-94.9	16.1-25.0	3.6-31.4	5.0-7.7	1.7-3.2	0.2-4.5
CV%§	30.8	25.5	30.7	12.8	2.3	37.5	4.5	2.7	22.7

3.3.1.3. Interactions between the Two Locations

The results of the interactions between the two locations, Beaumont and Eagle Lake, as presented in Tables 3.13 and 3.14, demonstrates that some morphological and agronomic traits (such as the days to 50% heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle, number of filled grains per panicle, grain yield per plant, and chalky seed percentage) varied significantly (Least Significant Difference, LSD, 0.01) among the locations. Several morphological and agronomic traits such as number of grains per panicle, sterility percentage, and fertility percentage varied significantly (LSD 0.05) among the locations. In addition, the results demonstrate no significant differences for four morphological and agronomic traits across locations: unfilled grains per panicle, thousand-grain weight, seed length, and seed width. The cultivars demonstrated highly significant differences in all morphological and agronomic traits, and the interactions between locations and cultivars showed highly significant differences for many traits such as the days to 50% heading, plant height, ligule length, unfilled grains per panicle, sterility percentage, fertility percentage, thousand-grain weight and chalky seed percentage. Additionally, while significant differences exist for some traits (such as number of tillers per plant, flag leaf area, panicle length, number of panicles per plant, number of grains per panicle), five morphological and agronomic traits (number of branches per panicle, number of filled grains per panicle, grain yield per plant, seed length, and seed width) had no significant variation in terms of interactions between locations and cultivars.

Table 3.13. The multi-location means squares of the ANOVA for days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at Beaumont and Eagle Lake, Texas in 2017.

Source	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicles Per plant	Number of Branches per Panicle	Number of Grains per Panicle (g)
Location	238.80**	15050.84**	241.08**	2244.82**	11.74**	280.82^{**}	18.25**	152.49**	1711.13*
Cultivar	146.12**	625.03**	11.59^{**}	109.87**	56.70^{**}	12.12^{**}	8.76^{**}	17.32^{**}	1439.92**
Location*Cultivar	29.49^{**}	79.98^{**}	4.72^{*}	43.48^{*}	3.52^{**}	3.93*	3.65 [*]	6.23 ^{ns}	780.54^{*}
Error	4.24	16.08	2.14	21.49	1.34	1.99	1.71	3.95	380.88

† ns = Non-significant; * = P<0.05; ** = P<0.01

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

Means of traits shown in Table 3.13 are included in Appendix 1.

Table 3.14. The multi-location ANOVA for number of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, seed length seed width and chalky seed percentage of rice lines and cultivars tested at Beaumont and Eagle Lake, Texas in 2017.

Source	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
Location	314.55 ^{ns}	3492.96**	386.82*	386.82*	0.13 ^{ns}	490.52**	0.02 ^{ns}	0.002 ^{ns}	4.19**
Cultivar	663.26**	990.14**	264.59**	264.59**	7.85^{**}	33.66*	0.49^{*}	0.08^{**}	1.27^{**}
Location*Cultivar	373.64**	519.92 ^{ns}	138.75^{**}	138.75^{**}	3.89**	18.77 ^{ns}	0.01 ^{ns}	0.003 ^{ns}	0.31**
Error	98.65	365.76	52.84	52.84	0.85	16.0	0.05	0.003	0.11

† ns = Non-significant; * = P<0.05; ** = P<0.01 ‡ % = Percentage; g = Gram; mm = Millimeter

Means of traits shown in Table 3.14 are included in Appendix 2.

3.3.2. Mapping Quantitative Trait Loci and Associated Genes in Aromatic Rice in a

Recombinant Inbred Lines Population

Eighteen linkage groups were generated, covering all 12 chromosomes of the rice genome in JoinMap. Twenty-six QTLs associated with 21 different traits were identified in the Amber 33-PI x Antonio population (see Figure 3.4).

3.3.2.1. Mapping Quantitative Trait Loci for Plant Height

Three QTLs in chromosome 1 associated with plant height, qHP1.1, qHP1.2, and qHP1.3, had LOD scores of 4.26, 5.49 and 7.89, respectively, and individually variance explained 15.1%, 19%, and 26.1% of the variation, respectively. Sarma et al. (2017) found the QTL on chromosome 1 in one of this locations that explained largest amount of variation amongst the QTLs for plant height detected. Han et al. (2017) found three QTLs for plant height, with the major ones being qHd7.1, qHd7.2 found in chromosome 7 and only one in chromosome 1.

3.3.2.2. Mapping Quantitative Trait Loci for Heading Date/ Flowering

Six QTLs in chromosome 3 associated with days to 50% heading were qDH3.1, qDH3.2, qDH3.3, qDH3.4, qDH3.5, and qDH3.6, which had LOD scores of 4.73, 8.67, 8.21, 9.17, 13.31, and 14.08, respectively, and individually variance explained 16.6%, 28.3%, 27%, 29.7%, 40%, and 41.7% of the variation, respectively. Talukdar et al. (2017) found eight QTLs to be associated with the time of flowering on chromosomes 3, 5, 7, 8, 10 and 12, and among which the QTL linked to chromosome 3 explained the highest differences. Han et al. (2017) found eight QTLs for heading date but qHd8 in chromosome 8 was the major QTL.

3.3.2.3. Mapping Quantitative Trait Loci for Aroma

Six QTL associated with aroma having a LOD score higher than 3.0 were identified in the Amber 33-PI x Antonio population. Four major effect QTLs (qAR8.1, qAR8.2, qAR8.3, and qAR8.4) were identified in chromosome 8 with LOD scores of 11.81, 30.8, 28.34 and 20.51, respectively and they explained 36.4%, 69.3%, 66.3% and 54.5% of phenotypic variance in aroma. Two minor effect QTLs (qAR10.1 and qAR10.2) were identified in chromosome 10 with LOD scores of, 4.74, and 4.62, respectively, and they explained 16.6%, and 16.2% of the total phenotypic variance in aroma, respectively.

The QTLs identified in this study appear to be congruent with the results from previous genetic mapping studies. Sarma et al. (2017) identified three QTLs for aroma, one QTL on chromosome 5 and two QTLs on chromosome 8. In addition, Talukdar et al., (2017) found a major QTL for aroma in Joha rice on chromosome 8.

This finding is also consistent with Hashemi et al. (2015) who identified two QTLs on chromosomes 4 and 8 that explained from 3.2% to 39.3% of the total aroma phenotypic variance. Talukdar et al. (2017) as mentioned above found a major QTL for aroma in Joha rice on chromosome 8, and four markers had significant association for aroma on three chromosomes, namely 7, 8 and 10, but among which RM214 on chromosome 7 explained the highest variation of 19.61. In addition, Sarma et al. (2017) identified three QTLs for aroma, one on chromosome 5 and two on chromosome 8, out of which the QTLs between Aro1-BAD2 was in similar position with aroma gene of Basmati rice. Results across these studies and ours show chromosome 8 has the largest effect on aroma. It is reported that using prominent markers such as RM23120 on chromosome 8, which is linked to aroma in Basmati rice cultivars, is advantageous to MAB (Sun et al., 2008).

3.3.2.4. Mapping Quantitative Trait Loci for Fertility/ Sterility

Three QTLs in chromosome 5 associated with fertility percentage and sterility percentage traits were qFP5.1, qFP5.2, and qFP5.3 (for fertility percentage) and qSP5.1, qSP5.2, and qSP5.3 (for sterility percentage), which means both traits share the same location and had similar LOD scores and percentages of variance explained. LOD score was 5.47, 4.91, and 4.7, respectively; individually variance explained 18.9%, 17.2%, and 16.5%, respectively. Zhao et al. (2016) found eleven related QTLs for four traits, with two QTLs, qSFht2 and qSFht4.2, for spikelet fertility on chromosomes 2 and 4.

3.3.2.5. Mapping Quantitative Trait Loci for Seed Traits (Full Seed, Seed Length)

Single QTL in chromosome 12 associated with full seed (filled grain) trait, qFS12, had a LOD score of 4.11, and individually variance explained 14.6% of the trait variation. For seed length, four QTLs were located in chromosome 7, qSL7.1, qSL7.2, qSL7.3, and qSL7.4 that can explain 23.3, 14.4, 15.0 and 18.5% of the variation, respectively. Qi et al. (2017) found the QTLs of grain length associated on chromosomes 3, 4 and 12, qGL3.2, qGL4, qGL12.1, and qGL12.2, were consistently detected across two to four environments, and qGL12.2 was mapped to chromosome 12 with a phenotypic variation explained (PVE) of 16.54%.

This study demonstrated that the highest variance percentages among seven morphological and agronomic traits and 26 QTLs were found in four aroma QTLs (qAR8.2, qAR8.3, qAR8.4 and qAR8.1) in chromosome 8, with 69.3%, 66.3%, 54.5% and 36.4% variances, respectively.

The present study showed that the largest QTLs for each trait, out of 26 QTLs in seven morphological and agronomic traits, including days to 50% heading, plant height, fertility percentage, sterility percentage, full seed, seed length and aroma, were qDH3.6, qHP1.3, qFP5.1, qSP5.1, qFS12, qSL7.1 and qAR8.2 respectively. Han et al. (2017) found eight QTLs for heading

date and three QTLs for plant height, with the major ones being qHd7.1, qHd7.2 and qHd8 for heading date, and qPh1 and qPh7.1 for plant height. Zhao et al. (2016) found eleven related QTLs for four traits, with two QTLs qSFht2 and qSFht4.2 for spikelet fertility on chromosomes 2 and 4.

The QTLs related to day to 50% heading, plant height, fertility percentage, sterility percentage, full seed, seed length and aroma can be fine mapped, and markers associated with them can be developed and validated and used in MAS and MAB to improve gain from selection and improve the selection efficiency. A total of 26 QTLs affecting seven morphological and agronomic traits were identified by MapQTL 6 software with the powerful MQM mapping, using its composite interval mapping (CIM) function at 4.0 cut off LOD score. Using MapQTL 6 is the regression approximation to maximum probability MQM mapping and interval. These markers can be developed based on the sequences of specific markers related with each trait. This will be followed by utilizing the sequences to make primers for SNP genotyping using the KASP assay at the AgriGenomics Lab for MAS and MAB. These results provided an opportunity for developing markers associated with the aroma (qAR8.2, qAR8.3 and qAR10.1) and full seed (qFS12) QTLs, thus providing an opportunity for combining yield improvement, tolerance to biotic and abiotic stresses and the aroma in Iraqi aromatic rice.

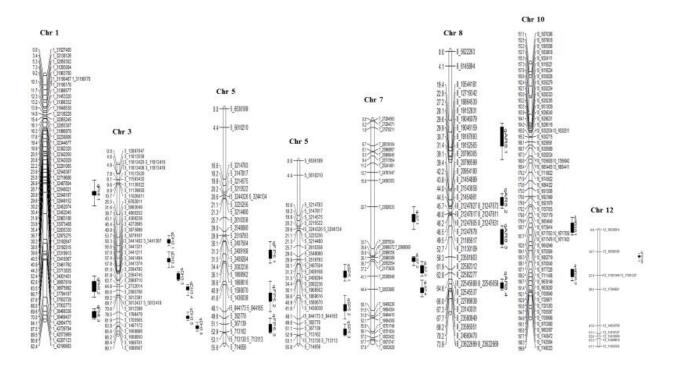


Figure 3.4. Distribution of 26 QTLs for seven traits in the molecular linkage map of Recombinant Inbred Lines (RILs) population. QTLs are indicated in bold at right side of the chromosomes and names of the markers. The numbers the left side are the genetic distance between markers in centimorgans (cM).

	Days to	Plant	Number of	Flag Leaf	Ligule	Panicle	Number	Number of	Number of
Lines	50%‡	Height	Tillers per	Area	Length	Length	of	Branches	Grains per
Lines	Heading	(cm)	Plant	(cm^2)	(mm)	(cm)	Panicles	per Panicle	Panicle (g)
							Per plant		
F6-1¥	98.5	88.7	11.5	30.6	15.5	21.9	10.8	18.8	174.2
F6-2	98.0	128.7	10.5	24.8	14.0	24.6	9.8	16.0	146.2
F6-3	97.5	125.2	4.0	19.5	8.5	24.0	4.0	13.7	134.7
F6-4	95.0	112.8	7.0	33.4	9.5	26.3	5.3	18.4	172.9
F6-5	83.0	144.4	7.0	22.2	8.5	20.8	7.0	9.9	95.9
F6-6	80.5	106.3	12.0	26.0	9.5	23.4	7.8	13.0	118.3
F6-7	86.5	159.9	8.0	37.8	11.5	28.0	8.0	15.2	160.2
F6-8	92.5	100.1	9.0	16.2	9.5	22.5	6.5	13.7	122.6
F6-9	74.5	109.6	4.0	19.7	11.5	23.9	4.3	14.0	133.9
F6-10	79.0	104.1	7.0	12.6	8.2	18.9	6.8	10.4	93.1
F6-11	86.0	97.2	6.0	17.4	7.0	23.6	5.8	11.0	100.4
F6-12	96.5	137.5	7.8	24.0	8.7	28.0	5.8	10.3	121.7
F6-13	91.5	89.5	12.5	27.5	7.5	20.6	8.5	13.3	124.1
F6-14	78.0	97.4	7.0	29.4	23.0	20.8	5.8	12.9	106.3
F6-15	100.0	92.3	8.0	24.9	18.7	20.5	7.8	13.5	96.3
F6-16	96.5	95.2	8.0	27.2	11.5	22.2	6.8	14.7	140.8
F6-17	94.5	86.4	10.0	21.4	3.5	23.3	7.3	12.5	140.2
F6-18	95.0	105.4	6.0	26.8	20.0	25.2	4.3	16.8	145.6
F6-19	89.0	89.9	7.0	25.2	9.5	21.7	6.5	11.9	120.4
F6-20	91.0	116.3	5.0	28.9	7.5	22.8	5.3	12.9	103.1
F6-21	81.0	125.1	10.0	20.0	7.5	23.8	7.5	12.9	127.1
F6-22	89.5	104.0	6.5	20.0	19.5	24.4	7.3	13.3	126.1

Table 3.15. Means of agronomic traits of days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines tested at multi-location, Texas in 2017.

