ASSESSMENT OF THE MARKER-TRAIT ASSOCIATION BETWEEN YIELD PARAMETERS AND POLYMORPHISM OF THE GHD7 LOCUS IN A CORE SET OF RICE GENOTYPES GROWN IN SRI LANKA

By

N.D.U.S. NAKANDALA

A REPORT

In Partial Fulfillment of the Requirement of the Degree of Bachelor of Science Honors in Molecular Biology and Biotechnology

of

UNIVERSITY OF PERADENIYA SRI LANKA

2017

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2017

DECLARATION

I do hereby declare that the work reported in this project report was exclusively carried out by me under the supervision of Prof. S.D.S.S. Sooriyapathirana, Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya. It describes the results of my own independent research expect where due references have been made in the text. No part of this research has been submitted earlier or concurrently for the same or any other degree.

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Rice yield improvement is a major priority in many breeding programs. *Ghd7* is a pleiotropic gene which regulates rice grain yield, plant height and heading date. Although Ghd7 has previously cloned and sequenced in several other rice genotypes, it has not yet been studied in Sri Lankan rice germplasm. Therefore, the objectives of this study were to screen 12 rice genotypes for morphometric measurements in *Maha* and *Yala* seasons, to detect the genetic polymorphism of *Ghd7* locus and to analyze the marker-trait association. The breeder seeds were obtained from RRDI, Bathalagoda, and were established in a greenhouse, at Peradeniva, Sri Lanka in Maha and Yala seasons, 2017. Morphometric measurements were recorded, analyzed by General Linear Model (GLM) using SAS 9.1.3. They were genotyped by 12 DNA markers linked to Ghd7 using PCR, and size separated by agarose gel electrophoresis. The association of each marker allele with yield class and nine yield traits was analyzed. All the traits except number of tillers, leaf blade width, yield and seed number were significantly different among the genotypes (P < 0.05). The overall grain yield was not significantly different among the genotypes in Yala season. However, Suwadhal recorded a low yield in Maha season (3.77 g). The genetic polymorphism reflected a total of 20 alleles with an average of 1.67 alleles per locus three main clusters and ten marker haplotypes.. The maximum number of yield trait associations with Seq 7-8 and RM5346 reveal their usefulness in Marker Assisted Selection (MAS).

Keywords: Rice breeding, DNA markers, grain yield, haplotypes, *pleiotropic* gene, polymorphic marker loci

DEDICATION

I dedicate this thesis to my beloved parents, Mr. Ramyalal Weerawardana, Mrs. Pabasara Rathnayaka, my loving twin sister, Piumi Nakandala and to my grandmother who offered immense support, encouragement, love and affection throughout this work. They have been a great source of inspiration to build my motivation.

ACKNOWLEDEMENTS

Rice is the major staple food crop in worldwide. This research was conducted in the purpose of enhancing the rice yield in order to compensate the high demand for rice. An exponential rise in global population has led to the high consumption of rice across the world. Many novel research studies are undergoing to improve the rice yield. Conventional methods in which the superior genotypes are selected through field observations are cumbersome. However with the advancements of molecular breeding, researchers have put forward a novel concept which is known as marker assisted selection. In this study, we focused on 12 Sri Lankan rice genotypes belonging to three types of yield categories and both the morphological and molecular analyses were performed. *Ghd7* which is a pleiotropic QTL regulates an array of yield related traits. Therefore, our main purpose was to detect the marker haplotypes of *Ghd7* QTL in selected 12 rice genotypes and to carry out a marker trait association analysis for them. This would further give an insight into the marker assisted selection and marker assisted breeding.

At the beginning of this thesis, I wish to extend my sincere thanks for all without whom this project would never have been possible. Many people have contributed in their own particular way to make this research a success and I take this opportunity to give all of them special thanks. First and foremost I wish to express my profound gratitude to my supervisor Prof. S.D.S.S. Sooriyapathirana (Head, Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka) for his patient guidance, constant support, valuable advice and comments, motivation, caring and great encouragement throughout the work of this study. He has been support in writing the thesis should be greatly acknowledged. I benefitted from many fruitful discussions related to my study throughout this period. I also owe my deepest gratitude to my supervisor for his suggestions and corrections on the initial drafts. I have been extremely lucky to have a supervisor who cared so much about my work and this work would not have been possible without his immense guidance.

I am particularly thankful for Dr. Amitha P. Bentota [Director, Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka] and Dr. U.A.K.S. Udawela (Additional Director, RRDI, Bathalagoda, Sri Lanka) for their support in selecting the rice

varieties for the research. Also I owe my gratitude for some people of outstanding importance for my research. Each of the members of the temporary academic staff is greatly acknowledged for their immense support and guidance in providing the chemicals, consumables and handling of machines and equipment in the laboratory. Besides the current temporary academic staff, my sincere thanks also go to Ms. Ruwani Dissanayaka, Ms. Kasuni Daundasekara, Mr. Terrence Sylvester, Ms. Nadeeshani Karannagoda and Ms. Indeewari Dissanayaka (former temporary academic staff) for their valuable support in many ways. The support given by Mrs. Manoja Dayananda (Technical Officer) and Mr. Gamini Chandrarathne (Nonacademic staff) are greatly acknowledged for their valuable support when working in the laboratory and greenhouse respectively. Furthermore I must express my gratitude for the staff members of the Department of Physics, for providing me vernier-calipers whenever I needed.

My family members are acknowledged with heartfelt gratitude for leading me to intellectual pursuits. My beloved father, mother, wonderful twin sister and siblings deserve special thanks for their everlasting encouragement and support. They supported me spiritually throughout my life and always helped me to explore my potential and to achieve my goals. Last, but not least, my thanks also go for my lab mates Hashan, Gayathri, Upendra and Sujani for their greater support and inspiration. It was a pleasure to work with such a nice team. I am ever grateful for all of them for being with me all the time and this work would not have been possible without their remarkable cooperation. I am particularly thankful for other colleagues for sharing the time, resources as well as the knowledge with me throughout this year.

