Deciphering the genetic basis of head rice yield, % chalkiness, grain dimension and different textural attributes to enhance food security along with different consumer preferences

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vorgelegt von
Herrn Gopal Misra,
geb. am 7.10.1982 in Indien

Gutachter:
1) Prof. Dr. A. Graner
2) Prof. Dr. M. Frei

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1. General Introduction

Milled rice (*Oryza sativa*) is obtained upon polishing after removing the bran, and it provides calories to more than half of the world's population. Demand is increasing to improve varieties with higher milling quality, appearance quality, and textural standards. Therefore, continued efforts are needed to develop high-yielding rice cultivars with premium quality standards to meet the demand of current and future food security targets for the growing human population. Ensuring superior grain quality will also enable meeting consumer demand, thereby providing the opportunity for farmers to earn higher revenues due to the higher premium offered for varieties having superior grain quality (Anacleto et al. 2015; Prosekov et al. 2018; Ramesh et al. 2000).
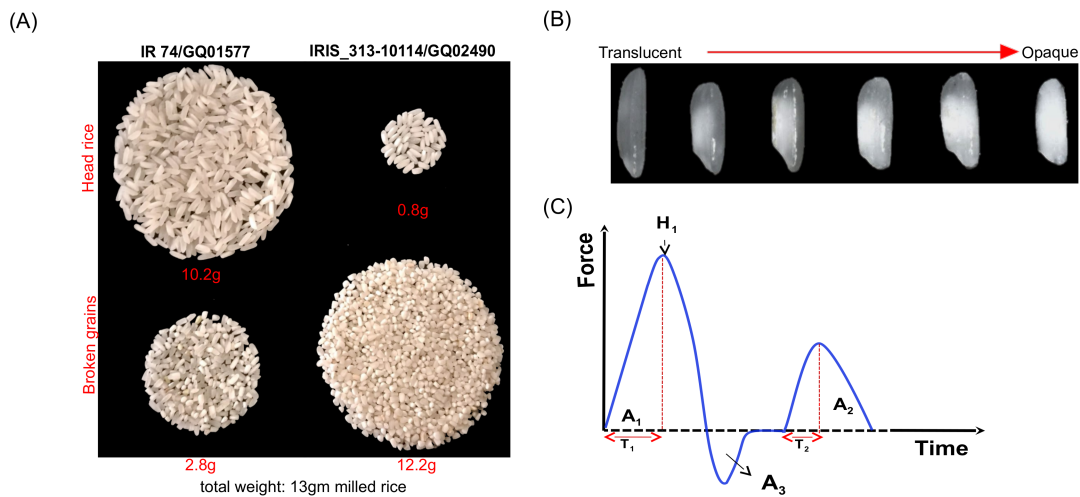
Figure 1: Characteristic features of rice grain quality. (A) Total of 13 g of rice paddy milled from two different varieties: IR74 and IRIS_313-10114. IR74 has very high head rice yield as the proportion of broken rice is small for this variety. (B) Transition from translucent to opaque grain depicting the chalky area in the grain. (C) Different textural attributes measured in a texture profile analyzer. The process mimics the first two bites after ingestion of cooked rice in the mouth and measures different forces. The negative force area under the first bite (A3) is referred to as adhesiveness. The maximum force required (presented as H1) for the first compression cycle simulates the force required to squeeze the grain using the molars. The peak force of the first compression measured by the height of the first curve is regarded as hardness. The ratio of the positive force area under the second compression to that of the first one (A2/A1) is considered as cohesiveness. Springiness (represented as T2/T1) is the height that the food recovers during the time that elapses from the upstroke to the peak in the second curve (T2) to the time elapsed from the starting point to the peak of the first curve (T1), representing degree of sample height recovery after partial compression.

Percent head rice yield represents the fraction of intact, undamaged grains, which determines the profit of millers and thereby affects the sustainability of farmers (Butardo et al. 2019; www.fao.org). Huge phenotypic variation exists for head rice yield across high-yielding rice varieties, with very few meeting the set criteria of millers. Some mega-varieties, which have been adopted over two decades, were perceived to possess higher milling quality and stable appearance quality in different environments, and thus remained preferred by farmers and millers for a long time. For instance, IR64, a mega-variety in various parts of Asia, which has good appearance and high milling quality (Champagne et al. 2010), is still grown on a large area even more than 30 years after its release, reflecting its popularity among farmers and consumers. A wider level of consumer preference and acceptability could turn a variety into a mega-variety, for which the criteria for good milling and appearance quality along with favourable textural characteristics play a key role (Cuevas et al. 2016; Custodio et al. 2019).

More than 30% of the total paddy produced is lost during the process of milling upon dehusking and removal of bran. Milling yield includes broken and intact grains. To meet the demand of consumers, millers separate the broken fraction from the unbroken whole grain yield. Milling quality is assessed with different quality parameters such as percent brown rice, percent milled rice, and percent head rice yield. Percent head rice yield refers to the fraction of intact grains that survive milling (Figure 1A). Hence, millers have adopted the criteria of assessing the performance of a variety to match higher head rice yield with lower chalkiness (percentage of opaque area in translucent endosperm, Figure 1B), and they are ready to pay a premium to farmers who grow these varieties (Butardo et al. 2019; Cnossen et al. 2003; Fitzgerald et al. 2009; Siebenmorgen et al. 2007; Qiu et al. 2015).

During post-harvest processing, various factors affect head rice yield: namely, storage, drying, milling machinery, and the degree and time of milling leading to differential breakage. In addition, numerous physiological factors affect head rice yield, including (a) harvest moisture content impacting fissure formation in rice kernels (Siebenmorgen et al. 2007) and (b) the switch from glassy to rubbery stage due to variations in the extrinsic factors temperature and moisture content (Cnossen et al. 2003). Grain shape is another quality parameter (ratio of grain length to grain width) that determines the slenderness of the grain (Butardo et al. 2019; Lisle et al. 2000; Qiu et al. 2015; Zhou et al. 2015) and also affects head rice yield (Buggenhout et al. 2013; Siebenmorgen et al. 2013; Siebenmorgen et al. 2011). Grain length, the primary constituent of grain shape, was negatively correlated with percent head rice yield, which means that increased grain length causes higher grain breakage (Buggenhout et al. 2013; Zhao and

Fitzgerald, 2013; Zheng et al. 2007; Zhou et al. 2015). In addition, environmental factors such as the occurrence of high night temperatures during seed development have a direct effect on percent head rice yield (Cooper et al. 2008). Therefore, ensuring the stability of head rice yield quality is rather challenging.