F6 = Filial six generation

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

Table 3.15.	Continued
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Lines	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicles Per plant	Number of Branches per Panicle	Number of Grains per Panicle (g)
F6-23	99.5	93.4	9.0	34.3	12.0	26.0	9.3	15.2	158.3
F6-24	80.5	128.2	5.0	20.9	4.5	22.8	4.8	15.2	149.9
F6-25	79.5	108.3	5.0	15.4	9.5	21.4	4.8	15.0	108.2
F6-26	79.5	128.7	9.0	13.4	9.5	24.1	8.5	10.8	100.2
F6-27	86.5	116.5	7.0	21.2	11.0	25.8	6.5	15.5	172.6
F6-28	98.0	105.9	6.0	23.1	10.5	26.6	7.0	15.9	137.2
F6-29	95.0	115.9	6.0	39.8	16.0	27.5	6.3	17.8	166.3
F6-30	74.0	92.9	6.0	26.1	11.5	22.3	6.3	13.8	106.6
F6-31	90.0	85.9	5.5	24.7	5.0	22.0	5.8	10.7	93.4
F6-32	95.0	118.0	6.0	14.3	3.5	20.9	6.3	10.7	102.2
F6-33	76.5	131.6	8.5	32.1	25.2	25.6	0.3 7.3	10.9	106.6
F6-34	85.5	131.0	8.5 9.5	22.7	5.5	25.3	8.0	10.9	108.8
F6-35	91.0	87.8	9.5 8.0	19.1	5.5 16.5	23.3 28.3	8.0 7.8	10.9	150.1
F6-36	96.5	129.1	8.5	33.9	3.5	28.3 25.9	8.8	13.0	100.4
F6-30 F6-37	90.3 77.5	129.1			9.5			13.0	92.6
			8.0	11.0		21.9	7.5		
F6-38	78.0	131.9	6.0	32.4	18.2	22.8	6.0	14.0	100.9
F6-39	97.0	127.1	8.5	23.1	13.5	23.0	8.5	14.3	117.6
F6-40	96.0	123.5	5.5	22.8	15.5	23.0	5.0	15.0	132.8
F6-41	97.0	121.4	5.0	30.2	10.5	24.9	4.0	12.7	136.9
F6-42	93.0	110.4	5.8	22.9	11.5	23.9	5.5	12.9	130.9
F6-43	106.5	126.6	6.0	36.8	9.5	26.1	6.0	18.9	176.8
F6-44	84.5	98.5	6.0	17.7	4.5	20.8	6.3	16.0	95.7
F6-45	87.0	100.7	9.5	19.8	7.5	22.1	8.8	14.4	109.6
F6-46	96.0	130.0	6.0	24.4	9.5	21.8	5.8	11.5	113.1
F6-47	99.5	115.6	13.5	29.9	27.2	27.0	12.0	17.4	147.6

Table 3.15.	Continued
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Lines	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicles Per plant	Number of Branches per Panicle	Number of Grains per Panicle (g)
F6-48	79.5	112.0	5.0	18.2	10.2	24.8	5.0	13.0	155.1
F6-49	96.5	124.4	6.0	24.9	11.7	22.5	6.0	14.3	124.9
F6-50	96.0	120.9	11.0	24.3	10.7	25.7	9.7	12.6	112.1
F6-51	90.0	122.5	6.0	28.5	15.7	23.7	5.7	14.5	173.5
F6-52	98.5	108.1	11.0	21.9	14.7	22.2	10.2	14.1	122.0
F6-53	93.0	130.2	7.0	27.1	3.2	25.6	6.2	18.3	149.0
F6-54	84.5	110.5	8.8	14.8	17.7	22.8	8.5	13.8	142.9
F6-55	88.0	106.2	11.5	16.4	7.7	20.9	11.0	14.4	131.0
F6-56	104.0	107.9	11.0	29.9	11.7	25.2	9.0	15.6	126.7
F6-57	102.5	115.9	6.0	20.2	19.2	23.2	4.5	12.1	126.1
F6-58	87.5	114.0	8.3	16.9	10.2	26.3	7.5	16.5	130.2
F6-59	97.5	106.2	10.0	21.0	11.7	24.9	9.0	19.6	135.2
F6-60	103.5	127.7	6.5	34.9	5.7	27.4	5.0	14.0	170.7
F6-61	103.0	105.0	7.0	31.2	11.7	26.9	5.2	13.1	149.9
F6-62	103.5	115.4	9.0	28.7	19.2	27.8	7.2	15.5	123.1
F6-63	86.0	137.8	5.0	27.9	14.7	24.9	3.7	12.6	126.2
F6-64	89.0	97.3	5.0	26.9	15.2	23.7	4.5	17.9	182.5
F6-65	93.0	110.4	5.0	18.6	9.7	22.5	4.5	14.0	128.4
F6-66	82.0	105.9	7.0	22.3	7.7	24.5	6.2	13.8	150.1
F6-67	99.0	88.4	9.0	17.2	7.7	21.2	7.5	13.9	109.2
F6-68	95.5	117.1	7.0	12.6	6.7	20.5	5.2	13.9	103.7
F6-69	100.5	100.8	10.0	18.1	9.7	26.4	9.0	13.3	142.6
F6-70	93.5	121.0	9.0	17.2	15.2	24.7	8.0	14.6	113.7
F6-71	86.5	90.0	9.0	9.2	5.7	24.9	8.7	17.8	144.0
F6-72	88.5	158.9	7.0	31.1	2.7	30.0	6.2	16.9	122.6

Table 3.15.	Continued
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Lines	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicles Por plant	Number of Branches per Panicle	Number of Grains per Panicle (g)
F6-73	97.0	135.4	7.0	10.1	9.7	23.8	Per plant	11.8	149.5
				19.1			6.0 7.0		
F6-74	100.5	119.4	7.0	28.9	9.7	22.5	7.0	19.6	187.0
F6-75	95.0	140.4	6.0	26.0	5.7	24.9	6.0	13.8	142.6
F6-76	98.5	127.8	11.0	31.8	14.7	25.8	8.2	13.4	128.6
F6-77	100.5	107.9	7.0	18.0	9.7	24.0	5.7	15.6	123.9
F6-78	97.0	128.1	11.0	24.2	20.2	25.1	10.5	14.8	135.6
F6-79	97.0	132.5	8.0	22.4	19.7	21.3	7.5	16.6	139.7
F6-80	92.0	132.1	5.0	22.1	4.7	26.5	5.0	13.6	108.2
F6-81	100.5	120.9	7.0	32.2	10.7	25.7	6.7	17.5	115.5
F6-82	91.5	114.3	9.0	15.1	15.7	18.8	8.7	14.0	141.7
F6-83	98.0	141.5	9.0	43.4	9.7	30.9	7.0	15.8	142.2
F6-84	94.0	85.4	10.0	16.9	6.7	23.9	9.7	13.3	126.1
F6-85	94.5	87.0	11.0	34.9	11.7	25.7	10.5	12.4	133.4
F6-86	101.5	113.9	7.0	27.1	9.7	23.0	6.5	13.4	129.2
F6-87	95.0	117.9	7.5	31.6	13.7	23.4	7.5	19.3	158.4
F6-88	100.0	122.8	6.5	17.2	12.7	24.8	7.0	12.3	137.2
F6-89	90.0	149.8	8.0	28.7	11.7	25.3	7.7	13.5	170.7
F6-90	97.5	124.4	6.0	19.3	25.2	20.6	6.0	10.8	115.9
F6-91	99.5	118.0	10.0	36.3	17.7	27.4	8.5	12.6	139.0
F6-92	91.0	120.7	5.5	32.0	23.7	26.8	5.2	14.9	165.9
F6-93	75.0	124.0	6.5	36.0	7.3	23.6	6.0	13.6	97.1
F6-94	99.5	94.6	6.5	30.6	13.3	23.0	5.8	23.0	174.3
F6-95	96.0	116.3	5.0	21.9	6.3	23.1	4.5	10.2	74.3
F6-96	89.0	132.8	8.0	27.2	9.3	26.3	6.8	14.1	135.8
F6-97	91.0	89.0	8.0	21.2	7.3	21.1	7.8	12.0	99.5

Tabl	e 3.15.	Continu	ed
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Lines	Days to 50%‡ Heading	Plant Height (cm)	Number of Tillers per Plant	Flag Leaf Area (cm ²)	Ligule Length (mm)	Panicle Length (cm)	Number of Panicles Per plant	Number of Branches per Panicle	Number of Grains per Panicle (g)
F6-98	105.0	106.3	7.5	34.9	7.8	24.0	7.3	16.3	155.3
F6-99	94.0	131.3	11.0	30.4	9.3	25.9	10.5	13.7	151.1
F6-100	89.0	125.3	11.0	25.7	12.8	24.4	8.8	10.3	110.2
F6-101	88.0	116.5	6.0	31.5	9.3	22.7	5.0	12.1	78.1
F6-102	94.5	98.9	7.0	20.7	10.3	23.7	6.5	12.1	115.1
F6-103	87.5	128.5	8.0	27.4	20.8	23.5	6.5	11.8	154.5
F6-104	85.5	124.8	9.0	16.0	8.3	24.3	6.3	10.2	82.3
F6-105	83.0	111.5	8.0	20.0	21.8	26.2	6.8	14.5	139.2
F6-106	98.0	123.9	6.5	24.5	11.3	22.4	6.3	14.2	157.0
F6-107	90.0	105.9	5.0	30.0	9.3	25.6	4.3	13.3	166.8
F6-108	85.0	157.0	5.0	30.5	5.3	27.4	4.5	14.7	166.0
F6-109	91.0	91.5	7.0	23.6	7.3	22.9	5.8	12.8	123.6
F6-110	96.0	119.3	6.0	32.6	11.3	25.8	5.0	17.1	168.0
F6-111	99.0	114.2	10.0	27.4	4.3	26.2	9.3	16.1	157.0
F6-112	81.5	121.9	5.0	25.8	9.8	24.2	5.3	14.8	134.1
F6-113	91.5	142.2	9.0	20.8	5.3	23.9	7.3	14.6	116.8
F6-114	94.0	107.5	8.5	16.9	9.8	24.8	7.3	14.8	105.7
F6-115	79.0	142.1	8.0	29.0	22.8	23.9	8.5	9.8	112.1
F6-116	78.5	111.1	7.5	29.4	17.3	22.9	7.0	11.2	121.3
F6-117	75.5	93.9	7.0	24.8	12.3	22.0	6.5	13.2	114.3
F6-118	100.0	104.8	10.3	24.8	8.3	21.4	8.3	10.3	95.5
F6-119	84.0	100.7	6.0	24.1	8.3	21.1	5.5	17.3	145.2
F6-120	102.5	117.7	9.0	23.9	9.3	22.0	8.8	16.6	120.8

Table 3.16. Means of agronomic traits of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, grain yield per line, rice milling, and seed length of rice lines tested at multilocation, Texas in 2017.