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LIST OF ABBREVIATIONS

100 EW	100 endosperm weight
100 SW	100 seed weight
At	Ambalanthota
Bg	Bathalagoda
Bw	Bombuwela
cDNA	complementary DNA
CL	Culm length
cM	centiMorgan
CRD	Complete randomize design
DCS	Department of Census and Statistics
DOA	Department of Agriculture
EL	Endosperm length
EW	Endosperm width
FAO	Food and Agricultural Organization
FLL	Flag leaf length
FLW	Flag leaf width
Ghd7	Grain number, Plant height and Heading date 7
IRRI	International Rice Research Institute
IUCN	International Union for Conservation of Nature
L	Landrace
LD	Long day
LL	Lowland
MAS	Marker assisted selection
miRNA	micro RNA
MOP	Muriate of Potash
NI	Newly Improved
NIL	Near-Isogenic Lines
NT	Number of tillers
NT PCR	Number of tillers Polymerase chain reaction
NT PCR PIH	Number of tillers Polymerase chain reaction Plant height
NT PCR PIH QTL	Number of tillers Polymerase chain reaction Plant height Quantitative trait loci

RRDI	Rice Research and Development Institute
SD	Short day
SL	Seed length
SN	Seed number
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
TSP	Tripple super phosphate
UL	Upland

1. INTRODUCTION

Rice is the staple food crop for nearly half of the world's population (FAO, 2004). It is cultivated in different locations across the world (IRRI, 2012). The statistical records have shown that there would be a 1.7 billion increase in the global population from 2003 to 2025 (Khush, 2004). This increase in global population has led to more industrialization throughout the world. As a consequence of that, many agricultural lands have widely been used for construction work (Goode and Chadwick, 2001). Also, this exponential rise in global population has led to the increased consumption of rice. To compensate this demand, an increase in rice production (Abdullah *et al.*, 2004) or area expanding would be required. However, the fact that Sri Lanka doesn't have additional area to be bought under rice is well known. Therefore, the only logical solution is to improve the rice yield and this is the major goal of almost all the rice breeding programs.

In the aspect of improving rice yield, both the phenotype-based and genotype-based selection methods have been used. The phenotype-based selection methods are conducted through field observations (Khush, 2004) and therefore they are cumbersome. Genotype-based selection is much popular with its high efficiency in plant breeding (Collard and Mackill, 2008). In this approach, the phenotypic traits are improved by studying at the DNA level (Acquaah, 2012). An important concept of molecular markers has been presented by scientists and their mapping in relevant linkage groups with the relative genomic distances is a requisite in molecular breeding (Jones *et al.*, 1997). Use of these DNA/molecular markers in molecular breeding is known as marker assisted selection (Collard and Mackill, 2008).

Rice yield is a complex agronomic trait governed by multiple loci quantitative trait loci (QTLs). These traits show continuous variation in segregating populations. Individual loci/genes have minor effects on overall phenotype of a particular trait (Yano and Sasaki, 1997). Rice yield is governed by both the genetic constitution of the plant and the environment (Yan *et al.*, 2011). Grain yield, height of the plant and heading date are three important traits which collectively influence the rice yield (Xue *et al.*, 2008). Previous studies have been conducted to study of a number of genes which distinctly control the grain yield (Ashikari *et al.*, 2005; Song *et al.*, 2007), heading date (Yano *et al.*, 2000; Takahashi *et al.*, 2001; Kojima *et al.*, 2002; Doi *et al.*, 2004) and height of the plant (Sasaki *et al.*, 2002)

in rice. Grain yield is mainly determined by number of panicles per plant, number of grains per panicle and grain weight (Huang *et al.*, 2009). Plant height is important in determining plant architecture and heading date is another important parameter which involves in environmental adaptation of rice (Xue *et al.*, 2008). These three parameters collectively have greater contribution on rice yield.

Ghd7 has been identified as a QTL which plays a key role in improving yield by simultaneous regulation of plant height, grain number and heading date (Xue *et al.*, 2008). *Ghd7* is comprised of a nucleotide sequence which is 3,917bp in length and the cDNA sequence is 1,014bp in length (Xing *et al.*, 2014). The protein encoded by *Ghd7* contains 257 amino acids (Xue *et al.*, 2008). The gene contains 2 exons (Xing *et al.*, 2014). High expression of *Ghd7* is responsible for increased number of grains, plant height and delaying of heading date. This QTL encodes a CCT domain protein (Xue *et al.*, 2008) and was identified in both the indica rice varieties such as Minghui 63 and japonica rice varieties such as Nipponbare (Xue *et al.*, 2008; Lu *et al.*, 2012). In addition to the above major traits, it also involves in controlling plant architecture, branching of tillers, hormone metabolism and plant responses to various environmental stresses. The expression of *Ghd7* is influenced by many environmental signals (Weng *et al.*, 2014).

Ghd7 has previously cloned and mapped to a position of 0.4 cM in chromosome 7 in rice using *Minghui 63* and *Zhenshan 97* cultivars (Xing *et al.*, 2014). Studies also show that the *Ghd7* mediated flowering is not only found in rice, but also found in other grass species such as maize, sorghum and *Brachypodium distachyon* (Yang *et al.*, 2012). Previous studies have studied the features of *Ghd7* QTL in many rice germplasms belonging to indica and japonica rice varieties. Haplotypes based on SNP mutations and protein diversity of *Ghd7* also have been studied (Xue *et al.*, 2008; Lu *et al.*, 2012). Also the involvement of *Ghd7* in regulating various other physiological parameters (Weng *et al.*, 2014) and its effects on other genes in flowering pathway of rice have studied in detail (Itoh *et al.*, 2010; Matsubara *et al.*, 2011).

However, none of the studies have been conducted on *Ghd7* for Sri Lankan rice germplasm. This work basically studied about selected 12 Sri Lankan rice varieties (hereafter mentioned as genotypes) including At 362, Bg 300, Bg 366, Bg 90-2, At 307, Bg 310, Bg 352, Bw 367, Bg 250, Bw 272-6b, *Pachchaperumal* and *Suwadhal*. Some of the genotypes considering in this study are high yielding and some are moderate yielding while the rest are low yielding.