Percent grain chalkiness and grain shape constitute further key parameters of grain quality. Several studies demonstrated that percent grain chalkiness is negatively correlated with head rice yield. The probability of breaks in the rice kernel increased with a higher percentage of grain chalkiness (Lyman et al. 2013; Fitzgerald et al. 2009). Seed lots with chalky grains do not obtain the required market value. Higher percentage of intact grains and grains having less than 2% of chalkiness considered as superior quality (Chun et al. 2009). Incidences of chalkiness in different rice cultivars were reported to be caused by various forms of environmental stresses. However, some varieties retain high grain quality even in variable environments (Zhao et al. 2016). Chalky grains have differences in packing of starch granules and therefore many studies were carried out focusing on the implications of starch composition for chalk formation. Airspaces created between malformed granules of starch lead to a chalky appearance of the grain. Chalkiness alters cooking quality because airspaces between starch granules increase water absorption during cooking and change the texture in an undesired way (Chun et al. 2009; Lisle et al. 2000).

The textural properties of the grain are primarily affected by the ratio of amylose to amylopectin. They can be described through the attributes adhesiveness, cohesiveness, hardness, and springiness (Figure 1C). Adhesiveness refers to the degree a sample adheres inside the oral cavity. Hardness denotes the tooth force needed to compress the sample during chewing. The extent a sample sticks together after chewing is described as cohesiveness. The propensity of a sample to return to its original state after decompression is described as springiness (Lawless and Heymann, 1999; Meullenet et al. 1998; Stokes et al. 2013).

1.1. The genetic basis of percent head rice yield

Few studies have attempted to decipher the genetic basis of milling quality traits. Quantitative trait loci (QTL) mapping in a population created from *indica* and *japonica* parents helped to identify three minor QTLs for percent head rice yield. Among those, the largest QTL accounts for 5.9% of the observed variation (Zheng et al. 2007). In another study, recombinant inbred lines (RILs) derived from crosses between *indica* and *japonica* parents led to the identification of novel chromosomal regions. Two QTLs explaining more than 10% of the phenotypic

variation were mapped on chromosome 6 and chromosome 9. RILs derived from the cross between two breeding lines also helped to identify QTL regions for head rice yield, which were independent of kernel shape (Nelson et al. 2011). Moreover, important QTLs were mapped in a population obtained from an inter-specific cross between *O. sativa* (IR64) and *O. rufipogon,* for which favourable alleles were contributed from IR64 (Septiningsih et al. 2003). Furthermore, earlier studies demonstrated that grain moisture content at harvest influences head rice yield (Cnossen et al. 2003; Siebenmorgen et al. 2007). This implies that initial moisture content at harvest and during drying might affect head rice yield. However, no systematic study has been carried out to understand the underlying genetics of percent head rice yield under moisture stress conditions. Thus, a detailed systematic study based on high-density genotyping data provides an opportunity to scrutinize the genetic basis of percent head rice yield under moisture stress conditions.

1.2. Genetic basis of chalky endosperm

Grain chalkiness lowers appearance quality and in addition decreases the percentage of head rice (Fitzgerald et al. 2009; Lyman et al. 2013). If some of the chalky grains sustain breakage, such seed lots do not obtain the required market value as superior quality, which is primarily scored based on the both traits, percentage of broken grains and percentage of chalkiness (Chun et al. 2009; Yamakawa et al. 2007). Grain chalkiness is a polygenic trait. Hundreds of QTLs have been identified across the rice genome. Many of these QTLs were also pinpointed to the level of candidate genes (Fitzgerald et al. 2009; Sreenivasulu et al. 2015). These include pyruvate orthophosphate dikinase (Kang et al. 2005), starch synthase IIIa (Fujita et al. 2007), cell wall invertase (Wang et al. 2008), UDP-glucose pyrophosphorylase (Woo et al. 2008), and grain size 9 (Zhao et al. 2018). A mutation was reported in the gene *GIF1*, which leads to incomplete grain filling and increases percent grain chalkiness (Wang et al. 2008). A major QTL from chromosome 5 referred to as *Chalk 5*, which encodes a vacuolar H+ translocating pyrophosphatase (V-PPase), was cloned and characterized (Li Y et al. 2014). However, *Chalk 5* was observed to be inadequate in conferring lower chalkiness when examining the effect of *NIL-Chalk 5* (Zhao et al. 2016). Owing to the small effects of the identified QTLs, it is tempting to further explore the genetic basis in diverse germplasm to reveal novel QTLs that explain moderate to high phenotypic variation for chalkiness, especially in *indica* germplasm.

1.3. Genetics of grain size and shape

The genetic basis of grain size and shape in rice has been widely studied during the past couple of decades. Raw grain shape contributes to milling quality; mainly via percent head rice yield, as revealed in earlier studies (Buggenhout et al. 2013; Zhou et al. 2015; Goodman and Rao, 1985; Fan et al. 2000). Moreover, grain shape affects grain weight and thereby grain yield. Conversely, the shape of cooked grain defines grain expansion in terms of its length and breadth during cooking, which has a greater impact on consumer preferences across different regions of the globe (Huang et al. 2013; Fitzgerald et al. 2009; Xing and Zhang, 2010; Mottaleb and Mishra, 2016). Several QTLs for grain shape (GS), grain width (GW), and grain length (GL) have been mapped using populations created by crosses between/within *indica* and *japonica* subspecies (Miura et al. 2011; Sreenivasulu et al. 2015). Of these, the role of *GS3* (a putative transmembrane protein) in grain length has been confirmed using three different bi-parental populations (*indica* × *indica, tropical japonica* × *Oryza rufipogon*, and *temperate japonica* × *indica*). Analysis of a random sub-population from the BC_3F_2 progeny created from successive crossing and backcrossing between Minghui 63 (large grain) and Chuan 7 (small grain) revealed that *GS3* explained more than 80% of the variation for grain length. A functional single-nucleotide polymorphism snp_03_16733441 (C > A) revealed that the A allele was abundantly present in tropical *japonica* and *indica* rice, leading to a long and thin grain phenotype, whereas the C allele that is mainly present in *temperate japonica* and *aus* confers shorter grain (Fan et al. 2006; Takano-Kai et al. 2009; Nan et al. 2018). The gene *qGL3* encodes a putative protein phosphatase. The combined effect of *GS3* with *qGL3* increased grain length significantly when compared to a single QTL effect (Gao et al. 2015; Zhang YD et al. 2016).

The gene *Grain width 5* (*GW5*) located in the recombination hotspot region on chromosome 5 encodes a calmodulin-binding protein having substantial control over grain width and grain weight (Weng et al. 2008). A 1212-bp deletion, 5 kb upstream of *GW5*, manifested a significant variation between the two subspecies of *Oryza sativa* (Gong et al. 2017; Liu et al. 2017). Moreover, *GS5* was identified approximately 2 Mb upstream of *GW5,* substantially contributing to the phenotypic variation of grain shape. Highly expressed *GS5* resulted in larger grain size (Li et al. 2011). A single base pair deletion in exon 4 of *GW2* located on chromosome 2 and encoding a RING-type protein with E3 ubiquitin ligase activity, results in a truncated protein with increased grain width (Li N et al. 2014; Song et al. 2007). Similarly, a 10-bp deletion in the promoter of *GW8* encoding a protein that is a positive regulator of cell proliferation produced long slender grains (Wang et al. 2012). A mutant study reported for the gene *SRS5*

(*small and round seed 5*) encoding alpha-tubulin identified one non-synonymous polymorphism (Arg > Leu), which leads to short and round seeds (Segami et al. 2012). Two crucial genes on chromosome 7 and chromosome 8 designated as *GW7* and *GW8*, respectively, contribute to grain shape independently or by interaction between them (Wang et al. 2015). In combination, *GW8* negatively regulates grain shape by binding at the promoter of *GW7* (Ngangkham et al. 2018; Wang et al. 2015). Although several studies addressed the shape of raw grains, limited studies have focused on defining the genetic basis of cooked grain shape. Therefore, delineating the genetic relationship between raw and cooked grain shape was prioritized in this thesis.