Lines	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Grain Yield per line† (g)	Rice Milling† (%)	Seed Length (mm)
¥F6-1	63.5	112.0	36.5	63.5	16.6	12.9	79.6	66.6	5.3
F6-2	52.2	95.3	34.9	65.1	18.4	13.1	122.5	66.0	6.6
F6-3	43.7	92.3	30.2	69.8	20.7	8.8	220.1	54.2	6.2
F6-4	61.5	112.8	34.4	65.6	19.7	10.2	186.1	59.9	6.3
F6-5	39.6	57.6	39.4	60.6	18.4	9.4	128.1	61.6	6.1
F6-6	42.8	76.8	34.8	65.2	16.8	9.8	237.3	62.6	5.7
F6-7	33.6	127.8	18.9	81.1	20.3	17.8	216.8	69.4	5.8
F6-8	10.4	113.4	8.6	91.4	17.9	10.5	310.7	66.4	5.7
F6-9	36.8	98.5	28.6	71.5	20.3	12.9	108.4	62.2	6.4
F6-10	30.7	63.7	31.3	68.7	21.2	10.5	151.4	63.4	6.8
F6-11	51.6	50.1	49.1	50.9	20.8	7.9	174.4	56.8	5.8
F6-12	39.0	84.0	31.2	68.8	21.9	10.2	169.3	64.4	6.9
F6-13	81.5	43.9	63.5	36.5	23.0	7.6	44.1	66.7	6.5
F6-14	25.1	82.5	23.4	76.6	21.2	12.0	195.4	60.8	6.5
F6-15	39.8	57.8	39.1	61.0	24.3	11.5	180.8	66.1	6.7
F6-16	49.5	92.6	33.6	66.4	19.9	12.2	83.9	69.9	5.9
F6-17	73.2	68.3	50.5	49.6	22.3	13.3	135.8	66.7	6.1
F6-18	62.1	84.7	39.2	60.8	19.7	10.7	104.9	67.0	5.6
F6-19	57.3	64.5	45.6	54.4	22.4	9.6	172.1	63.7	6.0
F6-20	37.5	66.9	34.9	65.1	22.8	9.2	76.7	65.1	7.1

F6 = Filial six generation

‡ % = Percentage; g = Gram; mm = Millimeter † Trait measured in one location

Table 3.16. Continued

	Number of	Number of	Sterility	Fertility	Thousand	Grain	Grain	Rice	Seed Length
Lines	Unfilled	Filled Grains	Percentage	Percentage	Grain	Yield per	Yield per	Milling [†]	(mm)
	Grains per	per Panicle	(%)‡	(%)	Weight	Plant (g)	line† (g)	(%)	
FC 01	Panicle	00.0	07.7	72.2	(g)	11.0	215.0	<i>c</i> 0 <i>c</i>	<u> </u>
F6-21	38.5	89.8	27.7	72.3	23.4	11.0	315.9	60.5	6.9
F6-22	40.3	87.1	31.0	69.0	16.1	9.2	200.7	68.1	6.3
F6-23	49.9	109.7	27.8	72.2	21.4	18.3	233.5	61.9	6.5
F6-24	75.0	76.3	47.9	52.1	21.1	9.4	194.9	60.4	6.4
F6-25	28.7	80.8	23.8	76.2	21.0	10.6	260.7	66.1	6.7
F6-26	16.3	85.4	15.8	84.2	24.1	14.3	209.4	65.3	7.1
F6-27	59.3	114.5	33.2	66.8	21.1	19.5	382.1	64.8	6.7
F6-28	27.9	110.6	19.7	80.4	18.9	16.2	120.2	64.7	6.3
F6-29	63.1	104.5	38.0	62.0	21.5	12.5	275.1	66.8	6.7
F6-30	29.9	78.0	27.0	73.0	23.1	12.5	312.0	56.8	6.5
F6-31	23.8	70.9	24.8	75.2	22.1	8.2	120.5	60.5	6.2
F6-32	21.7	81.7	20.7	79.3	23.0	7.5	180.7	56.8	6.1
F6-33	21.5	86.3	17.4	82.6	22.8	16.9	241.2	54.1	6.4
F6-34	44.0	66.1	37.3	62.7	19.6	8.1	244.3	62.3	6.4
F6-35	39.2	112.2	27.5	72.5	20.3	11.5	193.1	65.2	6.4
F6-36	37.7	64.0	37.6	62.4	19.0	13.7	160.0	60.7	7.0
F6-37	27.9	66.0	29.1	70.9	22.9	7.9	149.3	57.7	6.6
F6-38	33.3	68.9	31.4	68.6	23.7	11.4	141.2	61.3	6.8
F6-39	38.4	80.5	32.7	67.3	23.1	12.2	236.9	65.1	7.1
F6-40	42.1	92.0	29.2	70.8	20.2	10.1	163.3	66.1	6.2
F6-41	20.2	118.1	14.3	85.7	20.4	9.6	327.2	65.7	6.1
F6-42	25.1	107.1	18.5	81.5	22.3	11.7	240.2	69.1	6.1
F6-43	84.4	93.7	48.0	52.0	22.5	9.1	101.5	68.9	7.2
F6-44	42.2	54.8	41.2	58.8	19.5	10.8	121.8	69.3	6.7
F6-45	67.7	43.1	59.7	40.3	21.7	8.6	112.1	58.2	6.6
F6-46	28.8	85.5	24.5	75.5	18.3	10.6	334.6	64.6	6.0

Table 3.16. Continued

Lines	Number of Unfilled Grains per	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight	Grain Yield per Plant (g)	Grain Yield per line† (g)	Rice Milling† (%)	Seed Length (mm)
F6-47	Panicle 70.6	75.9	49.0	51.0	(g) 20.4	18.0	267.5	65.9	6.0
F6-47 F6-48	25.8	128.2	49.0 16.6	83.4	20.4 20.3	18.0	238.8	66.6	6.1
F0-48 F6-49	23.8 33.2	90.6	27.5	83.4 72.5	20.3	12.2	238.8 99.7	63.5	6.4
F6-50	37.4	73.7	33.9	66.1	22.4	11.7	205.5	60.5	7.2
F6-51	15.3	157.2	8.8	91.2	18.2	11.9	203.4	63.9	5.1
F6-52	22.2	98.7	18.7	81.3	19.5	13.0	153.6	65.1	5.9
F6-53	43.1	104.9	29.4	70.7	21.3	11.3	264.4	63.2	6.7
F6-54	49.9	91.9	35.4	64.6	20.5	13.5	172.3	69.3	6.4
F6-55	87.1	42.8	64.9	35.1	22.2	8.2	101.4	63.5	6.4
F6-56	39.3	86.3	31.1	68.9	21.8	9.8	271.0	59.7	6.5
F6-57	37.6	87.4	31.3	68.7	20.6	4.7	84.7	67.1	6.4
F6-58	37.4	91.8	27.9	72.1	22.2	11.4	246.4	58.4	6.9
F6-59	47.1	87.1	34.8	65.2	20.6	9.4	233.7	52.3	6.1
F6-60	72.8	96.9	43.2	56.8	23.3	8.0	164.4	60.8	7.2
F6-61	74.2	74.6	50.9	49.1	22.2	6.5	175.5	62.3	6.1
F6-62	44.0	78.1	36.1	63.9	23.1	9.7	202.6	59.8	6.4
F6-63	45.2	80.0	36.2	63.8	22.9	9.0	201.6	61.5	7.3
F6-64	37.5	143.9	20.9	79.1	19.2	9.0	155.9	69.8	5.3
F6-65	43.9	83.4	35.0	65.1	20.9	8.1	117.0	63.6	6.8
F6-66	61.8	87.3	41.3	58.7	21.0	14.7	264.9	64.7	6.3
F6-67	36.4	71.7	33.9	66.1	20.6	9.2	134.7	65.8	5.8
F6-68	49.4	53.2	52.2	47.8	20.5	6.9	126.8	64.2	6.2
F6-69	56.9	84.6	42.1	57.9	23.2	10.1	248.3	66.5	6.9
F6-70	18.5	94.2	16.2	83.8	20.7	13.2	269.2	65.0	6.7
F6-71	62.9	80.1	44.4	55.6	21.2	13.3	142.0	66.0	6.4
F6-72	44.5	77.1	37.2	62.8	22.4	14.1	145.0	60.9	7.1

Table 3.16. Continued

	Number of Unfilled	Number of Filled Grains	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield per	Grain Yield per	Rice Milling†	Seed Length (mm)
Lines	Grains per	per Panicle	(%) ‡	(%)	Weight	Plant (g)	line [†] (g)	(%)	(IIIII)
	Panicle	per i unicie	(/0)+	(70)	(g)	1 Iuni (5)	inite (g)	(/0)	
F6-73	67.2	81.2	44.2	55.8	23.8	21.5	275.5	65.7	7.0
F6-74	86.1	99.8	46.3	53.7	18.8	12.8	112.4	65.2	6.0
F6-75	27.2	114.3	19.1	80.9	23.0	12.1	223.1	60.3	6.6
F6-76	24.6	103.0	19.5	80.6	20.6	11.6	165.1	59.1	6.2
F6-77	64.0	58.8	55.4	44.6	23.2	8.4	116.7	66.7	6.4
F6-78	26.9	107.7	21.1	78.9	20.2	14.0	166.1	62.2	6.2
F6-79	41.1	97.6	27.9	72.1	18.5	8.9	133.8	68.9	5.8
F6-80	27.3	79.9	25.0	75.1	22.7	9.9	167.5	67.3	6.9
F6-81	33.0	81.5	29.1	70.9	20.2	10.2	224.4	63.4	6.8
F6-82	62.1	78.6	43.6	56.4	20.4	19.0	230.6	65.8	6.3
F6-83	42.9	98.3	30.7	69.4	23.4	14.2	88.6	63.8	6.2
F6-84	51.6	73.5	42.0	58.0	24.4	16.9	189.5	56.3	7.1
F6-85	54.4	77.9	41.6	58.5	23.9	20.8	101.6	59.3	7.0
F6-86	19.7	108.5	15.2	84.8	20.6	16.8	228.4	65.0	6.2
F6-87	59.9	97.5	38.2	61.8	19.9	17.6	254.8	66.5	6.1
F6-88	63.2	73.0	47.6	52.4	20.2	7.4	194.6	61.8	6.8
F6-89	80.8	88.9	46.7	53.3	17.6	7.8	70.0	60.5	6.0
F6-90	21.4	93.4	18.9	81.2	23.1	23.1	191.0	63.1	6.5
F6-91	35.0	102.9	25.6	74.4	21.7	14.3	261.6	58.6	6.5
F6-92	71.3	93.5	44.2	55.8	20.4	10.0	172.1	53.9	6.5
F6-93	43.0	53.9	45.2	54.8	21.0	6.3	160.4	55.1	6.4
F6-94	57.3	116.8	32.8	67.2	16.0	13.0	123.7	71.6	6.0
F6-95	13.4	60.8	17.9	82.1	22.5	9.6	244.0	64.4	6.6
F6-96	21.5	114.1	16.4	83.6	21.5	12.7	136.5	63.2	6.4
F6-97	18.8	80.4	19.6	80.5	23.9	11.8	344.3	71.1	6.3
F6-98	42.6	112.6	28.5	71.5	21.2	18.5	97.9	65.8	6.6

Table 3.16. Continued

Lines	Number of Unfilled Grains per	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight	Grain Yield per Plant (g)	Grain Yield per line† (g)	Rice Milling† (%)	Seed Length (mm)
E (00	Panicle	100 7	14.0	05.7	(g)	17.1	2267	(2.2	<u> </u>
F6-99	21.2	129.7	14.3	85.7	18.7	17.1	236.7	63.3	6.3
F6-100	36.3	73.7	33.5	66.5	19.0	12.0	325.9	61.6	6.3
F6-101	13.2	64.7	17.5	82.5	21.8	10.8	176.4	60.1	6.8
F6-102	28.6	86.3	25.2	74.8	20.9	9.9	130.8	64.9	6.8
F6-103	46.5	107.7	30.7	69.3	21.0	13.7	279.2	66.0	6.2
F6-104	15.7	66.4	19.6	80.4	21.4	10.0	169.4	60.5	6.7
F6-105	52.7	86.3	38.2	61.9	21.8	11.5	108.0	58.1	6.5
F6-106	48.1	108.6	27.8	72.2	22.8	9.4	264.0	64.5	6.4
F6-107	87.1	79.5	52.6	47.4	18.5	6.9	205.8	63.2	6.2
F6-108	40.3	125.4	24.0	76.0	21.3	11.8	164.9	58.2	6.8
F6-109	37.4	85.9	29.3	70.7	21.2	8.4	206.6	63.6	6.5
F6-110	45.7	122.1	27.4	72.6	22.9	15.0	267.7	69.2	6.8
F6-111	46.4	110.3	28.3	71.7	16.8	10.1	157.4	67.5	6.1
F6-112	31.2	102.7	22.4	77.6	22.9	10.9	315.1	65.3	7.2
F6-113	44.1	72.5	38.3	61.8	21.6	9.4	165.3	63.5	7.0
F6-114	21.4	84.1	20.5	79.5	21.0	10.1	169.0	67.6	6.5
F6-115	31.7	80.1	29.7	70.3	23.5	16.3	211.5	60.4	6.3
F6-116	21.6	99.5	18.8	81.3	21.1	14.6	374.6	67.6	6.3
F6-117	23.8	90.3	21.1	78.9	23.2	9.6	324.4	54.2	6.3
F6-118	34.9	60.4	39.1	60.9	22.3	6.5	153.7	62.7	6.2
F6-119	67.2	77.8	46.6	53.4	19.9	11.1	196.8	61.6	6.4
F6-120	35.3	85.3	28.8	71.2	22.5	13.8	124.3	66.5	7.1

Lines	Seed Width (mm)‡	Chalky Seed (%)	Aroma (2AP)†
F6-1¥	2.5	1.6	0.00
F6-2	2.2	1.4	0.05
F6-3	2.4	1.5	1.31
F6-4	2.2	1.0	0.01
F6-5	2.4	1.8	0.94
F6-6	2.2	1.7	0.01
F6-7	2.4	1.0	0.00
F6-8	2.4	2.1	0.00
F6-9	2.1	0.7	0.00
F6-10	2.2	1.0	0.68
F6-11	2.1	1.7	0.83
F6-12	2.2	1.3	0.90
F6-13	2.4	1.8	0.00
F6-14	2.2	0.4	0.00
F6-15	2.3	0.4	0.00
F6-16	2.3	1.1	0.00
F6-17	2.4	1.9	0.00
F6-18	2.2	1.4	0.65
F6-19	2.4	1.6	0.00
F6-20	2.3	1.7	0.00
F6-21	2.4	0.8	0.06
F6-22	2.2	0.5	0.51
F6-23	2.5	1.6	0.00
F6-24	2.3	0.6	0.46
F6-25	2.0	4.6	0.57
F6-26	2.3	1.4	0.40
F6-25	2.0 2.3	4.6	0.57

Table 3.17. Means of agronomic traits of seed width, chalky seed and aroma of rice lines tested at multi-location, Texas in 2017.