One of the main objectives of this work was to screen the 12 rice genotypes for yield parameters including vegetative, flowering and harvested measurements in both the *Maha* and *Yala* seasons. Another objective was to detect the genetic polymorphism of *Ghd7* locus among 12 rice genotypes using 12 DNA markers associated with the particular locus. Thereby it provides the access to study the correlation of aforementioned marker positions with the yield related traits of rice which in turn gives insight into the study of the marker trait association. This is important since the selection of genotypes based on genetic markers is more accurate and efficient over the phenotypic based selection. This would be beneficial since the high yielding rice genotypes can be selected at the seedling stage by analyzing the marker data, without being waiting for the ultimate harvest.

2. REVIEW OF LITERATURE

2.1 Rice production status in global and Sri Lankan contexts

Rice is produced globally in different locations including wettest and driest regions (IRRI, 2012). It is the staple food crop for nearly half of the world's population (FAO, 2004). Total 154 million hectares are annually cultivated for rice production (Khush, 2004). It accounts for 27% caloric intake and 20% protein supply in the diet (FAO, 2004). Rice production has increased in greater amounts particularly in Asian countries (Muthayya *et al.*, 2014) while a striking increase of 140% has been recorded worldwide since the green revolution. China and India are the two major countries which account for a markedly increased rice production. Bangladesh, Myanmar, Indonesia, Vietnam and Thailand are the next larger rice producers in the world. In addition to these top seven countries which provide 80% of the world rice production, Philippines, Africa, Egypt, Nigeria and Madagascar are the next leading countries while Russia, Italy and Spain are the leading European rice producers. Apart from this, United States accounted for nine million tons of rice production in 2006-2008 (IRRI, 2012). Although all of these countries contribute noticeably for the total supply, both the rice production and consumption are dominated by Asian countries (Muthayya *et al.*, 2014).

Rice is the staple food crop in Sri Lanka and it is cultivated in all the districts as a wetland crop. Currently 708,000 hectares have been allocated for rice production (DCS, 2016). During 1960-1980, there was a noteworthy rice production in Sri Lanka. However, since then expected yield was not attained due to various reasons including low yield potential of varieties and other associated limitations (Jayawardene, 2000). In 2015/2016 *Maha* season, a 13% paddy production was reported from *Anuradapura* District and 10%, 11% and 12% productions were reported from *Polonnaruwa*, *Kurunegala*, *Ampara* Districts respectively (DCS, 2016). Currently the country produces 90% of the entire need of rice for the 20 million population (Jayawardene, 2000). In the country, a greater amount of rice yield has been recorded in the areas with major irrigation systems and conversely the minor irrigation systems contribute to a less amount for the overall rice yield (Weerakoon *et al.*, 2005).

2.2 Need for improving rice yield

The statistical records have shown that there would be a 1.7billion increase in the global population from 2003 to 2025 (Khush, 2004). This increase in the population would be

mainly observed in developing countries. Data further revealed that South Asia will account for 31% of the total increase in global population while Sub-Saharan Africa will contribute only to a 28% increase. This increase in global population has resulted in more industrialization throughout the world and consequently, agricultural lands have widely been used for the construction of houses, roads and factories (Goode and Chadwick, 2001). It has been estimated that this increased population would lead to 493.5 million metric tons of rice consumption by 2030 and to meet this demand, 5.25 million tons would be required by 2050 (Abdullah *et al.*, 2004). Loss of land resources can be compensated by enhancing the cropping intensity as well as improving the overall rice yield (Weerakoon *et al.*, 2005). This will in turn results in alleviating the poverty of farmers while giving benefits for agricultural laborers in addition to solving the food crisis globally (Sombilla *et al.*, 2002). All of these facts explain the necessity of improving the rice yield globally.

2.3 Strategies for improving rice yield

Improving rice yield can be achieved by many different types of strategies. Basically this can be accomplished through improving on-farm yields, integrated crop and pest management, decreasing the post-harvest losses and bridging the yield gap (Singh *et al.*, 2001). In addition, field management practices including improving the organic matter content in the soil, well puddling, proper leveling in preparation of seed beds, management of losses of nutrients and water are significant in improving yield. Assisting the growth of roots by proper tillage is also beneficial for this purpose. Weed management strategies are also important to improve yield which can be facilitated by the addition of crop residues (Weerakoon *et al.*, 2005). Improving the quality of grain, product value and the diversification of rice system are the other different approaches to achieve high yields. More importantly, the natural resources such as water, emission of greenhouse gases, soil health, bio-diversity and plant nutrition can be integrated in a more productive and ecologically friendly way in the ultimate purpose of improving yield (Singh *et al.*, 2001).

Variety selection is also another important approach in improving yield. Varieties should be selected in a way that they are appropriate for the particular climatic and soil factors. In addition, the availability of irrigation schemes, duration of cropping and better management practices should be considered important in achieving improved yield (Weerakoon *et al.*, 2005). Despite the advantages of aforementioned strategies, sustained yield of rice can further be enhanced through conventional hybridization and selection procedures (Peng *et*

al., 2000), heterosis breeding (Virmani *et al.*, 1982), ideotype breeding (Peng *et al.*, 2004) and wide hybridization in which the crop varieties are hybridized with weedy races and wild species (Xiao *et al.*, 1996). In addition, currently, genetic engineering approaches are widely used for the incorporation of desired genes into the rice genome with the purpose of improving yield. This involves the use of strategies such as protoplast based and *Agrobacterium*-mediated DNA transferring (Datta *et al.*, 1990; Hiei *et al.*, 1994). Molecular marker assisted breeding is also much popular worldwide (Collard and Mackill, 2008; McCouch *et al.*, 1997).