1.4. Textural attributes reliant on regional preferences

Texture is an essential facet of rice quality because it is highly related to consumer acceptability. Different forms of texture, such as sticky, soft, dry, firm, and hard, attract various target consumers from different parts of the world (Champagne et al. 2010). Rice consumers from India and Pakistan, United States, South America and Middle East mainly prefer non-sticky and fluffy rice varieties, while in Bangladesh and parts of Myanmar people prefer hard-textured rice. India, Pakistan and Thailand consumers also prefer long grain fragrant rice with soft and fluffy texture (Champagne et al. 2010; Lyon et al. 2000). Whereas, consumers from China and Japan favour bold or long grain with sticky or soft texture. For textural attributes, amylose content has been treated as a trait of paramount importance in breeding programs (Bao et al. 2004; Champagne et al. 2004). Amylose content has been considered as a key determinant of rice cooking quality, with no or low amylose being correlated to sticky and soft textural phenotypes (Zhao et al. 2013; Cuevas et al. 2016). Nevertheless, high-amylose rice lines are shown to possess both hard and soft textural attributes (Champagne et al. 1999, 2010); thus, the difference in consumer preferences cannot be solely explained based on amylose content. Different textural attributes such as adhesiveness, hardness, cohesiveness, and springiness can play an important role in further defining texture in the same amylose content classes. Although sensory-based understanding of textural characteristics using a trained panel of testers represents a promising method (Cuevas et al. 2018), only limited numbers of samples can be tested by this approach, which limits its usefulness for large-scale testing of breeding material (Champagne et al. 1999). By contrast, an instrument-based texture profile analyser is high-throughput and can be deployed to estimate detailed textural parameters (Cuevas et al. 2018; Zhu et al. 2013; Chen et al. 2018). Using this technique, textural attributes can be analysed based on simulation of the first two bites in the mouth on rice grains (Stokes et al. 2013).

Previous attempts had been made to identify genomic regions significantly associated with different textural attributes. Cho et al. (2010) mapped QTLs based on a recombinant inbred population for adhesiveness on chromosomes 1 and 7, hardness on chromosomes 4 and 5, and springiness on chromosome 8. However, further fine mapping of these QTLs or discovering novel associations from different diversity panels is needed to validate the underlying genomic regions and identify candidate genes or to detect novel QTLs.

1.5. Association mapping and genomics tools

The existence of diverse genetic resources is the primary requirement for crop improvement to secure food for the future. Associating the milling, grain size and cooking quality data with the available sequence information could lay the foundation to dissect the genetics of target traits and also provide future strategies to improve varieties with good grain quality. Different computational techniques evolved over time to understand the phenotype-genotype association of complex traits with advantages and disadvantages. Classical QTL mapping has been used for the past couple of decades to identify the causal genetic regions useful for marker-assisted breeding (McCouch et al. 2016; Dwivedi et al. 2007; Wang et al. 2018; Xu et al. 2017). Association mapping or linkage disequilibrium (LD) mapping has received more attention in the past few years to dissect the complex traits in several crop species (Liu and Yan, 2019). In comparison to QTL mapping, association mapping benefits from a higher genetic resolution since it relies on a natural population whose genomes have been homogenized by a large number of meiotic events. On the other hand, it is difficult to detect the rare significant regions in association mapping due to filtering criteria for minor allele frequency, whereas classical QTL mapping can detect rare variations provided one of the parents is a carrier of the alleles (Verdeprado et al. 2018; Xu et al. 2017). The use of association mapping needs proper considerations of population structure and linkage disequilibrium (LD) between causal and non-causal variants to avoid any false discovery (Zhang P et al. 2016; Xu et al. 2017). Mixed linear model-based approaches emerged as the most appropriate solution to account for population structure and to control the false discovery rate (Yang et al. 2014; Kang et al. 2010). Until recently, array-based genotype data or genotype-by-sequencing (GBS)-based SNPs were used extensively to identify the allelic variants/haplotypes to be deployed for marker-assisted selection (Crowell et al. 2016, Furuta et al. 2017; McCouch et al. 2016). However, low coverage of markers in the genic region may pose a limitation for GBS in identifying the causal association of candidate genes (Bhatia et al. 2013).

The conserved gene order in the physical co-localisation of genetic loci between or within species is called synteny or collinearity (Tang et al. 2008). A high amount of genomic variation existed between *japonica* and *indica* for grain quality phenotypes, which could be used in a genome-wide association study (GWAS) by considering the population structure (Fitzgerald et al. 2009; Han and Xue, 2003; Molina et al. 2011). Since *indica* and *japonica* are the widely cultivated subspecies of rice, the underlying genetics of grain quality traits present in the syntenic region could be easily deployed to improve the breeding programs for both subspecies.

Sixty years of IRRI breeding have produced many important varieties, including IR64 and IR8 that helped to diminish hunger and poverty globally (www.irri.org). In order to meet the emerging consumer demand, there is a need to develop high-yielding varieties and also meet the demand of millers and traders by introgressing favourable alleles for grain quality. Therefore, novel genetic variation has to be identified from genebank collection resources, novel genes have to be identified, and beneficial alleles have to be deployed in the IRRI breeding program with newly gained knowledge and tools. This PhD thesis aims at advancing our knowledge regarding the genetic determinants of rice milling, appearance quality, and different textural attributes. To this end, I dissected the genetic architecture of various grain quality traits by using whole-genome sequence-based ultra-dense SNP resources and array-based genotyping data. Significant allelic and haplotype associations were identified that conferred higher head rice yield, low chalkiness, improved cooked grain size and shape, as well as advantageous textural traits. The presence of these beneficial superior alleles was scrutinized in selected lines from the IRRI breeding program. The results of this study will help to advance rice breeding programs to improve grain quality and to satisfy consumer preferences in a high-yielding background.

1.6. Objectives

The purpose of this study was to understand the genetic basis of important grain quality traits by studying hitherto untapped natural diversity. To this end, genome-wide association mapping was carried out in order to

ⅰ) decipher the genetic basis of head rice yield and percent grain chalkiness based on whole-genome sequence resources (Misra et al. 2019);

ⅱ) understand the genetic architecture of cooked grain shape of rice based on a whole-genome sequence approach (Misra et al. 2017), and

ⅲ) dissect the genetics of textural attributes based on an array-based SNP genotyping approach (Misra et al. 2018).