¥ F6 = Filial six generation ‡ mm = Millimeter; % = Percentage; 2AP = 2-Acetyl-1-pyrroline

⁺ Trait measured in one location

Table 3.17. Continued

Lines	Seed Width (mm);	Chalky Seed (%)	Aroma (2AP)†
F6-27	2.1	0.5	0.01
F6-28	2.1	1.6	0.04
F6-29	2.3	1.1	0.03
F6-30	2.4	1.4	0.01
F6-31	2.5	1.5	0.55
F6-32	2.2	1.9	0.01
F6-33	2.3	1.1	0.43
F6-34	2.2	1.5	0.71
F6-35	2.2	0.6	0.01
F6-36	2.1	1.9	0.65
F6-37	2.2	1.5	0.30
F6-38	2.2	2.0	0.02
F6-39	2.3	1.1	0.02
F6-40	2.2	0.7	0.31
F6-41	2.3	0.3	0.03
F6-42	2.5	1.9	0.18
F6-43	2.3	2.1	0.01
F6-44	2.1	1.8	0.23
F6-45	2.1	1.8	0.18
F6-46	2.3	0.4	0.03
F6-47	2.3	2.1	0.04
F6-48	2.3	0.6	0.28
F6-49	2.4	1.1	0.78
F6-50	2.1	1.3	0.68
F6-51	2.2	0.8	0.02
F6-52	2.2	0.9	0.66
F6-53	2.1	1.8	0.42
F6-54	2.2	2.0	0.01

Table 3.17. Continued

Lines	Seed Width (mm):	Chalky Seed (%)	Aroma (2AP)†
F6-55	2.1	0.6	0.03
F6-56	2.3	3.3	0.21
F6-57	2.0	1.8	0.10
F6-58	2.1	1.6	0.03
F6-59	2.1	0.4	0.00
F6-60	2.1	1.9	0.00
F6-61	2.4	2.2	0.55
F6-62	2.4	3.0	0.22
F6-63	2.2	0.6	0.72
F6-64	2.3	0.3	0.02
F6-65	2.3	1.6	0.03
F6-66	2.2	1.2	0.41
F6-67	2.7	1.7	0.80
F6-68	2.3	0.7	0.10
F6-69	2.4	1.7	1.23
F6-70	2.1	1.0	0.75
F6-71	2.2	1.5	0.02
F6-72	2.0	1.5	0.69
F6-73	2.3	1.1	0.71
F6-74	2.2	0.9	0.68
F6-75	2.2	0.6	0.04
F6-76	2.2	2.3	0.67
F6-77	2.4	1.5	0.64
F6-78	2.2	2.3	0.63
F6-79	2.2	1.1	0.49
F6-80	2.1	2.6	0.78
F6-81	2.2	1.0	0.04
F6-82	2.2	1.2	0.08
F6-83	2.6	2.2	0.66

Table 3.17. Continued

Lines	Seed Width (mm)	Chalky Seed (%)	Aroma (2AP)†
F6-84	2.2	1.6	0.01
F6-85	2.3	1.3	0.00
F6-86	2.1	2.6	0.44
F6-87	2.5	1.3	0.66
F6-88	2.3	3.5	0.21
F6-89	2.1	3.3	0.01
F6-90	2.4	2.0	0.58
F6-91	2.2	2.4	0.68
F6-92	2.3	0.6	0.03
F6-93	2.2	0.6	0.00
F6-94	2.2	1.3	0.02
F6-95	2.3	1.6	0.03
F6-96	2.1	0.8	0.03
F6-97	2.4	0.6	0.01
F6-98	2.6	1.3	0.01
F6-99	2.1	0.9	0.69
F6-100	2.1	1.5	0.84
F6-101	2.3	1.3	0.21
F6-102	2.1	1.0	0.00
F6-103	2.2	2.4	0.01
F6-104	2.1	2.0	0.19
F6-105	2.3	1.6	0.03
F6-106	2.4	1.1	0.75
F6-107	2.2	1.7	0.55
F6-108	2.0	1.9	0.02
F6-109	2.4	1.3	0.53
F6-110	2.3	0.6	0.02
F6-111	2.1	2.2	0.00
F6-112	2.1	2.5	0.00

Table 3.17. Continued

Lines	Seed Width (mm)‡	Chalky Seed (%)	Aroma (2AP)†
F6-113	2.0	1.4	0.00
F6-114	2.2	1.1	0.14
F6-115	2.2	0.8	0.61
F6-116	2.2	0.8	0.26
F6-117	2.5	2.8	0.02
F6-118	2.4	2.6	0.02
F6-119	2.2	1.0	0.36
F6-120	2.2	0.6	0.02

3.4. Conclusions

The conclusion to these results are:

- Rice lines such as F6-3, F6-69, F6-5, F6-12, F6-100, F6-11, F6-67, F6-80, F6-49, F6-106 and F6-70, had 1.31, 1.23, 0.94, 0.90, 0.84, 0.83, 0.80, 0.78, 0.78, 0.75 and 0.75 concentration (2AP), respectively, were much superior in aroma than Amber 33-PI, female parent, which had 0.75 concentration (2AP) and Antonio, male parent, which is non-aromatic. This clearly demonstrates the presence of positive transgressive segregation and possibility to improve aroma even in aromatic-by-non-aromatic crosses.
- The interactions between locations and cultivars showed highly significant differences for many traits such as the days to 50% heading, plant height, ligule length, unfilled grains per panicle, sterility percentage, fertility percentage, thousand-grain weight and chalky seed percentage. Additionally, while significant differences exist for some traits (such as number of tillers per plant, flag leaf area, panicle length, number of panicles per plant, number of grains per panicle), five morphological and agronomic traits (number of branches per panicle, number of filled grains per panicle, grain yield per plant, seed length, and seed width) had no significant variation in terms of interactions between locations and cultivars.
- Six QTLs associated with aroma were detected including four in chromosome 8 (qAR8.1, qAR8.2, qAR8.3, and qAR8.4) and two in chromosome 10 (qAR10.1 and qAR10.2). Three QTLs in chromosome 1 were associated with plant height, qHP1.1, qHP1.2, and qHP1.3. Six QTLs in chromosome 3 associated with days to 50% heading were detected, namely qDH3.1, qDH3.2, qDH3.3, qDH3.4, qDH3.5, and qDH3.6. Three QTLs in chromosome 5 associated with fertility and sterility percentage traits were qFP5.1, qFP5.2, and qFP5.3

(for fertility percentage) and qSP5.1, qSP5.2, and qSP5.3 (for sterility percentage), which means both traits share the same location. A single detected QTL in chromosome 12 (qFS12) was found to be associated with full seed trait. Four QTLs in chromosome 7 were associated with seed length, qSL7.1, qSL7.2, qLS7.3 and qSL7.4.

3.5. References

- Ahamadi J., M.H. Fotokian, and S. Fabriki-Orang. 2008. Detection of QTLs influencing panicle length, panicle grain number and panicle grain sterility in rice (Oryza sativa L.). J. Crop Sci. Biotech. 11(3): 163-170.
- Ahn, S.N., C.N. Bollich, and S.D. Tanksley. 1992. RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84: 825–828.
- Amarawathi, Y., R. Singh, A.K. Singh, V.P. Singh, T. Mohapatra, T.R. Sharma, and N.K. Singh. 2008. Mapping of quantitative trait loci for basmati quality traits in rice. Mol. Breed. 21: 49-65.
- Asins, M. 2002. Present and future of quantitative trait locus analysis in plant breeding. Plant Breed. 121: 281–291.
- Bundock, P.C., M.J. Cross, F.M. Shapter, and R.J. Henry 2006. Robust allele-specific polymerase chain reaction markers developed for single nucleotide polymorphisms in expressed barley sequences. Theor. Appl. Genet. 112: 358-65.
- Bullard, R.W. and G. Holguin.1977. 'Volatile components of unprocessed rice (Oryza sativa)'. J. Agric. Food Chem. 25: 99.
- Chiapparino, E., D. Lee, and P. Donini. 2004. Genotyping single nucleotide polymorphisms in barley by tetra-primer ARMS-PCR. Genome 47: 414-20.
- Choudhury, D. and A.K. Ghosh. 1978. Evaluation of agronomic and physiochemical characteristics of five scented rice varieties. Indian J.f Agric. Sci. 48: 573-528.
- Dhulappanavar, C.V. 1976. Inheritance of scent in rice. Euphytica. 25: 659-662.
- Doyle, J.J. and J.E. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.
- Fitzgerald, M.A., N.R.S. Hamilton, M.N. Calingacion, H.A. Verhoeven, and V.B. Butardo. 2008. Is there a second fragrance gene in rice? Plant Biotechnol. J. 6: 416–423.

- Ghose, R.L.M. and W.T. Butany. 1952. Studies of the inheritance of some characteristics in rice (Oryza sativa L.). Indian J. Genet. and Plant Breed. 12: 26-30.
- Golam, F., Y.Y. Hui, A. Masitah, N. Afnierna, A. M. Nazia, K. Norzulaani, and O. Mohamad. 2011. Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment, Aust. J. of Crop Sci. 5(11): 1318-1325.
- Han, Z., W. Hu, C. Tan, and Y. Xing. 2017. QTLs for heading date and plant height under multiple environments in rice. Genetica 145: 67–77. doi: 10.1007/s10709-016-9946-6
- Hang, N.T., Q. Lin, L. Liu, X. Liu, S. Liu, W. Wang, L. Li, N. He, Z. Liu, L. Jiang, and J. Wan. 2015. Mapping QTLs related to rice seed storability under natural and artificial aging storage conditions. Euphytica, 203: 673-681. <u>https://doi.org/10.1007/s10681-014-1304-0</u>
- Hayashi, K., N. Hashimoto, M. Daigen, and I. Ashikawa. 2004. Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. Theor. Appl. Genet. 108: 1212-20.
- Hashemi, F. S. G., M. Y. Rafi, M. R. Ismail, M. T. M. Mohamed, H. A. Rahim, M. A. Latif, and F. Aslani. 2015. The genetic and molecular origin of natural variation for the fragrance trait in an elite Malaysian aromatic rice through quantitative trait loci mapping using SSR and gene-based markers. Gene 555: 101–107.
- International Rice Research Institute. 1972. Annual Report for 1971. Los Baños, Philippines. 238 p.
- Jiang, S.K., X.J. Zhang, C. Huang, Y.N. Xing, Z.J. Xu, and W.F. Chen. 2010. Comparison of genetic linkage map and QTLs controlling flag leaf traits based on F2 and F2:6 populations derived from japonica rice. Chin. J. Rice Sci. 24: 372-378.
- Leung, A., J.D. Craske, and M. Wootton. 1998. Volatile aroma compounds in green tissues of Australian rice plants at different stages of maturity and different levels of nitrogen fertilization. 48th Australian Cereal Chemistry Conference: 278-281.
- Liu, T., D. Shao. and M.R. Kovi. 2010. Mapping and validation of quantitative trait loci for spikelets per panicle and 1,000-grain weight in rice (Oryza sativa L.). Theor. Appl. Genet. 120: 933-942.
- Liu, S., C. Griffey, M. Hall, A. McKendry, J. Chen, W. Brooks, G. Brown-Guedira, D.V. Sanford, and D.G. Schmale. 2013. Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. Theor. Appl. Genet. 126: 2485-2498. doi:10.1007/s00122-013-2149-y.
- Mahajan, G., M. Amar, S. Rajbir, P.S. Vijai, and S.C. Bhagirath. 2018. Basmati rice in the Indian subcontinent: Strategies to boost production and quality traits. Adv. Agron. 151: 1-55.