2.3.1 Conventional breeding

Plant breeding mainly improves the varieties by choosing the best ones among many individuals which have the ability of growing well with a better productivity in a particular environment (McCouch, 2004). The breeders had the idea of increasing the yield of food crops over the past years (Peng *et al.*, 2004). Two individuals with desirable characteristics are selected for a particular cross as parents and during the cross, the genes are swapped between the two genomes giving rise to a diversity in the subsequent progeny generations (McCouch, 2004). The selection of superior individuals basically depends on both the genotype and the surrounding environment (Hallauer, 2011). In this way the individuals bearing a combination of desired features of parents are selected and further bred (McCouch, 2004). There are some conventional breeding methods which are practiced by researchers including conventional hybridization and selection procedure in which the individuals with the desirable features are selected based on yield trials and field observations (Khush, 2004), hybrid breeding (Cassman, 1994), artificial pollination, wide crosses and tissue/embryo culture. Use of techniques such as chromosome doubling and protoplast fusion is also practiced (Acquaah, 2012).

2.3.2 Molecular breeding

Molecular breeding is the exploitation of biotechnological tools including functional genomics, proteomics, metabolic profiling and genomic analysis in order to improve the expression of a number of phenotypic traits at the DNA level. Molecular breeding is facilitated by genome mapping which gives insight into the location of the genes within the chromosome (Acquaah, 2012). In molecular breeding, scientists have put forwarded another important concept of molecular markers (DNA markers), which are variable sites positioned within the chromosomes. Mapping of these markers in particular linkage groups with their

relative genomic distances is also needed in this process (Jones *et al.*, 1997). Marker assisted selection (MAS) which takes the advantage of DNA markers is a mass revolution in molecular breeding with enhanced efficiency of plant breeding techniques. Identification of homozygotes, heterozygotes, selection of individual plants on the basis of their genotype, simplicity, saving the resources, time and effort, selection at the seedling stage are the major benefits of MAS (Collard and Mackill, 2008). The MAS can further be used in marker assisted breeding (Acquaah, 2012; Hospital and Charcosset, 1997) and marker assisted pyramiding (Hittalmani *et al.*, 2000).

2.3.2.1 Graphical genotyping

Graphical genotypes express the complete genome in the form of a distinct graphical image thereby providing a more comprehensive way to express the individual genetic composition (Young and Tanksley, 1989). The numerical discrete locus data are expressed in a visually alluring way in graphical genotypes (Van Eck *et al.*, 2017). The numerical locus data are confined into the linkage groups of an individual, reflecting the independent assortment and recombination. Graphical genotypes provide many advantages over the methods used previously. It facilitates the 'whole genome selection' which would in turn provide access to manipulate the complete genome of a particular individual. The way of reducing a large set of numerical data into a graphical image is astonishing in plant breeding programs as well as in polygenic trait analysis in animals. In plant breeding programs, during the following backcross generations, the donor and recurrent parental genome proportions and their positions are displayed in graphical genotypes (Young and Tanksley, 1989).

It also facilitates the rapid data assessment and the recognition of singletons thereby modifies the inaccurate genotyping results (Van Os *et al.*, 2005; Van Eck *et al.*, 2017). The recombinants and the trait values of them can be graphically analyzed to support the mapping of loci, associated with main effects at high resolution (Finkers-Tomczak *et al.*, 2009; Van Eck *et al.*, 2017). It also facilitates the positioning of specific plant trait associated genes with the help of molecular markers and linkage analysis (Hayano-Saito *et al.*, 1998). Also it allows the detection of genetic similarity between the individual genotypes (Macaulay *et al.*, 2001). Graphical genotyping was previously proposed as a useful tool in plant breeding programs. However, it is also applicable in humans, especially in studies of complex heritable genetic diseases (Lander and Botstein, 1986). Graphical genotypes can be displayed using a number of software and programs including Flapjack (Milne *et al.*, 2010), GGT (Van Berloo, 2008), Apple-Macintosh based program (Young and Tanksley, 1989). These facts support that the graphical genotyping is an essential tool in genomic studies.

2.3.2.2 Haplotype diversity based breeding

Haplotype diversity based analyses are useful in plant breeding programs. High density SNPs can be used as one of the means of representing the haplotype diversity in regions of the genome. A reduction in the polymorphism often results in a reduction of haplotype diversity (Yonemaru *et al.*, 2012). Studies based on pre-harvest sprouting (PHS) QTLs of wheat revealed, that the information derived from haplotype diversity of SSRs linked to these QTLs is useful in incorporating the PHS into cultivated wheat genotypes (Ogbonnaya *et al.*, 2007). The haplotype information along with the markers could be useful in developing novel varieties with enhanced resistance to PHS, by subsequent breeding programs (Shorinola *et al.*, 2017).

Based on the SSR marker polymorphism in selected rice genotypes, the salinity resistant and tolerant haplotypes can be compared in rice. This would be useful in developing novel varieties with salinity resistance (Islam *et al.*, 2012). Haplotype diversity, derived from the information of SSRs linked to *rym11* locus is useful in plant breeding, to develop mosaic virus resistance in Barely (Sedlacek and Marik, 2010). Haplotype diversity of a gene in grapevine (*Vitis vinifera* L), homologous to Arabidopsis *Terminal flower 1* can be correlated to the morphological variation in inflorescence and reproductive traits (Fernandez *et al.*, 2014). Despite the potential of haplotype diversity analysis in plant breeding programs, it is also a powerful strategy in animal breeding (Calus *et al.*, 2008). These facts reveal that the haplotype diversity information derived from SNPs and SSRs can be effectively used in plant and animal breeding strategies.

2.4 Rice yield related QTLs for molecular breeding

Grain yield is a complex agronomic trait which is determined by many yield attributing traits. Yield is regulated by many types of genes each may contribute to a number of traits (Kolta *et al.*, 2013). Yield is a quantitative trait in which the expression is influenced by an array of genes and other environmental effects making it more complex (Zhou *et al.*, 2013). The detection of QTLs underlying the major yield traits is a pre-requisite in the development of high yielding genotypes (Kolta *et al.*, 2013). The advances of molecular genomics and the availableness of entire genome sequence of rice are major breakthroughs in detection and

further characterization of yield attributing QTLs (Marri *et al.*, 2005). In order for them to be utilized in molecular breeding, the detection of interaction between QTLs and that with the environment is needed (Takai *et al.*, 2017). Many previous studies have reported the mapping of yield related QTLs (Guteirrez *et al.*, 2010; Fan *et al.*, 2005; Tian *et al.*, 2006; Zou *et al.*, 2005). Rice yield QTLs such as *GW2*, *GW5*, *Gn1a*, *GS3*, *TGW6*, *qGL3*, *APO1*, *GW8*, *G1F1*, *Ghd8*, *DEP1*, *PROG1*, *SRS3*, *EP2*, *MOC1*, *IPA1* have previously been mapped and cloned (Table 2.1).