2. Peer-reviewed scientific articles

2.1. Dissecting the genome-wide genetic variants of milling and appearance quality traits in rice

Gopal Misra, Roslen Anacleto, Saurabh Badoni, Vito Butardo Jr., Lilia Molina, Andreas Graner, Matty Demont, Matthew K Morell, Nese Sreenivasulu

Abstract: Higher head rice yield (whole grain yield), which represents the proportion of intact grains that survive milling, and lower grain chalkiness (opacity) are key quality traits. We investigated the genetic basis of percent head rice yield and chalkiness in 320 diverse resequenced accessions of *indica* rice with integrated single- and multi-locus genome-wide association studies using 2.26 million single-nucleotide polymorphisms. We identified novel haplotypes that underly higher percent head rice yield on chromosomes 3, 6, 8, and 11, and lower grain chalkiness in a fine-mapped region on chromosome 5. Whole-genome sequencing of 92 IRRI breeding lines was performed to identify the genetic variants of percent head rice yield and chalkiness. Rare and novel haplotypes were found for lowering chalkiness, but missing alleles hindered progress towards enhancing percent head rice yield in breeding material. The novel haplotypes that we identified have potential use in future breeding programs aimed at improving these important traits in the rice crop.

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2.2. Whole genome sequencing based association study to unravel genetic architecture of cooked grain width and length traits in rice cooked grain width and length traits in rice

Gopal Misra, Saurabh Badoni, Roslen Anacleto, Andreas Graner, Nickolai Alexandrov, Nese Sreenivasulu

Abstract: In this study, we used 2.9 million single nucleotide polymorphisms (SNP) and 393,429 indels derived from whole genome sequences of 591 rice landraces to determine the genetic basis of cooked and raw grain length, width and shape using genome-wide association study (GWAS). We identified a unique fine-mapped genetic region GWi7.1 significantly associated with cooked and raw grain width. Additionally, GWi7.2 that harbors *GL7*/*GW7* a cloned gene for grain dimension was found. Novel regions in chromosomes 10 and 11 were also found to be associated with cooked grain shape and raw grain width, respectively. The indel-based GWAS identified fine-mapped genetic regions GL3.1 and GWi5.1 that matched synteny breakpoints between *indica* and *japonica*. GL3.1 was positioned a few kilobases away from *GS*3, a cloned gene for cooked and raw grain lengths in *indica*. GWi5.1 found to be significantly associated with cooked and raw grain width. It anchors upstream of cloned gene *GW*5, which varied between *indica* and *japonica* accessions. GWi11.1 is present inside the 3′-UTR of a functional gene in *indica* that corresponds to a syntenic break in chromosome 11 of *japonica*. Our results identified novel allelic structural variants and haplotypes confirmed using single locus and multilocus SNP and indel-based GWAS.

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2.3. Deciphering the Genetic Architecture of Cooked Rice Texture

Gopal Misra, Saurabh Badoni, Cyril John Domingo, Rosa Paula O. Cuevas, Cindy Llorente, Edwige Gaby Nkouaya Mbanjo, Nese Sreenivasulu

Abstract: The textural attributes of cooked rice determine palatability and consumer acceptance. Henceforth, understanding the underlying genetic basis is pivotal for the genetic improvement of preferred textural attributes in breeding programs. We characterized diverse set of 236 *Indica* accessions from 37 countries for textural attributes, which includes adhesiveness, hardness, springiness, and cohesiveness as well as amylose content. A set of 147,692 high quality SNPs resulting from genotyping data of 700K high Density Rice Array (HDRA) derived from the *Indica* diversity panels of 218 lines were retained for marker-trait associations of textural attributes using single-locus (SL) genome wide association studies (GWAS) which resulted in identifying hotspot on chromosome 6 for Amylose content and Adhesiveness attributes. Four independent multi-locus approaches (ML-GWAS) including FASTmrEMMA, pLARmEB, mrMLM, and ISIS_EM-BLASSO were implemented to dissect additional loci of major/minor effects influencing the rice texture and to overcome limitations of SL-based GWAS approach. In total 224 significant quantitative trait nucleotide (QTNs) were identified using ML-GWAS, of which 97 were validated with at least two out of the four multi-locus methods. The GWAS results were in accordance with the very significant negative correlation ($r = -0.83$) observed between AC and ADH, and the significant correlation exhibited by AC ($r < 0.4$) with Hardness, Springiness, and Cohesiveness. The novel haplotypes and putative candidate genes influencing textural properties beyond AC will be a useful resource for deployment into the marker assisted program to capture consumer preferences influencing rice texture and palatability.

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3. General discussion

3.1 Milling quality traits that define a premium for rice farmers

High HRY (with ≥55%) and low chalk (≤2%) are key target traits that determine the milling quality of rice, which contributes to enhancing revenues for farmers and millers. The latest trends of limited genetic gain in yield, in combination with lower HRY and an increase in percent chalkiness in newly developed varieties, have made it difficult to replace widely adopted rice mega-varieties (Laborte et al. 2015; Sreenivasulu et al. 2015). Studies on market trends conducted among 886 rice farmers in Cambodia, the Philippines, Bangladesh, and Eastern India suggested that farmers prioritize to invest for HRY in a range of 0–14% of their total investment depending on the geographic location, the season, and the rice mega-variety intended to be replaced. Slender varieties such as Phka Rumdoul in Cambodia (14%), SL-8 in the Philippines (12%), BRRI Dhan-50 in Bangladesh (11%), and Miniket in Eastern India (7%) attracted the highest investment in HRY and some of these varieties are typically grown in the dry season (Sreethong et al. 2019; Maligalig 2018; Ynion et al. 2015). In addition, the lower percent head rice yield observed during the dry season across different zones was in accordance with the observations made by the rice industry, which evidenced the link between the increasing temperature during the dry season and the higher incidence of grain breakage during milling (Custodio et al. 2016; Zohoun et al. 2018).