- Mall, A.K., J.D.P. Babu, and G.S. Babu. 2005. Estimation of genetic variability in rice. J. Maharashtra Agric. Univ.30(2): 166-168.
- Murai, M. and T. Kinoshita. 1986. Diallel analysis of traits concerning yield in rice. Jpn. J. Breed. 36: 7-15.
- Nagaraji, M., D. Chaudhry, and M.J.B.K. Rao. 1975. A simple technique to identify scent in rice and inheritance pattern of scent. Curr. Sci. 44: 599-602.
- Pérez-Pérez, J.M., D. Esteve-Bruna, and J.L. Micol. 2010. QTL analysis of leaf architecture. J Plant Res. 123: 15–23.
- Qi, L., Y. Sun, J. Li, L. Su, X. Zheng, X. Wang, K. Li, Q. Yang, and W. Qiao. 2017. Identify QTLs for grain size and weight in common wild rice using chromosome segment substitution lines across six environments. Breeding Sci. 67(5): 472–482. http://doi.org/10.1270/jsbbs.16082.
- Saha, P., M. Islam, M. Islam, and M. Salam. 2015. Analysis of yield components and aroma of small grain aromatic rice (Oryza sativa L.) in Bangladesh. The Agriculturists 13(2): 17-24.
- Sarma, R.N., P.R. Talukdar, S. Rathi, K. Pathak, S. Chetia, A.R. Baruah, and R.N. Sarma. 2017. QTL analysis in aromatic rice of Assam, India. J. Rice Res. 5: 186. Doi: 10.4172/2375-4338.1000186.
- Selvaraj, C. I., P. Nagarajan, K. Thiyagarajan, M. Bharathi, and R. Rabindran. 2011. Genetic parameters of variability, correlation and path-coefficient studies for grain yield and other yield attributes among rice blast disease resistant genotypes of rice (Oryza sativa L.). Afr. J. Biotechnol., 10(17): 3322-3334.
- Soleimani, V.D., B.R. Baum, and D.A. Jhonson. 2003. Efficient validation of single nucleotide polymorphisms in plants by allele-specific PCR, with an example from barley. Plant Mol. Bio. Rep. 21: 281-288.
- Sood, B.G. and E.A. Siddiq. 1978. A rapid technique for scent determination in rice. Indian J. Gen. Plant Breed. 38: 268-271.
- Sourosh, H. R., M. Mesbah, A. Hossainzadeh, and R. Bozorgipour 2004. Genetic and phenotypic variability and cluster analysis for quantitative and qualitative traits of rice. Seed Plant, 20(2): 167-182.
- Siddiq, E.A., L.R. Vemireddy, and J. Nagaraju. 2012. Basmati rices: genetics, breeding and trade. Agric. Res. 1: 25-36.

- Sun, S.X., F.Y. Gao, X.J. Lu, X.J. Wu, X.D. Wang, G.J. Ren, and H. Luo. 2008. Genetic analysis and gene fine mapping of aroma in rice (Oryza sativa L. Cyperales, Poaceae). Genet. Mol. Biol, 31(2): 532–538.
- Tabien, R. E., C. L. Harper, and P. M. Frank. 2015. Registration of 'Antonio', a very early maturing and high yielding U.S. conventional long grain rice cultivar. J. Plant Reg. 9: 53-59. doi:10.3198/jpr2014.02.0005crc.
- Talukdar, P.R., S. Rathi, K. Pathak, S.K. Chetia, and R.N. Sarma. 2017. Population structure and marker-trait association in indigenous aromatic rice. Rice Sci. 24: 45-154.
- Tan, L., P. Zhang, F. Liu, G. Wang, S. Ye, Z. Zhu, Y. Fu, H. Cai, and C. Sun. 2008. Quantitative trait loci underlying domestication and yield-related traits in an Oryza sativa x Oryza rufipogon advanced backcross population. Genome 51: 692-704.
- Vanavichit, A., W. Kamolsukyurnyong, S. Wanchana, S. Wongpornchai, S. Ruengphayak, T. Toojinda, and S. Tragoonrung 2004. Discovering genes for rice grain aroma. In: Proc. of the 1st International Conference on Rice for the Future, 31 August–3 September, 2004: 71–80. Kasetsart University, Bangkok, Thailand.
- Vanavichit, A., S. Tragoonrung, T. Theerayut, S. Wanchana, and W. Kamolsukyunyong. 2005. Transgenic rice plants with reduced expression of Os2AP and elevated levels of 2-acetyl-1-pyrroline. United States Patent, Patent No. US 7,319,181 B2.
- Wei, X., J. Xu, H. Guo, L. Jiang, S. Chen, C. Yu, Z. Zhou, P. Hu, H. Zhai, and J. Wan. 2010. DTH8 suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiol. 153: 1747–1758.
- Wang, F., F. Cheng, and G. Zhang. 2007. Difference in grain yield and quality among tillers in rice genotypes differing in tillering capacity. Rice Sci. 14(2): 135-140.
- Wang, Y., L.R. Cheng, T.Q. Zheng, Y. Sun, Z. Zhou, J. Yang, Z.J. Xu, and J.L. Xu. 2009. Response of main effect qtl for plant height and flag leaf width to artificial selection in rice. Chin. J. Rice Sci. 23: 363-370.
- Yaobin, Q., P. Cheng, Y. Cheng, Y. Feng, D. Huang, T. Huang, X. Song, and J. Ying. 2018. QTL-Seq identified a major QTL for grain length and weight in rice using near isogenic F 2 population. Rice Sci. 25(3): 121-131.
- Ye, S., S. Dhillon, X. Ke, A.R. Collins, and I.N.M. Day. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res. 29: e88-8.
- Yue, B., W.Y. Xue, L.J. Luo, and Y.Z. Xing. 2006. QTL Analysis for flag leaf characteristics and their relationships with yield and yield traits in rice. Acta Genet. Sin. 33: 824-832.

Yoshida, S. 1972. Physiological aspects of grain yield. Ann. Rev. Plant Physiol. 23: 437-464.

- Zhao, L., J. Lei, Y. Huang, S. Zhu, H. Chen, R. Huang, Z. Peng, Q. Tu, X. Shen, and S. Yan. 2016. Mapping quantitative trait loci for heat tolerance at anthesis in rice using chromosomal segment substitution lines. Breeding Sci. 66(3): 358–366. <u>http://doi.org/10.1270/jsbbs.15084</u>
- Zhang, W., M.C. Gianibelli, W. Ma, L. Rampling, and K.R. Gale. 2003. Identification of SNPs and development of allele-specific PCR markers for γ-gliadin alleles in Triticum aestivum. Theor. Appl. Genet. 107: 130-138.
- Zhu, L.H., D.B. Zhong, J.L. Xu, S.B. Yu, and Z.K. Li. 2008. Differential expression of lodging resistance related QTLs in rice (Oryza sativa L.). Plant Sci. 175: 898–905.

CHAPTER IV

SUMMARY AND GENERAL CONCLUSIONS

This dissertation focused on two different aspects of Iraqi aromatic rice breeding, covering morphological characterization and a genetic analysis of rice. We used a cluster dendrogram and mapped quantitative trait loci (QTL) using a genotyping-by-sequencing (GBS) platform. The F_{2:6} recombinant inbred line (RIL) population used in these studies was derived from a cross between Amber 33-PI (aromatic) and Antonio (non-aromatic) cultivar.

In Chapter II, we found the number of days needed to reach the 50% heading differed among the cultivars: Anber 33, Amber 33-GSOR, Amber-GSOR, Amber 33-PI, and Amber-PI needed between 103 and 125 days to reach the 50% heading, and these were the longest periods recorded for the flowering times. The heights of Amber 43-PI, Amber-PI, Amber-GSOR, Amber 43-GSOR, Amber 33-PI, Amber 33-GSOR, and Amber Coarse were the tallest, with measurements ranging between 148 cm and 168 cm. In regard to tillers per square meter, Amber Coarse-GSOR and Amber Coarse-PI had the highest numbers of tillers per square meter. The flagleaf area of the aromatic rice cultivar Amber 33-PI was 55.6 cm², which was the largest flag-leaf areas. Amber 33-PI, Amber-GSOR, Amber 33-GSOR, Amber-PI, and Anber 33 had the longest panicle lengths, which ranged between 24 cm and 29 cm. One thousand-grain weight of Amber Coarse-IP weighed 27.1 g, which was considerably heavier than the weights of the Amber-PI sample (19.9 g). The highest concentration of 2-Acetyl-1-pyrroline (2AP), a trait associated with aroma, were recorded for the aromatic rice cultivars Amber 33-GSOR and Amber 33-PI, which had concentrations of 0.78 2AP and 0.75 2AP, respectively. The aroma trait was related to the grain yields. For example, cultivars such as Amber, Amber 33, and Anber 33 produced lower grain

yields (kg ha⁻¹) with higher aromas, and Amber Coarse and Amber 43 produced higher grain yields (kg ha⁻¹) with lower aromas. A highly positive correlation between the number of panicles per plant and number of tillers per plant was observed. Aroma was associated with specific agronomic, morphological, and physiological traits. The high positive and indirect effect was observed for aroma via panicle length and aroma via days to the 50% heading. In this study, cultivars such as Amber, Amber 33, and Anber 33 exhibited similarities in their agronomic, botanical, phenological, morphological, and physiological traits. In addition, cultivars such as Amber Coarse and Amber 43 showed similarities in their agronomic, botanical, phenological, morphological, and physiological traits. The 27 accessions were grouped into five distinct clusters. The first group had 4 accessions (Basmati PI-385456, Amber 43-GSOR, Amber Coarse-GSOR, and Amber Coarse-PI), the second had 2 accessions (Amber 43-PI and Basmati-PI-385471), the third had 13 accessions (Amber-PI, Amber 33-PI, Anber 33, Amber 33-GSOR, Amber-GSOR, Basmati 5853-PI, Basmati 6313-PI, Basmati-PI-431251, Basmati 37-PI, Basmati 5874-PI, Basmati Pardar-PI, Basmati Medium-PI, and Basmati-PI-385817), the fourth had 2 accessions (Basmati T3-PI and Scented A-PI), and the fifth had 6 accessions (Antonio, Presidio, Dellmont-PI, Della-Clor, Della, and Jazzman). Iraqi aromatic-rice cultivars had two groups for aroma trait. Those in the first group had higher aromas, such as Amber, Amber 33, and Anber 33, and those in the second group had lower aromas, such as Amber Coarse and Amber 43. The Iraqi aromatic accessions were distributed among three clusters. The majority of the accessions were placed in the third cluster, a few were placed in the first, and only one was placed in the second. The results of the principal coordinate analysis (PCoA) showed that most of the genotypic variance within the data could be explained by the first three principal components (PCo1 = 45%, PCo2 = 11%, and PCo3 = 6%). The PCoA results obtained for the cultivars divided them into five clusters, which included two

clusters of Amber rice cultivars, two clusters of Basmati rice cultivars, and one cluster of U.S. cultivars. The results of the PCA showed that most of the phenotypic variance within the data could be explained by the first three principal components (PC1 = 23%, PC2 = 21%, and PC3 = 16%). The first three PCs were plotted in a three-dimensional scatterplot to visualize the grouping and relations among the cultivars.

As shown in Chapter III, rice lines such as F6-3, F6-69, F6-5, F6-12, F6-100, F6-11, F6-67, F6-80, F6-49, F6-70 and F6-106 had 1.308, 1.225, 0.937, 0.903, 0.843, 0.827, 0.796, 0.784, 0.775, 0.751, and 0.750 concentrations of 2AP, respectively; Amber 33-PI, the female parent with the superior aroma, had a 0.750 concentration of 2AP; and Antonio, the male parent a non-aromatic rice, had a zero concentration of 2AP. Some morphological and agronomic attributes (such as the days to the 50% heading, plant heights, tillers per plant, flag-leaf areas, ligule lengths, panicle lengths, panicles per plant, branches per panicle, the filled grains per panicle, grain yields per plant, and chalky seed percentages) varied significantly (Least Significant Difference, LSD, 0.01) among the locations. Several morphological and agronomic traits (such as the grains per panicle, sterility percentages, and fertility percentages) varied significantly (LSD 0.05) among the locations. Twenty-six QTL associated with 21 different traits were identified in the Amber 33-PI x Antonio population. In chromosome 1, the three QTL that were associated with plant height were qHP1.1, qHP1.2, and qHP1.3. In chromosome 3, the six QTL that were associated with days to the 50% heading were qDH3.1, qDH3.2, qDH3.3, qDH3.4, qDH3.5, and qDH3.6. Six QTL associated with aroma and four major-effect QTL (qAR8.1, qAR8.2, qAR8.3, and qAR8.4) were identified in chromosome 8, and two minor-effect QTL (qAR10.1 and qAR10.2) were identified in chromosome 10. In chromosome 5, the three QTL that were associated with the fertility-percentage and sterility-percentage traits were qFP5.1, qFP5.2, and qFP5.3 (for fertility percentage) and

qSP5.1, qSP5.2, and qSP5.3 (for sterility percentage), and these results mean that both traits shared the same location and had similar LOD scores and percentages of variance explained. In chromosome 12, the single QTL that was associated with the full-seed trait was qFS12. Four QTLs in chromosome 7 were associated with length seed, qSL7.1, qSL7.2, qSL7.3 and qSL7.4.

This study identified several QTL for aroma and other morphological and agronomic traits with sufficient variation that can result into the development single nucleotide polymorphic (SNP) markers that can be used in marker-assisted selection and marker-assisted breeding to increasing the efficiency of breeding programs focusing on developing aromatic rice cultivars.

APPENDIX A

Table Appendix 1. Means of agronomic traits of days to 50 % heading, plant height, number of tillers per plant, flag leaf area, ligule length, panicle length, number of panicles per plant, number of branches per panicle and number of grains per panicle of rice lines and cultivars tested at multi-location, Texas in 2017.