2.4.1 QTLs for grain number

Studies reveal that the QTL *Gn1a*, which is mapped on Chromosome one encodes cytokinin oxidase protein, which is responsible for increasing the grain number (Ashikari *et al.*, 2005). Also it has been shown that the *DEP1* which encodes a PEBP-like domain protein is responsible for increasing the grain number subsequently resulting in high yield of rice (Huang *et al.*, 2009). The number of grains per panicle is regulated by *IPA1*. It is also responsible for lowering the number of tillers and gives rise to a strong culm. It is positioned within the Chromosome 8 and it is responsible for encoding the protein OsSPL14 (Jiao *et al.*, 2010; Miura *et al.*, 2010). Studies have identified *WFP* as a yield related QTL which is capable of inducing panicle branching simultaneously increasing the grain yield (Miura *et al.*, 2010). Also the researches have revealed that *APO1* is an important gene in determining panicle size, grain number per panicle and the number of primary rachis branches in rice (Terao *et al.*, 2010; Ikeda *et al.*, 2007). All these QTLs enhance the rice yield by increasing the number of grains.

QTL/Gene	Trait	Chromosome	Encoded protein	Causal mutation	Allele attributing high yield	Reference
GW2	Grain filling, width, weight	2	Ring type ubiquitin E3 ligase	Premature stop	Loss of function	Song <i>et al.</i> , (2007)
GW5/qSW5	Grain width, weight	5	Unknown	Deletion	Loss of function	Weng et al., (2008)
Gnla	Grain number	1	Cytokinin oxidase	Deletion	Low expression	Ashikari et al., (2005)
GS3	Grain length, weight	3	Putative PEBP-like domain protein	Premature stop	Loss of function	Fan et al., (2006)
TGW6	Grain length, weight	6	Unknown	Frame-shift	Loss of function	Ishimaru et al., (2013)
qGL3	Grain length, width, thickness, thousand grain weight	3	Putative protein phosphatase with OsPPKL1 domain	SNP1	Low expression	Zhang et al., (2012)
APO1	Grain number, panicle size,	6	F-box protein	Substitution	Gain of function	Terao <i>et al.</i> , (2010) Ikeda <i>et al.</i> (2007)
GW8	Grain filling, width	8	Transcription factor	Deletion	High expression	Wang <i>et al.</i> , (2012)
G1F1	Grain filling, weight	4	Cell wall invertase	Premature stop	Over expression	Wang et al., (2008)
Ghd8/DTH8	Plant height, Grain number, heading date	8	CCAAT-box binding transcription factor	Frame-shift	High expression	Wei <i>et al.</i> , (2010) Yan <i>et al.</i> , (2011)
DEP1	Grain number, panicle	9	PEBP-like domain protein	Premature stop	Loss of function	Huang et al., (2009)
PROG1	Tiller number, grain number	7	Zinc finger nuclear transcription factor	Substitution	Loss of function	Jin <i>et al.</i> , (2008) Tan <i>et al.</i> , (2008)
SRS3	Grain weight, size	5	Kinesin motor domain protein	Substitution	Loss of function	Kitagawa <i>et al.</i> , (2010)
EP2	Panicle length, grain size	7	Unknown	Deletion	Loss of function	Zhu et al., (2010)
MOC1	Tiller number, Grain number	6	Putative GRAS family nuclear protein	Premature stop	Slight over expression	Li et al., (2003)
IPA1	Tiller number, grain number	8	OsSPL14	Substitution	Higher expression	Jiao <i>et al.</i> , (2010) Miura <i>et al.</i> , (2010)

Table 2.1 Major QTLs/genes responsible for rice yield traits

2.4.2 QTLs for grain weight, size, width, length and grain filling

Yield of rice is enhanced by increasing both the grain length and weight in the presence of *GS3* QTL (Fan *et al.*, 2006). The overall size of the rice grain is improved by increasing both the grain width and weight by *GW2*. In addition, it also controls an array of yield related traits including number of panicles, length of the panicle and heading date. It encodes a protein containing a deletion of 310 amino acids, giving rise to a wider hull of the spikelet which in turn increases the milk filling rate of rice grain (Song *et al.*, 2007). The grain width and weight are also regulated by *GW5* via ubiquitin proteasome pathway (Weng *et al.*, 2008). *TGW6* is another gene which is found to be responsible for increasing the rice grain length. Functionless *Kasalath* allele of *TGW6* accounts for increasing grain weight which in turn plays a significant role in enhancing rice grain yield (Ishimaru *et al.*, 2013). Putative protein phosphatase encoding gene *qGL3* positively affects the length, thickness, weight and width of rice grain. This QTL has been mapped on to chromosome 3 and is useful in breeding programs which can improve the rice yield (Zhang *et al.*, 2012).