The experimental evidence also suggests that, over several generations of newly introduced varieties, head rice recovery has declined, while chalkiness has increased dramatically. This is consistent with farmers' prioritization of this trait in a high-yielding background. In a majority of the countries, premium long slender rice is widely preferred; thus, many breeding programs worldwide have been focusing on long slender rice types. This increasing demand for rice with long slender grain shape in Asian countries (Custodio et al. 2016) and African regions (Demont et al. 2017) has introduced trade-offs between breeding for slenderness and decreasing HRY, as both are generally considered as antagonistic traits because slender rice breaks more easily during milling. Improvement in HRY slowed down substantially in breeding programs due to the lack of intensive selection for HRY (Zhao et al. 2013; Zhou et al. 2015), the lack of availability of a robust high-throughput phenotyping method to score early-generation breeding material, the lack of stable QTLs, and also the trend in shifting preferences from bold grain to long slender grain from 1960 to 2010. HRY was found to be significantly higher (= ~60%) in varieties released in earlier decades (e.g., 1966-75). Furthermore, IRRI-released varieties

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targeted an increase in grain length in recent decades, which might have negatively affected HRY in varieties released in recent decades (Buggenhout et al. 2013). Using chalk as a proxy trait for improving milling quality resulted in improving chalk (average of 4%) in IRRI breeding programs; however, for HRY improvement, progress was limited because median HRY remains poor at 45% among the released breeding varieties. Chalkiness had been considered as a proxy measure for head rice yield, which evidently did not work (Sreethong et al. 2019; Zhou et al. 2015). This led to the conclusion that merely addressing chalkiness may not suffice to effectively overcome low milling quality. Therefore, addressing chalkiness as a proxy measure for HRY will not necessarily improve HRY, a common assumption that has prevented national rice breeding programs for decades from improving milling quality. Therefore, identifying the stable QTL regions that confer higher phenotypic variance for HRY is an important requirement for the rice industry.

3.2. Whole-genome-based high-density SNPs helped to identify the fine-mapped genetic regions for percent head rice yield

One of the major limitations of bi-parental population-based QTL mapping is the lack of high mapping resolution (Xu et al. 2017). The genomic regions identified for milling quality traits through bi-parental populations derived from crosses between *japonica* cultivars in earlier studies were minor QTLs with low phenotypic variance (Nelson et al. 2011; Nelson et al. 2012; Pinson et al. 2013). Bi-parental populations made from *indica* and *japonica* cultivars also identified minor QTLs for HRY (Zheng et al. 2007). To explore the higher mapping resolution in *indica*, whole-genome sequence-based high-density SNP data from an *indica* panel were prioritized in this study.

Since HRY is found to be a low-heritability trait influenced primarily by post-harvest practices and implied moisture stress in the grain (Nelson et al. 2011; Siebenmorgen et al. 2007; Siebenmorgen et al. 2013), Controlled drying conditions was employed to achieve ambient temperature during the transition from rubbery to glassy state (Cnossen et al. 2003). In this PhD study, 320 *indica* diversity lines and 300 *japonica* diversity lines (subsets from the 3,000 whole-genome sequence project) were studied to understand the genetic basis of HRY, chalkiness, and grain size traits to overcome the limited variability observed in existing IRRI breeding lines. The *indica* panel contained a broad range of variation for HRY (from 6.22% to 63.80%); in the *japonica* panel, HRY variation ranged from 24% to 70%. In the *indica* panel, HRY exhibited a significant seasonal effect with broad-sense heritability (H^2) of 0.56 between wet and dry

seasons. However, across the replications in the 2015 dry season, percent HRY showed H^2 of 0.88.

HRY data obtained from controlled drying conditions employing 320 re-sequenced *indica* diverse germplasm accessions with ultra-dense SNP genotyping provided the opportunity to identify fine-mapped genetic regions from chromosomes 6 and 8. In this study, the ultra-high SNP density was up to threefold higher than that of recently published reports of GWAS-based studies in rice (Crowell et al. 2016; McCouch et al. 2016; Wang et al. 2017). This valuable genomic resource enabled high-resolution mining of trait-specific allelic variants for higher HRY on chromosome 8 (23.89–24.03 Mb) under controlled drying, whereas additional genomic regions on chromosome 8 for stability were identified distant from the controlled HRY region. The stability value was calculated by dividing the HRY value under stress by the HRY value under control condition. The application of controlled drying conditions helped in identifying and fine mapping of genetic regions for HRY. A candidate gene, *LOC_Os08g39780*, identified on chromosome 8 forming a haplotype using all the non-synonymous SNPs, exhibited a median of more than 50% HRY. The fine-mapped candidate gene (*LOC_Os08g39780*) explained 9.37% of the phenotypic variation (PV). Furthermore, association mapping on HRY stability (calculated as the ratio of percent head rice yield obtained in control and stress) led to the identification of a prominent locus on chromosome 8, located 5.7 Mb distant from a prominent signal of percent HRY. This suggests the contribution of two individual regions from the same chromosome regulating percent HRY and percent HRY stability. All the identified regions were identified as novel in comparison to the already reported QTL regions based on the *indica* population (Qiu et al. 2015). These approaches proved to be advantageous in *indica*, wherein LD decays more rapidly than in *tropical* and *temperate japonica* (Mather et al. 2007; Huang et al. 2012a). Collinearity breaks in fine-mapped regions unveiled the presence of structural variations between the two major subspecies. The identified candidate gene for HRY needs to undergo further functional validation in future studies.

Re-sequencing of popular IRRI varieties and studying target regions of HRY provided opportunities to inspect the breeding signatures of divergent selections in breeding pools for allelic variations in the hotspot region for HRY on chromosome 8. Our study established that beneficial haplotypes responsible for high HRY in a very few of the ancestral accessions were typically clustered as group 1. It is highly likely that these underlying beneficial alleles have

not been selected during breeding selection cycles. Many breeding lines in groups 3 and 4 are characterized by inferior alleles with poor HRY. As a result, no significant genetic improvements were made for HRY in post-Green Revolution breeding programs. IR64 was found in group 2 with moderate HRY together with other modern varieties: IRRI109, IRRI135, IRRI156, and IRRI174. IR64 is a popular mega-variety widely planted in India, Indonesia, Pakistan, the Philippines, and Vietnam, possibly because of improved HRY coupled with a softer texture, resulting in wider preference by farmers and millers (Khush and Virk, 2005; Mackill and Khush, 2018).

3.3. A novel genetic region identified for percent grain chalkiness is independent of a significant region for head rice yield

Farmers generally sell their harvest to millers, who prefer less chalky rice grains as those are perceived to lessen the predisposition to grain breakage during dehulling and polishing (Champagne et al. 2004; Cooper et al. 2008; Del Rosario et al. 1968; Lisle et al. 2000; Wang et al. 2007), as previous studies pointed out the inverse relationship between percent grain chalkiness (PGC) and HRY (Buggenhout et al. 2013; Zhao et al. 2013). In this study, using most of the IRRI rice varieties and diversity rice lines, lower correlations were found between PGC and HRY. Moreover, grain chalkiness possessing high heritability (Wan et al. 2005; Zhao et al. 2016) was judged as a crucial determinant for grading export-quality rice.

Percent grain chalkiness scored from the core collection panels showed H^2 of 0.90 across different replications of the 2015 dry season for the *indica* panel. Heritability (H^2) decreased slightly (= 0.86) when compared between wet and dry seasons. Furthermore, appearance quality traits such as grain length, width, and shape possessed H^2 consistently higher than 0.90 across dry and wet seasons, with a good range of phenotypic variability.