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-1¥	98.5	88.7	11.5	30.6	15.5	21.9	10.8	18.8	174.2
F6-2	98.0	128.7	10.5	24.8	14.0	24.6	9.8	16.0	146.2
F6-3	97.5	125.2	4.0	19.5	8.5	24.0	4.0	13.7	134.7
F6-4	95.0	112.8	7.0	33.4	9.5	26.3	5.3	18.4	172.9
F6-5	83.0	144.4	7.0	22.2	8.5	20.8	7.0	9.9	95.9
F6-6	80.5	106.3	12.0	26.0	9.5	23.4	7.8	13.0	118.3
F6-7	86.5	159.9	8.0	37.8	11.5	28.0	8.0	15.2	160.2
F6-8	92.5	100.1	9.0	16.2	9.5	22.5	6.5	13.7	122.6
F6-9	74.5	109.6	4.0	19.7	11.5	23.9	4.3	14.0	133.9
F6-10	79.0	104.1	7.0	12.6	8.2	18.9	6.8	10.4	93.1
F6-11	86.0	97.2	6.0	17.4	7.0	23.6	5.8	11.0	100.4
F6-12	96.5	137.5	7.8	24.0	8.7	28.0	5.8	10.3	121.7
F6-13	91.5	89.5	12.5	27.5	7.5	20.6	8.5	13.3	124.1
F6-14	78.0	97.4	7.0	29.4	23.0	20.8	5.8	12.9	106.3
F6-15	100.0	92.3	8.0	24.9	18.7	20.5	7.8	13.5	96.3
F6-16	96.5	95.2	8.0	27.2	11.5	22.2	6.8	14.7	140.8
F6-17	94.5	86.4	10.0	21.4	3.5	23.3	7.3	12.5	140.2
F6-18	95.0	105.4	6.0	26.8	20.0	25.2	4.3	16.8	145.6
F6-19	89.0	89.9	7.0	25.2	9.5	21.7	6.5	11.9	120.4

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

 \ddagger % = Percentage; cm = Centimeter; cm² = Centimeter squared; mm = Millimeter; g = Gram

F6 = Filial six generation

Table Appendix 1. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-20	91.0	116.3	5.0	28.9	7.5	22.8	5.3	12.9	103.1
F6-21	81.0	125.1	10.0	20.0	7.5	23.8	7.5	12.9	127.1
F6-22	89.5	104.0	6.5	20.0	19.5	24.4	7.3	13.3	126.1
F6-23	99.5	93.4	9.0	34.3	12.0	26.0	9.3	15.2	158.3
F6-24	80.5	128.2	5.0	20.9	4.5	22.8	4.8	15.2	149.9
F6-25	79.5	108.3	5.0	15.4	9.5	21.4	4.8	15.0	108.2
F6-26	79.5	128.7	9.0	13.8	9.5	24.1	8.5	10.8	100.4
F6-27	86.5	116.5	7.0	21.2	11.0	25.8	6.5	15.5	172.6
F6-28	98.0	105.9	6.0	23.1	10.5	26.6	7.0	15.9	137.2
F6-29	95.0	115.9	6.0	39.8	16.0	27.5	6.3	17.8	166.3
F6-30	74.0	92.9	6.0	26.1	11.5	22.3	6.3	13.8	106.6
F6-31	90.0	85.9	5.5	24.7	5.0	22.0	5.8	10.7	93.4
F6-32	95.0	118.0	6.0	14.3	3.5	20.9	6.3	10.9	102.2
F6-33	76.5	131.6	8.5	32.1	25.2	25.6	7.3	10.9	106.6
F6-34	85.5	135.3	9.5	22.7	5.5	25.3	8.0	10.9	108.8
F6-35	91.0	87.8	8.0	19.1	16.5	28.3	7.8	13.3	150.1
F6-36	96.5	129.1	8.5	33.9	3.5	25.9	8.8	13.0	100.4
F6-37	77.5	100.2	8.0	11.0	9.5	21.9	7.5	13.4	92.6
F6-38	78.0	131.9	6.0	32.4	18.2	22.8	6.0	14.0	100.9
F6-39	97.0	127.1	8.5	23.1	13.5	23.0	8.5	14.3	117.6
F6-40	96.0	123.5	5.5	22.8	15.5	23.0	5.0	15.0	132.8
F6-41	97.0	121.4	5.0	30.2	10.5	24.9	4.0	12.7	136.9
F6-42	93.0	110.4	5.8	22.9	11.5	23.9	5.5	12.9	130.9
F6-43	106.5	126.6	6.0	36.8	9.5	26.1	6.0	18.9	176.8
F6-44	84.5	98.5	6.0	17.7	4.5	20.8	6.3	16.0	95.7
F6-45	87.0	100.7	9.5	19.8	7.5	22.1	8.8	14.4	109.6

Table Appendix 1. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-46	96.0	130.0	6.0	24.4	9.5	21.8	5.8	11.5	113.1
F6-47	99.5	115.6	13.5	29.9	27.2	27.0	12.0	17.4	147.6
F6-48	79.5	112.0	5.0	18.2	10.2	24.8	5.0	13.0	155.1
F6-49	96.5	124.4	6.0	24.9	11.7	22.5	6.0	14.3	124.9
F6-50	96.0	120.9	11.0	24.3	10.7	25.7	9.7	12.6	112.1
F6-51	90.0	122.5	6.0	28.5	15.7	23.7	5.7	14.5	173.5
F6-52	98.5	108.1	11.0	21.9	14.7	22.2	10.2	14.1	122.0
F6-53	93.0	130.2	7.0	27.1	3.2	25.6	6.2	18.3	149.0
F6-54	84.5	110.5	8.8	14.8	17.7	22.8	8.5	13.8	142.9
F6-55	88.0	106.2	11.5	16.4	7.7	20.9	11.0	14.4	131.0
F6-56	104.0	107.9	11.0	29.9	11.7	25.2	9.0	15.6	126.7
F6-57	102.5	115.9	6.0	20.2	19.2	23.2	4.5	12.1	126.1
F6-58	87.5	114.0	8.3	16.9	10.2	26.3	7.5	16.5	130.2
F6-59	97.5	106.2	10.0	21.0	11.7	24.9	9.0	19.6	135.2
F6-60	103.5	127.7	6.5	34.9	5.7	27.4	5.0	14.0	170.7
F6-61	103.0	105.0	7.0	31.2	11.7	26.9	5.2	13.1	149.9
F6-62	103.5	115.4	9.0	28.7	19.2	27.8	7.2	15.5	123.1
F6-63	86.0	137.8	5.0	27.9	14.7	24.9	3.7	12.6	126.2
F6-64	89.0	97.3	5.0	26.9	15.2	23.7	4.5	17.9	182.5
F6-65	93.0	110.4	5.0	18.6	9.7	22.5	4.5	14.0	128.4
F6-66	82.0	105.9	7.0	22.3	7.7	24.5	6.2	13.8	150.1
F6-67	99.0	88.4	9.0	17.2	7.7	21.2	7.5	13.9	109.2
F6-68	95.5	117.1	7.0	12.6	6.7	20.5	5.2	13.9	103.7
F6-69	100.5	100.8	10.0	18.1	9.7	26.4	9.0	13.3	142.6
F6-70	93.5	121.0	9.0	17.2	15.2	24.7	8.0	14.6	113.7
F6-71	86.5	90.0	9.0	9.2	5.7	24.9	8.7	17.8	144.0
F6-72	88.5	158.9	7.0	31.1	2.7	30.0	6.2	16.9	122.6

Table Appendix 1. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-73	97.0	135.4	7.0	19.1	9.7	23.8	6.0	11.8	149.5
F6-74	100.5	119.4	7.0	28.9	9.7	22.5	7.0	19.6	187.0
F6-75	95.0	140.4	6.0	26.0	5.7	24.9	6.0	13.8	142.6
F6-76	98.5	127.8	11.0	31.8	14.7	25.8	8.2	13.4	128.6
F6-77	100.5	107.9	7.0	18.0	9.7	24.0	5.7	15.6	123.9
F6-78	97.0	128.1	11.0	24.2	20.2	25.1	10.5	14.8	135.6
F6-79	97.0	132.5	8.0	22.4	19.7	21.3	7.5	16.6	139.7
F6-80	92.0	132.1	5.0	22.1	4.7	26.5	5.0	13.6	108.2
F6-81	100.5	120.9	7.0	32.2	10.7	25.7	6.7	17.5	115.5
F6-82	91.5	114.3	9.0	15.1	15.7	18.8	8.7	14.0	141.7
F6-83	98.0	141.5	9.0	43.4	9.7	30.9	7.0	15.8	142.2
F6-84	94.0	85.4	10.0	16.9	6.7	23.9	9.7	13.3	126.1
F6-85	94.5	87.0	11.0	34.9	11.7	25.7	10.5	12.4	133.4
F6-86	101.5	113.9	7.0	27.1	9.7	23.0	6.5	13.4	129.2
F6-87	95.0	117.9	7.5	31.6	13.7	23.4	7.5	19.3	158.4
F6-88	100.0	122.8	6.5	17.2	12.7	24.8	7.0	12.3	137.2
F6-89	90.0	149.8	8.0	28.7	11.7	25.3	7.7	13.5	170.7
F6-90	97.5	124.4	6.0	19.3	25.2	20.6	6.0	10.8	115.9
F6-91	99.5	118.0	10.0	36.3	17.7	27.4	8.5	12.6	139.0
F6-92	91.0	120.7	5.5	32.0	23.7	26.8	5.2	14.9	165.9
F6-93	75.0	124.0	6.5	36.0	7.3	23.6	6.0	13.6	97.1
F6-94	99.5	94.6	6.5	30.6	13.3	23.0	5.8	23.0	174.3
F6-95	96.0	116.3	5.0	21.9	6.3	23.1	4.5	10.2	74.3
F6-96	89.0	132.8	8.0	27.2	9.3	26.3	6.8	14.1	135.8
F6-97	91.0	89.0	8.0	21.3	7.3	21.1	7.8	12.0	99.5
F6-98	105.0	106.3	7.5	34.9	7.8	24.0	7.3	16.3	155.3
F6-99	94.0	131.3	11.0	30.4	9.3	25.9	10.5	13.7	151.1

Table Appendix 1. Continued

Lines and Cultivars	Days to	Plant	Number	Flag Leaf	Ligule	Panicle	Number of	Number of	Number of
	50%‡	Height	of Tillers	Area	Length	Length	Panicles	Branches	Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
F6-100	89.0	125.3	11.0	25.7	12.8	24.4	8.8	10.3	110.2
F6-101	88.0	116.5	6.0	31.5	9.3	22.7	5.0	12.1	78.1
F6-102	94.5	98.9	7.0	20.7	10.3	23.7	6.5	12.1	115.1
F6-103	87.5	128.5	8.0	27.4	20.8	23.5	6.5	11.8	154.5
F6-104	85.5	124.8	9.0	16.0	8.3	24.3	6.3	10.2	82.3
F6-105	83.0	111.5	8.0	20.0	21.8	26.2	6.8	14.5	139.2
F6-106	98.0	123.9	6.5	24.5	11.3	22.4	6.3	14.2	157.0
F6-107	90.0	105.9	5.0	30.0	9.3	25.6	4.3	13.3	166.8
F6-108	85.0	157.0	5.0	30.5	5.3	27.4	4.5	14.7	166.0
F6-109	91.0	91.5	7.0	23.6	7.3	22.9	5.8	12.8	123.6
F6-110	96.0	119.3	6.0	32.6	11.3	25.8	5.0	17.1	168.0
F6-111	99.0	114.2	10.0	27.4	4.3	26.2	9.3	16.1	157.0
F6-112	81.5	121.9	5.0	25.8	9.8	24.2	5.3	14.8	134.1
F6-113	91.5	142.2	9.0	20.8	5.3	23.9	7.3	14.6	116.8
F6-114	94.0	107.5	8.5	16.9	9.8	24.8	7.3	14.8	105.7
F6-115	79.0	142.1	8.0	29.0	22.8	23.9	8.5	9.8	112.1
F6-116	78.5	111.1	7.5	29.4	17.3	22.9	7.0	11.2	121.3
F6-117	75.5	93.9	7.0	24.8	12.3	22.0	6.5	13.2	114.3
F6-118	100.0	104.8	10.3	24.8	8.3	21.4	8.3	10.3	95.5
F6-119	84.0	100.7	6.0	24.1	8.3	21.1	5.5	17.3	145.2
F6-120	102.5	117.7	9.0	23.9	9.3	22.0	8.8	16.6	120.8
Amber33 PI†	99.1	138.3	11.8	43.2	17.3	25.9	10.7	12.1	132.3
Amber PI	102.5	135.7	11.0	35.0	19.7	26.6	9.5	11.7	138.7
Amber Coarse PI	91.0	113.0	13.5	28.7	11.2	19.5	12.5	8.6	58.6
Amber43 PI	91.5	153.9	11.0	41.6	8.9	21.6	10.0	9.3	84.0
Amber33 GSOR	98.0	135.2	13.5	35.2	13.4	23.8	10.5	11.2	101.3
Amber GSOR	99.5	140.6	12.0	33.8	17.9	25.9	9.3	11.8	116.5