Kinesin motor domain protein encoding gene *SRS3* possesses a coiled-coil structure which plays crucial roles in elongating cell length and regulating cell number in the formation of seeds (Kitagawa *et al.*, 2010). The size of the grain is regulated by *EP2* gene which is positioned on chromosome 7, thereby increases the rice yield. In addition, panicle architecture including the length of the panicle is controlled by an encoded protein with ten exons, nine introns and 1365 amino acids (Zhu *et al.*, 2010). Grain filling is another yield component trait of rice and it is a principle determinant in regulation of grain weight (Wang *et al.*, 2008). A significant role is played by *GW8* by increasing the grain filling and cell division rate consequently promoting the grain width and the overall rice yield (Wang *et al.*, 2012). A Cell wall invertase encoding gene *G1F1* controls grain filling by manipulating sugar levels in rice grains and is also involved in the development of grain through sucrose unloading. *G1F1* has been mapped within the chromosome 4 and a deletion in the coding region results in a premature stop codon giving rise to the predicted protein with 598 amino acids (Wang *et al.*, 2008). All these QTLs involve in the development of grain

2.4.3 QTLs for tillers per plant

Increased expression of *LRK1* gene has been identified in regulating a number of traits which involve in enhancing grain yield of rice. It increases the number of tillers per plant, panicle

number, spikelet number per panicle and also the number of grains per plant simultaneously decreasing the height of the plant. These all components play crucial roles in improving rice yield (Zha *et al.*, 2009).Previous studies have shown that *PROG1* gene encodes a zinc finger transcript factor and regulates the tiller number, tiller angle and architecture of the rice plant. This gene is mapped on chromosome 7 and increased expression of *PROG1* has been observed at the sites where the tiller buds and auxillary meristem are formed (Jin *et al.*, 2008). The inactive expression of *PROG1* will give rise to an increase in the number of grains and an erect growth of rice (Tan *et al.*, 2008). The tillering of rice is also regulated by *MOC1* gene which encodes a putative GRAS family nuclear protein and it involves mainly in the formation of auxillary buds (Li *et al.*, 2003).

2.4.4 QTLs for heading date

To improve the rice yield, not only the yield component traits, but also the plant architecture which is crucially determined by plant height and the number of days for flowering is also important. Latter facilitates the plants to exploit the available sunlight and temperature in a particular environment thereby enabling the plants to survive under various seasonal alterations (Xue *et al.*, 2008). A CONSTANS-like protein encoding QTL *Hd1* increases the flowering time specifically under short day conditions and conversely it has negative roles on heading date under long day conditions (Yano *et al.*, 2000). FT-like protein encoding *Hd3a*, on chromosome 6 (Kojima *et al.*, 2002) and *Ehd1* on chromosome 10 encoding a B-type response regulator involve in increasing flowering only under short day conditions (Doi *et al.*, 2004). *Ghd7* is a pleiotropic QTL which simultaneously increases plant height, grain number and heading date under short day conditions (Xue *et al.*, 2008). Previous findings also revealed that, *Ghd8/DTH8* QTL in chromosome 8 also involves in controlling heading date, grain number and plant height by elongating cells in internodes and keeping a stable culm thickness in rice plants (Wei *et al.*, 2010; Yan *et al.*, 2011).

2.4.5 Ghd7 QTL

Ghd7 QTL plays a major role in regulating plant height, grain yield and heading date by encoding a CCT (CO,CO-LIKE AND TIMING OF CAB1) domain protein and its enhanced expression results in increased plant height, panicle size and extended heading date (Xue *et al.*, 2008). It has been positioned to a region of 2300 kb at 0.4cM distance in Chromosome 7 in between *C1023* and *R1440* molecular markers (Xing *et al.*, 2014). This gene has been identified as a heterochromatic gene (Yang *et al.*, 2012). *Ghd7* has a sequence of 3917bp

which is comprised of two exons (Xing *et al.*, 2014). 1014bp lengthened cDNA is responsible for encoding 257 amino acids (Xue *et al.*, 2008). This *Ghd7* QTL was found to have repetitive elements in a high proportion and gene densities in a low proportion (Yang *et al.*, 2012). Mutations arose within *Ghd7* lead to a number of different haplotypes in both the Indica and Japonica rice varieties with varied functional effects subsequently leading to conspicuous phenotypic variations (Lu *et al.*, 2012). This QTL is responsible for a significant high yield of rice concurrently improving their adaptability into different locations globally (Xue *et al.*, 2008).

Ghd7 was identified in a number of varied rice populations including *IR64-Azucena* (Indica-Japonica cross population) (Li *et al.*, 2003), *Zhenshan 97-Minghui 63* (Indica-Indica cross population) (Xing *et al.*, 2002), *Nipponbare-Kasalath* (Japonica-Japonica cross population) (Lin *et al.*, 2003), and *IRGC* and *105491* (wild cultivated population) (Thomson *et al.*, 2003) and the genetic basis of *Ghd7* differs from one population to another (Xing *et al.*, 2014). The promoter region of *Ghd7* was detected with high variability with compared to that of the coding region and this enables the rice plants to acquire their developmental needs and to acclimatize into different geographical regions (Lu *et al.*, 2012). All the tissues were found to be expressed with *Ghd7* (Xing *et al.*, 2014) and the levels of expression was varied in different tissues regulating an array of phenotypic traits. Also the *Ghd7* protein was found to be expressed in the nucleus. Although the effects of *Ghd7* is differed from that of the other CCT domain protein encoding members (Xue *et al.*, 2008) the *Ghd7* mediated flowering pathway is also present in a number of other grass species such as sorghum and *Brachypodium distachyon* (Yang *et al.*, 2012).

2.4.5.1 Effect of *Ghd7* on other genes in flowering

Ghd7 significantly controls the expression pattern of *Ehd1* and *Hd3* differentially in short day and long day conditions. Under long day conditions, *Ghd7* represses the activity of *Ehd1* particularly during early morning periods and weakly encourages its expression under short day conditions within dark periods (Xue *et al.*, 2008). *Ghd7* negatively controls the transcription of *Hd3a*, which is known to have positive considerable effects on transition from vegetative to reproductive phases (Tamaki *et al.*, 2007). However, *Ghd7* has no effects on floral development of *Hd3a* (Okada *et al.*, 2017). This suggests that *Ghd7* has significant effects on flowering in rice through mediating the *Ehd1-Hd3a* pathway (Xue *et al.*, 2008;

Itoh *et al.*, 2010). Studies also showed that *Ehd3* which is a repressor of *Ghd7* acts upstream of *Ghd7* in *Ehd3-Ghd7-Ehd1* pathway of flowering. This in turn positively regulates the expression of *Hd3a* and *RFT1*, which are known to be FT-like genes. On the other hand, *Ehd3* enhances the expression of *Ehd1* without the involvement of *Ghd7* (Matsubara *et al.*, 2011).