Upon employing GWAS, highly significant association signal was detected from the 0.31-Mb hotspot region of chromosome 5 associated with PGC in rice and observed it consistently across multi-seasons/replications of field trials within *indica*. This further ruled out the major influence of environmental factors on the hotspot region. This region was spotted significantly distant (1.78 Mb) from earlier cloned gene *chalk5 V-PPase* with inorganic pyrophosphate (PPi) H^+-translocation activity (Li Y et al. 2014). The hotspot region regulating chalkiness detected in this study with the novel uncharacterized *chalk 5.1* gene is distinct from earlier reported regions (Chen et al. 2016; Gao et al. 2016; Peng et al. 2014; Yun et al. 2016; Zhao et al. 2016; Zhao et al. 2015; Zhu et al. 2018).

Targeted gene association studies in combination with GWAS resulted in narrowing down the hotspot region to a locus encompassing three candidate genes. One of those, *GW5*, is well known for regulating grain width. Here, a 1212-bp deletion upstream of *GW5* (LOC_Os05g09520) was already reported in earlier studies, which contributes to the higher grain width in *japonica* (Liu et al. 2017; Shomura et al. 2008). Recent studies also highlighted the role of *GW5* in influencing the chalkiness (Qiu et al. 2015; Yun et al. 2016). Another gene (LOC_Os05g09530) identified in this region encode an aspartate protease protein. Furthermore, SNP mining revealed that the topmost significant SNP overlapped with the genomic region lying 3 kb upstream of *GW5*. Upon subsequent analysis utilizing *in silico* gene prediction methods identified a candidate gene with unknown function, i.e. *chalk 5.1*, 5' UTR region of which is spanned by top most SNP identified in the study. This gene comprised a haplotype conferring low percent grain chalkiness compared with that of two other genes in this significant region. *Chalk 5.1* is an entirely distinct candidate gene from earlier reported genes/QTLs regulating PGC (Sreenivasulu et al. 2015; Yun et al. 2016; Zhao et al. 2016). The lowest PGC value confirmed by the haplotype of the *chalk 5.1* gene, particularly the 5' promoter region within the transcription binding sites, suggests its importance in lowering chalk phenotype. The topmost SNPs positioned within the 5' UTR of *chalk 5.1* overlapped with the binding site for trihelix transcription factor. A recent study on *Brachypodium distachyon* explored the probable role of trihelix transcription factor in the regulation of mixed-linkage glucan (MLG) (Fan et al. 2018). Other significant SNPs present in the promoter region that overlapped with different transcription factor binding sites demonstrated their role in expression and chalk phenotype. Many of the significant causal genomic variants were not found in the SNP set against low chalk reference genome Minghui 63 (Zhang J et al. 2016) (MH63, with lowest chalkiness) for all three candidate genes. The skewed distribution of the phenotype toward low chalkiness resulted in the absence of many genomic variants in the SNP set against the MH63 reference. Taken together, the multi-genome association approach was able to verify and validate the significance of the identified candidate region. In breeding lines, lower PVE (Percent phenotypic variation explained) values were noted for *chalk 5.1* (11.4%) over *chalk 5 V-PPase* (16.7%), which can be attributed to the low-frequency representation of *chalk 5.1* alleles in breeding material (Misra et al. 2019). Taken together, both chalk genes identified on chromosome 5 explain higher PV (26.12%), reflecting the likely potential benefits of their deployment in future national breeding initiatives to decrease chalk.

3.4. Genetic architecture of grain size and shape better defined using cooked rice grain

Grain shape/dimension is another key determinant of consumer preference and has always been in the focus of breeding to develop new favorable varieties (Anacleto et al. 2015; Fitzgerald et al. 2009; Huang et al. 2012b; Huang et al. 2013). Throughout the world, there are clear preferences among consumers for different grain shapes. For example, rice consumers in Japan, South Korea, and China highly prefer short and bold types of rice grains, whereas South Asian countries (including India and Bangladesh), Southeast Asian countries, and the United States prefer long slender rice grains (Juliano and Villareal, 1993). Several hundred QTLs have been mapped for grain size and shape, but only a few genes have been identified (Huang et al. 2013; Zheng et al. 2015). Although all mapping studies were carried out on raw grains only, grain size and shape after completion of the cooking process remain important to consumers. Therefore, this study focused on raw and cooked grains to understand commonalities and specific genetic regions. Since the two widely cultivated subspecies (*japonica* and *indica*) show apparent differences in their grain size/shape features (Civan et al. 2019), a GWAS was performed in a panel comprising genotypes from both subspecies to efficiently dissect and compare the genetic signatures in both *indica* and *japonica* rice germplasm. This approach identified two consecutive regions on chromosome 7 significantly associated with grain width. The first LD block, *Gwi7.1,* went from 22.1 to 22.8 Mb and the second one, *Gwi7.2,* from 23.3 to 25.2 Mb. Further, narrowing down this region helped to identify four candidate genes for *Gwi7.1*. Among those, one candidate gene identified as a NAC transcription factor affects the width of both raw and cooked grain. This finding is consistent with an earlier report describing an orthologue of this NAC transcription factor regulating grain width (Mathew et al. 2016). Previous studies on MAGIC and bi-parental populations describe a region on chromosome 7 from 22.7 to 26.0 Mb for raw grain width. This genetic region overlaps with the second LD block identified in this study. Several studies fine-mapped candidate genes such as *GL7* and *GLW7* from the second LD block, *Gwi7.2* (Yellari et al. 2008; Nonoy et al. 2013; Si et al. 2016; Wang et al. 2015). Importantly, the functional SNP situated at position 16733441 on chromosome 3 with C > A transition was already reported for *GS3* (Fan et al. 2006; McCouch et al. 2016). GWAS confirmed the significance of *GS3* for both raw and cooked grain. A significant region situated downstream of *GS3* resulted from the INDEL-based association mapped in this study. The underlying region possessed high LD decay. Two candidate genes were identified from this region based on associated INDEL. The pinpointing of *GW5* for grain width trait was consistent with earlier studies (Gong et al. 2017; Liu et al. 2017; Weng et al.

2008). *GW5* showed significant association for both cooked and raw grain width in our study. Comparing the significant regions for cooked and raw grain revealed only one novel significant region for cooked grain shape on chromosome 10. One single SNP overlapped with an E3 ubiquitin ligase (*LOC_Os10g35920*) gene, for which prior reports have already revealed the role of E3 ubiquitin ligases in grain width of rice and grain size in other crops (Li N et al. 2014; Song et al. 2007). Comparable results between cooked and raw grains suggest that most of the prominent candidate genes identified for raw grain size were confirmed to exhibit a similar phenotype for cooked grain size; thus, either of the phenotyping targets will be good enough to focus on for breeding selections.