Table Appendix 1. Continued

Lines and Cultivars	Days to 50%‡	Plant Height	Number of Tillers	Flag Leaf Area	Ligule Length	Panicle Length	Number of Panicles	Number of Branches	Number of Grains per
	Heading	(cm)	per Plant	(cm^2)	(mm)	(cm)	Per plant	per Panicle	Panicle (g)
Amber Coarse GSOR	89.0	133.5	14.8	30.6	11.8	19.4	14.3	8.7	62.6
Amber43 GSOR	92.5	127.6	11.0	27.1	8.9	19.5	9.8	8.5	65.8
Anber	100.5	142.0	13.0	37.7	17.6	27.1	10.3	14.0	134.7
Jazzman	100.8	93.6	5.8	32.9	8.0	21.9	5.6	17.6	145.5
Della	99.8	117.8	7.0	29.6	7.7	23.1	6.2	17.8	134.0
Antonio	78.5	91.1	5.1	20.6	10.6	20.4	5.0	15.9	148.1
Presidio	80.0	120.2	7.0	27.8	11.7	20.5	6.8	15.5	168.5
Della Clor	99.5	102.4	10.0	40.3	5.3	24.6	9.8	18.7	176.7
Basmati T3	70.5	106.0	9.0	36.0	5.3	20.3	8.8	12.7	133.2
Scented A	82.5	126.5	6.0	19.7	9.3	19.0	5.8	10.5	124.2
Basmati	76.0	131.9	9.0	29.2	9.3	21.8	8.8	8.7	63.5
Basmati	83.0	135.0	8.0	30.8	16.3	17.6	5.8	11.7	105.5
Basmati Pardar	87.5	129.0	9.0	27.8	19.3	24.2	6.3	12.2	157.2
Basmati Medium	87.0	126.4	4.0	25.7	22.3	20.8	3.8	9.5	119.0
Basmati	87.5	127.9	6.0	26.7	18.3	22.0	5.8	11.0	98.5
Basmati 6313	86.5	121.7	11.0	22.9	12.3	25.0	9.5	9.2	94.0
Basmati 37	104.5	113.0	8.0	25.7	19.3	27.7	6.8	9.2	119.7
Basmati 5853	88.0	119.4	7.0	35.5	16.3	21.6	6.8	9.5	107.0
Basmati 5874	101.5	111.5	6.0	27.6	13.3	25.9	5.8	9.5	112.2
Basmati	104.0	126.5	4.0	29.9	16.3	24.8	3.8	8.5	99.5
Dellmont	102.5	92.0	7.0	44.2	4.3	22.6	6.8	21.7	143.7

Table Appendix 2. Means of agronomic traits of number of unfilled grains per panicle, number of filled grains per panicle, sterility percentage, fertility percentage, thousand grains weight, grain yield per plant, seed length, seed width and chalky seed percentage of rice lines and cultivars tested at multi-location, Texas in 2017.

Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
F6-1¥	63.5	112.0	36.5	63.5	16.6	12.9	5.3	2.5	1.6
F6-2	52.2	95.3	34.9	65.1	18.4	13.1	6.6	2.2	1.4
F6-3	43.7	92.3	30.2	69.8	20.7	8.8	6.2	2.4	1.5
F6-4	61.5	112.8	34.4	65.6	19.7	10.2	6.3	2.2	1.0
F6-5	39.6	57.6	39.4	60.6	18.4	9.4	6.1	2.4	1.8
F6-6	42.8	76.8	34.8	65.2	16.8	9.8	5.7	2.2	1.7
F6-7	33.6	127.8	18.9	81.1	20.3	17.8	5.8	2.4	1.0
F6-8	10.4	113.4	8.6	91.4	17.9	10.5	5.7	2.4	2.1
F6-9	36.8	98.5	28.6	71.5	20.3	12.9	6.4	2.1	0.7
F6-10	30.7	63.7	31.3	68.7	21.2	10.5	6.8	2.2	1.0
F6-11	51.6	50.1	49.1	50.9	20.8	7.9	5.8	2.1	1.7
F6-12	39.0	84.0	31.2	68.8	21.9	10.2	6.9	2.2	1.3
F6-13	81.5	43.9	63.5	36.5	23.0	7.6	6.5	2.4	1.8
F6-14	25.1	82.5	23.4	76.6	21.2	12.0	6.5	2.2	0.4
F6-15	39.8	57.8	39.1	61.0	24.3	11.5	6.7	2.3	0.4
F6-16	49.5	92.6	33.6	66.4	19.9	12.2	5.9	2.3	1.1
F6-17	73.2	68.3	50.5	49.6	22.3	13.3	6.1	2.4	1.9
F6-18	62.1	84.7	39.2	60.8	19.7	10.7	5.6	2.2	1.4
F6-19	57.3	64.5	45.6	54.4	22.4	9.6	6.0	2.4	1.6
F6-20	37.5	66.9	34.9	65.1	22.8	9.2	7.1	2.3	1.7

† PI: plant introduction, GSOR: genetic stocks-oryza collection identification number; CIor: Cereal Investigation Oryza

§ CV = Coefficient of variation; LSD = Least significant difference at 5% level of significance

‡ % = Percentage; g = Gram; mm = Millimeter

F6 = Filial six generation

Table	Ap	pendix	2.	Co	ontinu	ed
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	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%) ‡	(%)	Weight	per Plant		(mm)	(%)
	Panicle	Panicle	(/0)*	(70)	(g)	(g)	(IIIII)	(IIIII)	(70)
F6-21	38.5	89.8	27.7	72.3	23.4	11.0	6.9	2.4	0.8
F6-22	40.3	87.1	31.0	69.0	16.1	9.2	6.3	2.2	0.5
F6-23	49.9	109.7	27.8	72.2	21.4	18.3	6.5 6.5	2.2	1.6
F6-24	75.0	76.3	47.9	52.1	21.1	9.4	6.4	2.3	0.6
F6-25	28.7	80.8	23.8	76.2	21.0	10.6	6.7	2.0	4.6
F6-26	16.3	85.4	15.8	84.2	24.1	14.3	7.1	2.3	1.4
F6-27	59.3	114.5	33.2	66.8	21.1	19.5	6.7	2.1	0.5
F6-28	27.9	110.6	19.7	80.4	18.9	16.2	6.3	2.1	1.6
F6-29	63.1	104.5	38.0	62.0	21.5	12.5	6.7	2.3	1.1
F6-30	29.9	78.0	27.0	73.0	23.1	12.5	6.5	2.4	1.4
F6-31	23.8	70.9	24.8	75.2	22.1	8.2	6.2	2.5	1.5
F6-32	21.7	81.7	20.7	79.3	23.0	7.5	6.1	2.2	1.9
F6-33	21.5	86.3	17.4	82.6	22.8	16.9	6.4	2.3	1.1
F6-34	44.0	66.1	37.3	62.7	19.6	8.1	6.4	2.2	1.5
F6-35	39.2	112.2	27.5	72.5	20.3	11.5	6.4	2.2	0.6
F6-36	37.7	64.0	37.6	62.4	19.0	13.7	7.0	2.1	1.9
F6-37	27.9	66.0	29.1	70.9	22.9	7.9	6.6	2.2	1.5
F6-38	33.3	68.9	31.4	68.6	23.7	11.4	6.8	2.2	2.0
F6-39	38.4	80.5	32.7	67.3	23.1	12.2	7.1	2.3	1.1
F6-40	42.1	92.0	29.2	70.8	20.2	10.1	6.2	2.2	0.7
F6-41	20.2	118.1	14.3	85.7	20.4	9.6	6.1	2.3	0.3
F6-42	25.1	107.1	18.5	81.5	22.3	11.7	6.1	2.5	1.9
F6-43	84.4	93.7	48.0	52.0	22.5	9.1	7.2	2.3	2.1
F6-44	42.2	54.8	41.2	58.8	19.5	10.8	6.7	2.1	1.8
F6-45	67.7	43.1	59.7	40.3	21.7	8.6	6.6	2.1	1.8
F6-46	28.8	85.5	24.5	75.5	18.3	10.6	6.0	2.3	0.4

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-	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%) ‡	(%)	Weight	per Plant		(mm)	(%)
	Panicle	Panicle	(70)+	(/0)	(g)	(g)	(IIIII)	(IIIII)	(/0)
F6-47	70.6	75.9	49.0	51.0	20.4	18.0	6.0	2.3	2.1
F6-48	25.8	128.2	16.6	83.4	20.3	12.2	6.1	2.3	0.6
F6-49	33.2	90.6	27.5	72.5	21.0	11.3	6.4	2.4	1.1
F6-50	37.4	73.7	33.9	66.1	22.4	11.7	7.2	2.1	1.3
F6-51	15.3	157.2	8.8	91.2	18.2	11.9	5.1	2.2	0.8
F6-52	22.2	98.7	18.7	81.3	19.5	13.0	5.9	2.2	0.9
F6-53	43.1	104.9	29.4	70.7	21.3	11.3	6.7	2.1	1.8
F6-54	49.9	91.9	35.4	64.6	20.5	13.5	6.4	2.2	2.0
F6-55	87.1	42.8	64.9	35.1	22.2	8.2	6.4	2.1	0.6
F6-56	39.3	86.3	31.1	68.9	21.8	9.8	6.5	2.3	3.3
F6-57	37.6	87.4	31.3	68.7	20.6	4.7	6.4	2.0	1.8
F6-58	37.4	91.8	27.9	72.1	22.2	11.4	6.9	2.1	1.6
F6-59	47.1	87.1	34.8	65.2	20.6	9.4	6.1	2.1	0.4
F6-60	72.8	96.9	43.2	56.8	23.3	8.0	7.2	2.1	1.9
F6-61	74.2	74.6	50.9	49.1	22.2	6.5	6.1	2.4	2.2
F6-62	44.0	78.1	36.1	63.9	23.1	9.7	6.4	2.4	3.0
F6-63	45.2	80.0	36.2	63.8	22.9	9.0	7.3	2.2	0.6
F6-64	37.5	143.9	20.9	79.1	19.2	9.0	5.3	2.3	0.3
F6-65	43.9	83.4	35.0	65.1	20.9	8.1	6.8	2.3	1.6
F6-66	61.8	87.3	41.3	58.7	21.0	14.7	6.3	2.2	1.2
F6-67	36.4	71.7	33.9	66.1	20.6	9.2	5.8	2.7	1.7
F6-68	49.4	53.2	52.2	47.8	20.5	6.9	6.2	2.3	0.7
F6-69	56.9	84.6	42.1	57.9	23.2	10.1	6.9	2.4	1.7
F6-70	18.5	94.2	16.2	83.8	20.7	13.2	6.7	2.1	1.0
F6-71	62.9	80.1	44.4	55.6	21.2	13.3	6.4	2.2	1.5
F6-72	44.5	77.1	37.2	62.8	22.4	14.1	7.1	2.0	1.5

Table	Ap	pendix	2.	Co	ontinu	ed
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	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cultivars	Grains per	Grains per	(%)‡	(%)	Weight	per Plant		(mm)	(%)
	Panicle	Panicle			(g)	(g)	~ /		~ /
F6-73	67.2	81.2	44.2	55.8	23.8	21.5	7.0	2.3	1.1
F6-74	86.1	99.8	46.3	53.7	18.8	12.8	6.0	2.2	0.9
F6-75	27.2	114.3	19.1	80.9	23.0	12.1	6.6	2.2	0.6
F6-76	24.6	103.0	19.5	80.6	20.6	11.6	6.2	2.2	2.3
F6-77	64.0	58.8	55.4	44.6	23.2	8.4	6.4	2.4	1.5
F6-78	26.9	107.7	21.1	78.9	20.2	14.0	6.2	2.2	2.3
F6-79	41.1	97.6	27.9	72.1	18.5	8.9	5.8	2.2	1.1
F6-80	27.3	79.9	25.0	75.1	22.7	9.9	6.9	2.1	2.6
F6-81	33.0	81.5	29.1	70.9	20.2	10.2	6.8	2.2	1.0
F6-82	62.1	78.6	43.6	56.4	20.4	19.0	6.3	2.2	1.2
F6-83	42.9	98.3	30.7	69.4	23.4	14.2	6.2	2.6	2.2
F6-84	51.6	73.5	42.0	58.0	24.4	16.9	7.1	2.2	1.6
F6-85	54.4	77.9	41.6	58.5	23.9	20.8	7.0	2.3	1.3
F6-86	19.7	108.5	15.2	84.8	20.6	16.8	6.2	2.1	2.6
F6-87	59.9	97.5	38.2	61.8	19.9	17.6	6.1	2.5	1.3
F6-88	63.2	73.0	47.6	52.4	20.2	7.4	6.8	2.3	3.5
F6-89	80.8	88.9	46.7	53.3	17.6	7.8	6.0	2.1	3.3
F6-90	21.4	93.4	18.9	81.2	23.1	23.1	6.5	2.4	2.0
F6-91	35.0	102.9	25.6	74.4	21.7	14.3	6.5	2.2	2.4
F6-92	71.3	93.5	44.2	55.8	20.4	10.0	6.5	2.3	0.6
F6-93	43.0	53.9	45.2	54.8	21.0	6.3	6.4	2.2	0.6
F6-94	57.3	116.8	32.8	67.2	16.0	13.0	6.0	2.2	1.3
F6-95	13.4	60.8	17.9	82.1	22.5	9.6	6.6	2.3	1.6
F6-96	21.5	114.1	16.4	83.6	21.5	12.7	6.4	2.1	0.8
F6-97	18.8	80.4	19.6	80.5	23.9	11.8	6.3	2.4	0.6
F6-98	42.6	112.6	28.5	71.5	21.2	18.5	6.6	2.6	1.3