Hd1 acts along with *Ghd7* in delaying of flowering by suppressing the *Ehd1* particularly under LD conditions. The expression of *Ehd1* is mainly mediated by forming a complex of *Hd1* and *Ghd7* which in turn binds the cis-regulatory elements found within *Ehd1* (Nemoto *et al.*, 2016). Under SD conditions, *Hd1* which is known to affect heading date significantly in Asian cultivars (Ebana *et al.*, 2011) promotes the expression of *RFT1* and *Hd3a* while repressing the rice florigens under LD conditions independently of *Ghd7* (Wu *et al.*, 2008). Studies also reveal that the activity of *Ghd7* is controlled through phosphorylation by *Hd16*. This subsequently involves in decreasing the expression of rice florigens including *Hd3a* and *RFT1* (Hori *et al.*, 2013). The reduced transcription of *Hd3a* is responsible for delayed flowering (Komiya *et al.*, 2009) and that of the *RFT1* also increases the heading date (YuanLi *et al.*, 2012) and negatively affects the floral transition (Komiya *et al.*, 2009). Proteins of *Ghd7* and *Hd16* are also known to interact with each other in vitro (Hori *et al.*, 2013). *Ghd7* has found to act downstream of *Hd16* (Kwon *et al.*, 2015). Reduced kinase activity of *Hd16* has no conspicuous effects on the interaction with *Ghd7* (Hori *et al.*, 2013).

Under both SD and LD conditions, *Ef7* which has been identified as an ortholog of Arabidopsis *ELF3* induces flowering by negatively controlling the expression of *Ghd7* (Saito *et al.*, 2012). *Hd5* also involves in regulating heading date in the presence of *Ghd7* (Fujino *et al.*, 2013). Moreover, *OsCOL10* which has relatively less effects on flowering is controlled by *Ghd7* under LD conditions. *Ghd7* is unable to bind the promoter region of *OsCOL10* directly and the presence of certain other proteins is a requirement in signal transduction in this process. *OsCOL10* also involves in controlling of *Hd3a* and *RFT1* by repressing the expression of *Ehd1* similar to *Ghd7* (Tan *et al.*, 2016). These facts give a clear perception that the involvement of *Ghd7* in rice flowering is mediated by many other flowering genes. Also, *Ghd7* interacts with them subsequently regulating their expression patterns in different photoperiodic pathways.

2.4.5.2 Role of *Ghd7* on other physiological traits

Besides of *Ghd7* gene marked effects on regulating the flowering time, plant height and grain number, it utilizes the signals perceived through the surrounding environment for the plant to monitor its growth and development, morphology, plant architecture and stress feedback. It has a significant influence in regulating numerous arrays of traits by retarding the shift from vegetative phase to reproductive phase and escalating the lateral organ development (Weng *et al.*, 2014). *Ghd7* has a noticeable effect on growth and development of stem of rice plant. The stems of the rice plants which carry the *Ghd7* allele are wider compared to that of the plants in which the allele is absent. This is mainly due to the presence of numerous layers of bigger cells present in the *Ghd7* positive plants. It also shows that *Ghd7* accounts for many nodes and an elongated topmost internode in the rice stem (Xue *et al.*, 2008).

Ghd7 has imperative effects on regulating the area, length and width of the flag leaf of rice with comparison for its negative counterparts (Cong *et al.*, 2012) and the flag leaf has an essential role in delivering carbohydrates to rice grains (Gladun and Karpov, 1993). During the flowering period of rice plant, *Ghd7* influences in controlling the amount of chlorophyll in leaf. By down-regulating the genes which are responsible for the synthesis of chloroplast and chlorophyll, *Ghd7* accounts for lowering the amount of chlorophyll content (Wang *et al.*, 2015). It also has been revealed that *Ghd7* is responsible for the lodging resistance and increased yield by raising the transportation ability and mechanical vigor acquired through developed vascular systems (Xue *et al.*, 2008). *Ghd7* also has an exclusive benefit over the other photoperiod- insensitive alleles due to its ability to diminish photoperiod sensitivity devoid of declining the number of tillers. This is more often beneficial in breeding of a photoperiod insensitive variety (Xu *et al.*, 2014).

Ghd7 manipulate the branching of both tillers (vegetative phase) and panicles (reproductive phase) in a density reliant manner which in turn controls the flexibility of development of branches in a way that it makes the plant more adaptable to diverse environmental circumstances. This also increases the reproductive success. PHYTOCHROME B (PHY B) regulates the activity of *Ghd7* in controlling this procedure (Weng *et al.*, 2014). *Ghd7* occupies in the growth of auxillary buds on the main shoot which subsequently develop into tillers (Xu *et al.*, 2014) and both the genetic issues and environmental conditions control tiller branching. A study has been conducted using NIL (mh7) which accounts for less number of tillers compared to that of NIL (zs7) where the former is relatively larger in size

in comparison to the latter. When the NIL (mh7) plants are cultivated in conditions of low density, they had considerably high number of tillers implying that the effects of *Ghd7* are determined by the density of the plant (Weng *et al.*, 2014). In regulating tiller branching *Ghd7* also suppresses the *OsTB1* expression (Lo *et al.*, 2008) by proceeding upstream of *OsTB1* (Weng *et al.*, 2014) and promoting a protein of *OsTB1* named as OsMADS57 (Guo *et al.*, 2013).

Panicle branching is correlated with the flowering time (Weng *et al.*, 2014) and the number of primary and secondary branches is regulated by *Ghd7* (Xue *et al.*, 2008). Also *Ghd7* plays crucial roles in the development of auxillary buds which are present on primary branches into secondary branches (Xu *et al.*, 2014). Several genes such as *TCP* genes, *SPL* genes and *YABBY* genes which are monitored by *Ghd7* are responsible in stipulating meristem and lateral organ distinctiveness (Weng *et al.*, 2014). Previously a study has been conducted on the time period of panicle differentiation in *Minghui 63* and *Zhenshan 97* where *Minghui 63* associating a wild type *Ghd7* allele has a 30 day time period while the latter shows only 23 days in which the *Ghd7* locus is utterly deleted. This reveals that the impact of *Ghd7* on the size of the panicle is interconnected to the time period of panicle differentiation. This is important because the number of spikelets per panicle is determined not only by the rate of spikelet differentiation, but also by the time period of panicle differentiation to the heading date, plant height and grain number in rice.