3.5 Impact of grain size traits on grain chalkiness

Grain size-related traits such as grain width are known to confer an unfavorable pleiotropic effect on grain chalkiness and thus grain width was found to be positively correlated with an increase in PGC (Zhao et al. 2016). Previous studies reported that the QTLs governing grain width (*qGW5*) and percent grain chalkiness (*qPGWC5*) were overlapping (Gong et al. 2017), and thus linked grain size genes could not be distinguished from chalk causal genes. Additional reports highlighted that chalk QTLs were found to overlap with grain width-related genes (i.e., *GW7*) on chromosome 7 (Chen et al. 2016; Gong et al. 2017). Interestingly, in our study, chromosome 5 region overlaps with known grain width-governing gene. Although the chromosome 5 region interval possesses a known candidate gene for grain width (*GW5)*, this did not directly signify association with chalk phenotype. In-depth analysis in indica germplasm revealed other SNPs present in this region away from the *GW5* (present upstream) significantly associated with chalk phenotype. Notably, identified SNPs were mapped on a putative candidate gene (*chalk 5.1*) encoding an unknown protein, present upstream of *GW5*, and influence grain chalkiness. Further LD analysis revealed *GW5* and *chalk 5.1* situated in different LD blocks. Some other fine-mapped target genes like *LOC_Os07g41200 (GW7)* for grain width did not show any significant association with chalkiness. Taken together, our results suggest that key genes influencing grain width are not causal factors in influencing chalk in our germplasm panel and these careful finer resolution studies using high-density SNPs can separate the pleiotropic effect that occurs between grain width and chalk.

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3.6. Textural attributes go beyond the control of amylose content

Amylose content may not be sufficient to be used as a sole indicator of the texture of cooked rice given that rice varieties with similar amylose content fall into different textural classifications (Champagne et al. 1999; Champagne et al. 2010). It was evident in the correlation study that only some of the textural attributes are tightly correlated with amylose content, lending strength to the hypothesis that some of the textural attributes are independent of amylose content. Amylose content has no significant correlation with cohesiveness and springiness. Previous studies with small sample sets manifested a significant positive correlation between amylose content and hardness, similar to our study (Cho et al. 2010). Improvement of textural attributes through breeding requires a direct selection technique for textural attributes. Texture Profile Analyzer (TPA)-based attributes such as adhesiveness, hardness, cohesiveness, and springiness were measured in divergent *indica* populations whose amylose content skewed toward intermediate to high. The high resolution of SNPs along with phenotype data guided us to obtain more concrete and sound outputs in this study. A very high narrow-sense heritability of amylose content along with all textural attributes was noticed across seasons. The low resolution of SNP data and limited recombination events in bi-parental populations were the two foremost limitations in earlier studies. Significant regions identified for different textural attributes were not fine-mapped and well characterized (Bao et al. 2004; Cho et al. 2010). The array-based SNP genotyping data used in this thesis work helped to dissect the significant regions using a GWAS approach. Single-locus and multi-locus GWAS conducted for amylose content with days to maturity as a covariate and without a covariate produced consistent significant regions. This potentially indicates that days to maturity do not affect amylose content and other textural attributes. Furthermore, the uniformity of the discovered significant regions across seasons negated any substantial environmental effect on amylose content, adhesiveness, hardness, cohesiveness, and springiness. Amylose content and adhesiveness overlapped the significant region on chromosome 6 (1545370-2038469 bp), with converse allelic effects, consistent with the trait correlations. Multiple multi-locus association mapping methods were used to overcome the shortcomings of mixed linear model-based single-locus association mapping, especially when trait expression is governed by several adjacent minor-effect loci (Tamba et al. 2017; Wang et al. 2016; Zhang et al. 2017). Confirmation of a considerable number of SNPs with more than one multi-locus GWAS method identified a total of 224 SNPs associated with various texture attributes that explained minor- to moderate-effect genomic loci. A total of 48 novel SNPs validated with multiple methods influenced the different

textural attributes. Besides *GBSSI,* multiple candidate genes (alpha/beta hydrolases and phosphotransferases) were identified from chromosome 6 for amylose content and adhesiveness present in the surrounding region. This evidence highlights the genetic control of textural characteristics independent of amylose content (Champagne et al. 1999; Champagne et al. 2010). This knowledge can now be leveraged for improving textural preferences in breeding lines.

4. Final Outlook

In summary, although the fine-mapped haplotypes of chromosome 5 identified for decreasing chalk have been successfully explored in IRRI breeding programs, the novel haplotypes identified for enhanced HRY were not deployed in the 92 IRRI breeding lines released from 1966 to 2015. Interestingly, superior alleles for high HRY and higher stability were not recombined among the studied diversity lines. To ensure future economic benefits for farmers, superior alleles contributing to high HRY and stable HRY from chromosome 8 and minimizing chalk from fine-mapped *chalk 5.1* genes need to be deployed in rice breeding programs to improve milling quality attributes for long slender *indica* varieties. Although some key mega-varieties such as IR64 typically harbour both slender grain and high HRY because of superior HRY alleles, many of the recently released varieties do not possess these superior haplotypes. The major QTLs identified for HRY, HRY stability, and chalk are in the syntenic break points; therefore, we expect that gene order for the important target genes identified in this study for key grain quality traits between *japonica* and *indica* germplasm is not conserved. Applying the knowledge from the significant markers identified from the core collection panel of genebank lines from the GWAS approach to improve milling and cooking quality traits in pre-breeding programs will be valuable. The important donor lines identified with superior haplotypes from genebank lines need to be used for pre-breeding and future selections should include the superior haplotypes for chalkiness and percent head rice yield. Current breeding programs took care when making selections to capture the target traits of grain size, chalk, and amylose content mainly through phenotyping, which could be complemented with marker-assisted selection by targeting superior haplotypes.

5. Summary

Until recent times, rice breeding had mainly focused on high yield with amylose content as a deciding factor to address regional preferences for grain quality. Improved milling and appearance quality will help farmers to better meet the demand of millers and to better address local preferences of textural attributes. Several QTLs for milling and appearance quality were identified and fine-mapped by using a dense panel of SNP markers. Untapped genomic variations in a natural population could be a resource to further fine-map already mapped QTLs and identify significant new genetic regions.

Several rice reference genomes representing different subspecies have been created with the gold standard of sequence quality in the recent past. Population genomics with a multi-reference genome approach helped to identify the correlation between phenotype and genotype with higher confidence. Furthermore, the overlaying of significant genomic regions with collinearity information between different subspecies helped to understand the structural variation within the vital genetic regions mapped with grain quality association peaks.

Milling quality mainly presented as percent head rice yield shows high variability with moisture content in seed. The significant regions denoted through controlled drying techniques and through moisture stress tests led to the identification of key genetic regions influencing high and stable head rice yield. Percent head rice yield and percent grain chalkiness showed a negative correlation with moderate r value. A novel candidate gene, *chalk 5.1,* was identified showing a high effect on percent grain chalkiness, especially in combination with the previously reported *chalk 5* gene. Furthermore, this was the first attempt for which the genetic basis of grain shape for cooked grain was dissected in detail, and key genomic regions for cooked rice shape were unravelled. In addition, other significant regions matched between raw and cooked grain were discovered. Thus, a study with a combined population of *indica* and *japonica* helped to fine-map the novel candidate genes for grain shape. Amylose content and adhesiveness were observed in very strong inter-correlation among all the textural attributes, whereas other textural attributes had independent genetic control different from the region governing amylose content.