Tab	le 4	Append	dix 2.	Conti	nued
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Lines and Cultivars	Number of Unfilled Grains per Panicle	Number of Filled Grains per Panicle	Sterility Percentage (%)‡	Fertility Percentage (%)	Thousand Grain Weight (g)	Grain Yield per Plant (g)	Seed Length (mm)	Seed Width (mm)	Chalky Seed (%)
F6-99	21.2	129.7	14.3	85.7	18.7	17.1	6.3	2.1	0.9
F6-100	36.3	73.7	33.5	66.5	19.0	12.0	6.3	2.1	1.5
F6-101	13.2	64.7	17.5	82.5	21.8	10.8	6.8	2.3	1.3
F6-102	28.6	86.3	25.2	74.8	20.9	9.9	6.8	2.1	1.0
F6-103	46.5	107.7	30.7	69.3	21.0	13.7	6.2	2.2	2.4
F6-104	15.7	66.4	19.6	80.4	21.4	10.0	6.7	2.1	2.0
F6-105	52.7	86.3	38.2	61.9	21.8	11.5	6.5	2.3	1.6
F6-106	48.1	108.6	27.8	72.2	22.8	9.4	6.4	2.4	1.1
F6-107	87.1	79.5	52.6	47.4	18.5	6.9	6.2	2.2	1.7
F6-108	40.3	125.4	24.0	76.0	21.3	11.8	6.8	2.0	1.9
F6-109	37.4	85.9	29.3	70.7	21.2	8.4	6.5	2.4	1.3
F6-110	45.7	122.1	27.4	72.6	22.9	15.0	6.8	2.3	0.6
F6-111	46.4	110.3	28.3	71.7	16.8	10.1	6.1	2.1	2.2
F6-112	31.2	102.7	22.4	77.6	22.9	10.9	7.2	2.1	2.5
F6-113	44.1	72.5	38.3	61.8	21.6	9.4	7.0	2.0	1.4
F6-114	21.4	84.1	20.5	79.5	21.0	10.1	6.5	2.2	1.1
F6-115	31.7	80.1	29.7	70.3	23.5	16.3	6.3	2.2	0.8
F6-116	21.6	99.5	18.8	81.3	21.1	14.6	6.3	2.2	0.8
F6-117	23.8	90.3	21.1	78.9	23.2	9.6	6.3	2.5	2.8
F6-118	34.9	60.4	39.1	60.9	22.3	6.5	6.2	2.4	2.6
F6-119	67.2	77.8	46.6	53.4	19.9	11.1	6.4	2.2	1.0
F6-120	35.3	85.3	28.8	71.2	22.5	13.8	7.1	2.2	0.6
Amber33 PI†	32.8	99.5	25.1	75.0	19.2	12.9	5.5	2.2	2.5
Amber PI	45.3	93.2	31.2	68.8	17.7	10.1	6.0	2.1	2.0
Amber Coarse PI	10.9	47.5	19.2	80.8	23.8	14.5	6.1	2.6	1.6
Amber43 PI	28.8	54.9	35.1	64.9	21.4	9.8	5.6	2.6	1.5

Table Appendix 2. Continued

Lines and Cultivars	Number of Unfilled	Number of Filled	Sterility Percentage	Fertility Percentage	Thousand Grain	Grain Yield	Seed Length	Seed Width	Chalky Seed
Lines and Cuttivars	Grains per	Grains per	(%)‡	(%)	Weight	per Plant	(mm)	(mm)	(%)
	Panicle	Panicle			(g)	(g)			
Amber33 GSOR	19.7	81.4	19.2	80.8	20.6	13.3	5.6	2.2	2.7
Amber GSOR	15.4	100.8	13.4	86.6	19.0	16.9	6.0	2.1	2.4
Amber Coarse GSOR	13.2	49.2	22.2	77.8	24.8	16.1	6.0	2.5	1.7
Amber43 GSOR	15.0	50.6	22.8	77.2	22.7	12.2	5.9	2.6	1.5
Anber	34.5	100.0	25.9	74.1	20.5	12.5	5.8	2.3	2.0
Jazzman	36.7	108.8	25.4	74.6	23.9	17.0	7.0	2.3	0.5
Della	35.0	98.9	26.5	73.5	22.5	17.4	6.7	2.3	0.6
Antonio	28.0	120.1	18.7	81.3	23.4	19.5	6.5	2.2	0.7
Presidio	30.4	137.8	18.2	81.8	22.7	19.4	6.8	2.2	1.8
Della Clor	46.8	129.7	26.5	73.5	22.6	23.8	6.8	2.2	0.3
Basmati T3	25.0	108.0	18.1	81.9	22.9	22.2	5.5	3.3	3.4
Scented A	19.7	104.3	15.1	84.9	22.6	19.8	5.4	3.0	1.8
Basmati	4.3	59.0	7.8	92.2	20.2	9.3	6.7	2.0	0.4
Basmati	44.7	60.5	38.6	61.4	21.8	8.5	5.3	2.8	1.6
Basmati Pardar	34.0	122.9	22.0	78.0	18.4	7.4	6.7	1.7	0.4
Basmati Medium	35.0	83.7	30.4	69.6	17.5	13.5	6.7	1.9	0.5
Basmati	15.3	82.9	16.0	84.0	18.1	18.3	6.7	1.9	0.4
Basmati 6313	16.7	77.1	18.5	81.5	22.1	15.9	7.7	2.0	1.3
Basmati 37	37.5	82.0	31.9	68.2	19.0	10.1	6.7	2.4	0.6
Basmati 5853	22.5	84.3	21.5	78.5	22.6	18.2	6.8	1.9	0.7
Basmati 5874	33.0	79.0	29.6	70.4	22.0	12.6	6.8	1.9	0.9
Basmati	28.7	70.6	27.8	72.3	20.4	10.1	6.8	2.0	1.1
Dellmont	30.1	113.4	20.5	79.5	24.7	10.3	6.8	2.4	1.5

Grain Yield Grain Yield per Aroma (2AP) Lines per line (g) Plant (g) ¥F6-1 17.5 79.6 0.00 F6-2 0.05 13.9 122.5 <mark>F6-3</mark> <mark>8.7</mark> <mark>220.1</mark> **1.31** F6-4 186.1 0.01 11.3 F6-5 <mark>9.2</mark> <mark>128.1</mark> <mark>0.94</mark> 8.2 237.3 F6-6 0.01 F6-7 23.4 216.8 0.00 F6-8 7.5 310.7 0.00 F6-9 9.2 108.4 0.00 F6-10 6.5 151.4 0.68 F6-11 <u>6.7</u> <mark>174.4</mark> 0.83 F6-12 <mark>9.5</mark> <mark>169.3</mark> <mark>0.90</mark> F6-13 7.4 44.1 0.00 F6-14 195.4 16.6 0.00 F6-15 15.6 180.8 0.00 F6-16 13.8 83.9 0.00 F6-17 10.8 135.8 0.00 F6-18 10.7 104.9 0.65 F6-19 9.3 172.1 0.00 F6-20 11.6 76.7 0.00 F6-21 8.8 315.9 0.06 F6-22 7.5 200.7 0.51 F6-23 16.7 233.5 0.00 F6-24 9.8 194.9 0.46

Table Appendix 3. Means of agronomic traits of grain yield per plant, grain yield per line and aroma of rice lines tested at Beaumont, Texas in 2017.

‡ g = Gram; 2AP = 2-Acetyl-1-pyrroline

F6 = Filial six generation

Table Appendix 3. Continued

	Grain Yield per	Grain Yield	Aroma (2AP)
Lines	Plant (g)	per line (g)	
F6-25	13.0	260.7	0.57
F6-26	16.6	209.4	0.40
F6-27	26.6	382.1	0.01
F6-28	15.9	120.2	0.04
F6-29	12.5	275.1	0.03
F6-30	13.9	312.0	0.01
F6-31	7.9	120.5	0.55
F6-32	7.0	180.7	0.01
F6-33	17.8	241.2	0.43
F6-34	8.5	244.3	0.71
F6-35	9.0	193.1	0.01
F6-36	9.4	160.0	0.65
F6-37	8.4	149.3	0.30
F6-38	9.8	141.2	0.02
F6-39	11.1	236.9	0.02
F6-40	10.1	163.3	0.31
F6-41	13.2	327.2	0.03
F6-42	12.3	240.2	0.18
F6-43	8.6	101.5	0.01
F6-44	12.6	121.8	0.23
F6-45	7.3	112.1	0.18
F6-46	9.1	334.6	0.03
F6-47	26.0	267.5	0.04
F6-48	15.0	238.8	0.28
<mark>F6-49</mark>	<mark>12.4</mark>	<mark>99.7</mark>	<mark>0.78</mark>
F6-50	14.5	205.5	0.68
F6-51	12.9	203.4	0.02
F6-52	11.9	153.6	0.66

Table Appendix 3. Continued

т.	Grain Yield per	Grain Yield	Aroma (2AP)
Lines	Plant (g)	per line (g)	
F6-53	11.8	264.4	0.42
F6-54	16.7	172.3	0.01
F6-55	11.1	101.4	0.03
F6-56	14.7	271.0	0.21
F6-57	5.6	84.7	0.10
F6-58	15.8	246.4	0.03
F6-59	12.2	233.7	0.00
F6-60	12.2	164.4	0.00
F6-61	8.1	175.5	0.55
F6-62	13.2	202.6	0.22
F6-63	6.7	201.6	0.72
F6-64	12.2	155.9	0.02
F6-65	9.8	117.0	0.03
F6-66	8.9	264.9	0.41
<mark>F6-67</mark>	<mark>10.0</mark>	<mark>134.7</mark>	<mark>0.80</mark>
F6-68	6.3	126.8	0.10
<mark>F6-69</mark>	<mark>11.7</mark>	<mark>248.3</mark>	<mark>1.23</mark>
<mark>F6-70</mark>	<mark>17.2</mark>	<mark>269.2</mark>	<mark>0.75</mark>
F6-71	20.4	142.0	0.02
F6-72	19.6	145.0	0.69
F6-73	11.4	275.5	0.71
F6-74	16.7	112.4	0.68
F6-75	13.7	223.1	0.04
F6-76	13.3	165.1	0.67
F6-77	8.9	116.7	0.64
F6-78	12.4	166.1	0.63
F6-79	13.7	133.8	0.49
<mark>F6-80</mark>	<mark>11.4</mark>	<mark>167.5</mark>	<mark>0.78</mark>

Table Appendix 3. Continued

T :	Grain Yield per	Grain Yield	Aroma (2AP)
Lines	Plant (g)	per line (g)	
F6-81	13.5	224.4	0.04
F6-82	19.5	230.6	0.08
F6-83	14.4	88.6	0.66
F6-84	21.3	189.5	0.01
F6-85	33.5	101.6	0.00
F6-86	23.8	228.4	0.44
F6-87	17.0	254.8	0.66
F6-88	6.9	194.6	0.21
F6-89	11.4	70.0	0.01
F6-90	18.0	191.0	0.58
F6-91	12.8	261.6	0.68
F6-92	12.2	172.1	0.03
F6-93	8.3	160.4	0.00
F6-94	16.8	123.7	0.02
F6-95	7.8	244.0	0.03
F6-96	10.4	136.5	0.03
F6-97	17.5	344.3	0.01
F6-98	30.3	97.9	0.01
F6-99	22.8	236.7	0.69
<mark>F6-100</mark>	<mark>10.9</mark>	<mark>325.9</mark>	<mark>0.84</mark>
F6-101	10.2	176.4	0.21
F6-102	11.1	130.8	0.00
F6-103	19.3	279.2	0.01
F6-104	10.0	169.4	0.19
F6-105	16.3	108.0	0.03
<mark>F6-106</mark>	<mark>11.0</mark>	<mark>264.0</mark>	<mark>0.75</mark>
F6-107	5.2	205.8	0.55
F6-108	11.4	164.9	0.02

Table Appendix 3. Continued

Lines	Grain Yield per Plant (g)	Grain Yield per line (g)	Aroma (2AP)
F6-109	11.9	206.6	0.53
F6-110	18.1	267.7	0.02
F6-111	11.4	157.4	0.00
F6-112	14.7	315.1	0.00
F6-113	9.5	165.3	0.00
F6-114	9.2	169.0	0.14
F6-115	22.7	211.5	0.61
F6-116	17.7	374.6	0.26
F6-117	12.1	324.4	0.02
F6-118	6.4	153.7	0.02
F6-119	11.4	196.8	0.36
F6-120	16.8	124.3	0.02