The gene discovery work implemented in this study showed the gaps in breeding programs and helped to discover novel alleles and candidate genes using untapped rice genetic diversity. Several novel regions identified for different grain quality traits could enter the future breeding pipeline after due validation. Deciphering the significant regions in breeding lines released during the past few decades helped to understand the progress made for traits such as grain

chalkiness and grain size traits, while genetic regions for crucial traits such as head rice yield were identified as gap traits.

6. Zusammenfassung

Bis vor kurzem konzentrierte sich die Reiszüchtung hauptsächlich auf hohe Erträge mit einem hohen Amylosegehalt als entscheidendem Faktor, um regionale Präferenzen für die Kornqualität zu berücksichtigen. Verbesserte Mahl- und Qualitätsmerkmale werden Landwirten helfen, die Anforderungen der Mühlen und der Verbraucher im Hinblick auf die regionale Präferenzen im Hinblick auf die Korntextur besser zu befriedigen. Im Rahmen der vorliegenden Arbeit wurden mehrere QTLs für die unterschiedliche Qualitätsparameter identifiziert und mit Hilfe eines dichten Panels von SNP-Markern feinkartiert. Die nachgewiesene genomische Variationen in einer natürlichen Population könnte eine Ressource zur weiteren Feinkartierung von QTLs und zur Identifizierung neuer genetischer Marker-Merkmals Assoziationen sein.

Mehrere qualitativ hochwertige Reis-Referenzgenome, die verschiedene Unterarten repräsentieren, wurden in der jüngsten Vergangenheit erstellt. Der im Rahmen der Studie eingesetzte populationsgenomische Ansatz ermöglichte es, Korrelationen zwischen Phänotyp und Genotyp mit hoher Sicherheit zu identifizieren. Darüber hinaus half die Überlagerung signifikanter genomischer Regionen mit Kollinearitätsinformationen zwischen verschiedenen Unterarten, die strukturelle Variation in chromosomalen Regionen zu verstehen, in denen wichtige QTLs für Kornqualität kartiert wurden.

Die Mahlqualität, dargestellt als relativer Anteil des verwertbaren Kornertrags (percent Head Rice Yield, HRY), zeigt eine hohe Variabilität in Abhängigkeit des Feuchtigkeitsgehalts des Saatguts. Die signifikanten Regionen, die durch kontrollierte Trocknungstechniken und durch Feuchtigkeitsstresstests ermittelt wurden, führten zur Identifizierung von genetischen Schlüsselregionen, die sowohl die Höhe als auch die Stabilität des Kopfreisertrags beeinflussen. Die Merkmale HRY und Mürbigkeit des Korns (Chalkiness) zeigten eine moderat negative Korrelation. Ein neues Kandidatengen, chalk 5.1, wurde identifiziert, das einen hohen Effekt auf die Ausprägung der Mürbigkeit zeigte, insbesondere in Kombination mit dem zuvor berichteten Gen, chalk 5. Darüber hinaus wurde in der vorliegenden Studie erstmalig die genetische Basis der Kornform anhand der Analyse von gekochtem Reis untersucht. Dabei

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wurden wichtige chromosomale Schlüsselregionen identifiziert. Darüber hinaus wurden weitere signifikante Marker-Merkmals Assoziationen nachgewiesen, die zwischen rohem und gekochtem Korn übereinstimmen. Die Untersuchung einer gemischten Population aus Indica- und Japonica-Reis half bei der Identifizierung und Feinkartierung neuer Kandidatengene für die Kornform. Der Amylosegehalt und die Klebrigkeit (Stickyness) des geschälten Korns waren stark mit einer Reihe weiterer Merkmale zur Korntextur korreliert, während andere Texturattribute eine unabhängige genetische Kontrolle aufwiesen, die sich von der Region, die den Amylosegehalt bestimmt, unterscheidet.

Die in dieser Studie aufgedeckte allelische Diversität zeigte die vorhandenen Lücken in den Züchtungsprogrammen auf und half bei der Entdeckung neuer Allele und Kandidatengene in bisher züchterisch ungenutzten genetischen Ressourcen. Mehrere neue Regionen, die für verschiedene Kornqualitätseigenschaften identifiziert wurden, könnten nach entsprechender Validierung in die zukünftige Züchtung einfließen. Die Entschlüsselung der signifikanten Regionen in Zuchtlinien und Sorten aus vergangenen Jahrzehnten ermöglichte es, den Fortschritt bei der Verbesserung von Merkmalen wie der Mürbigkeit des Korns zu verstehen. Umgekehrt gelang es neue genetische Regionen für wichtige Merkmale wie den verwertbaren Kornertrags zu identifizieren.

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8. Abbreviations

Abbreviation	Explaination
GS	Grain shape
GS3	*Grain shape 3*
GS5	*Grain shape 5*
GW	Grain width
GW2	*Grain width 2*
GW7	*Grain width 7*
GW8	*Grain width 8*
HDRA	High density rice array
HRY	Head rice yield
IRGC	International Rice Gene bank Collection
IRRI	International rice research institute
LD	Linkage disequilibrium
PGC	Percent grain chalkiness
PVE	Percent variation explained
QTL	Quantitative trait loci
RILS	Recombinant inbred lines
SNP	Single nucleotide polymorphism
SRS5	*Small and round seed 5*

9. Acknowledgements

10. Curriculum vitae

Name	Gopal Misra	
Birth	7th October, 1982	
Employment record	06/2015 – 06/2020	Doctorate candidate, International Rice research Institute. Los Banos, Philippines & Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
	08/2013-05/2015	Intern International Rice research Institute Los Banos, Philippines
	01/2010-07/2013	Senior Research Fellow National institute of plant genome research New Delhi, India
University education	2009	M.Sc. (Tech), Bioinformatics, West Bengal University of Technology, Kolkata, India
	2004	B.Sc. Botany, University of Calcutta, Kolkata, India
School education	2000	Higher Secondary, West Bengal board of secondary education, Kolkata, India
	1998	Secondary, West Bengal Council of Higher Education, Kolkata, India

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Signature
(Gopal Misra)

11. Eidesstattliche Erklärung / Declaration under Oath

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

_____   _____

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12. Erklärung über bestehende Vorstrafen und anhängige Ermittlungsverfahren / Declaration concerning Criminal Record and Pending Investigations

Hiermit erkläre ich, dass ich weder vorbestraft bin noch dass gegen mich Ermittlungsverfahren anhängig sind. / I hereby declare that I have no criminal record and that no preliminary investigations are pending against me.

_____   _____

Datum / Date   Unterschrift des Antragstellers / Signature of the applicant