2.4.5.2.1 Involvement of Ghd7 in stress response pathway and ROS homeostatis

Genes that are controlled by *Ghd7* were found to have crucial impacts on biotic and abiotic stress responses (Weng *et al.*, 2014). *OsDREB1A* which makes the monocots withstand to cold stresses, drought, high-salt concentrations (Dubouzet *et al.*, 2003) and *OsPR4*'s expression that is significantly regulated by abiotic stress conditions (Wang *et al.*, 2011) are up-regulated in *Ghd7* over expressed (OX-*Ghd7*) ^{HJ19} plants. *Ghd7* is also involved in reactive oxygen species (ROS) homeostatis in rice plants (Weng *et al.*, 2014). *OsMT2b* encoding metallothionein protein that eliminates the reactive oxygen species (ROS) from the cells (Wong *et al.*, 2004) is regulated in rice plants to which the *Ghd7* over expression (OX-*Ghd7*) ^{HJ19} and artificial miRNA (amiRNA) constructs have been incorporated (Weng *et al.*, 2014). Also the expression of *RACK1A* gene which is responsible for the production of ROS is decreased in (OX-*Ghd7*) ^{HJ19} plants (Nakashima *et al.*, 2008). These results clearly show

that the involvement of *Ghd7* is crucial on both the ROS homeostatis and stress response pathways in rice plants.

2.4.5.2.2 Role of *Ghd7* on regulating various transcriptome networks of vegetation and reproductive organs

OX-*Ghd7*^{HJ19} plants showed variations in MADS box genes expression in rice (Weng *et al.*, 2014). *OsMADS55* which is mainly expressed in grown up leaves suppresses the brassinossteroid (BR) responses which in turn promotes the stem elongation (Lee *et al.*, 2008). This was observed to be up regulated in OX-*Ghd7*^{HJ19} plants. *OsMADS14* and *OsMADS18* genes are known to be induced in the meristem during the period of transition from vegetative to reproductive stage by altering the shoot apical meristem phase into the inflorescence meristem phase (Kobayashi *et al.*, 2012). The expression of these genes has found to be decreased in OX-*Ghd7*^{HJ19} plants. Also, both the *OghoX1* and cytokinin oxidase genes which involved in the hormone metabolism are regulated in OX-*Ghd7*^{HJ19} plants (Weng *et al.*, 2014). Increased levels of *GA2OXS* which leads to decreased plant height, increased root biomass, flowering, production of grains and regulation of tillering (Lo *et al.*, 2008) are negatively regulated in OX-*Ghd7*^{HJ19} plants (Weng *et al.*, 2014). These reveal that *Ghd7* plays a significant role in regulating the transcription machineries in rice.

2.4.5.3 Regulation of Ghd7 expression

In the long day conditions, within the light periods, the *Ghd7* expression is much greater compared with that of the short day conditions and dark periods (Xue *et al.*, 2008). This is achieved through opened gates of *Ghd7* around day-break in long day conditions (Osugi *et al.*, 2011) and these gates are opened with a photo stimulating model which peaks at dawn (Itoh *et al.*, 2010). In short days the *Ghd7* expression was gated at around midnight (Osugi *et al.*, 2011) by altering the photo stimulating model by ten hours before day-break. This indicates that *Ghd7* regulation is manipulated by gated mechanism of phytochrome signaling (Itoh *et al.*, 2010). *Ghd7* expression is relevant to the transcript level and increased *Ghd7* transcript results in delay of flowering, increased plant height and increased grain number (Weng *et al.*, 2014).

More increased levels of *Ghd7* transcript expressed in many transgenic plants caused no flowering while some other plants showed inconspicuous delaying of flowering in rice. Also yield related traits maybe undesirably affected by much higher levels of *Ghd7* (Okada *et al.*,

2017). However, Nemoto *et al.*, (2016) shows that during early seedling development the *Ghd7* transcript level is much greater under long day conditions than that was observed under short day conditions. Osugi *et al.*, (2011) reveals that phyA/phyA heterodimer or the combined effect of phyB and phyC can cause enhanced expression of *Ghd7*. This is accomplished by probably through suppressing *Ehd1* activity. On the other hand, phyB alone reduces the expression of *Ghd7* by boosting the repressor activity of *Ghd7*.

The juvenile tissues of rice plant including apical meristem, developing leaves and the sheaths of leaves are the main tissues in which the gene is expressed (Xue *et al.*, 2008) and in the leaf, it was primarily discovered in the emerged leaf blade, but the pre emerged juvenile leaf blade covered by leaf sheath was noticed to be lack of the gene. Even in the emerged leaf blade, the *Ghd7* transcript was detected in high levels in the leaf tip than that of the leaf base and the transcript level was comparatively even in the leaf blade at the periods of vegetation, reproduction and ripening (Weng *et al.*, 2014). The gene is also expressed in young seedling, the root meristem, the epidermis of growing stems, branched primordial of developing panicles, vascular tissues in fully inflated leaves but weakly expressed in root and vascular tissues of leaf sheath (Xue *et al.*, 2008). This indicates that the expression of *Ghd7* is controlled by many factors.

2.4.5.3.1 Genetic background

The variability of the performance of NILs and transgenic progenies of *Hejiang 19*, *Mudanjiang 8* and *Nipponbare* support the idea that the expression of *Ghd7* depends on the genetic make-up. Comparative sequencing of *Ghd7* alleles reveals that *Minghui 63* has *Ghd7* allele which expresses powerful effects. The allele expressed in *Nipponbare* cultivar exhibits weak effects. *Hejiang 19* and *Mudanjiang 8* have lost the entire function of *Ghd7* allele due to a premature termination thereby considered as nonfunctional. Another particular genotype is found in *Teqing* varieties in which the allele has stronger functions and the *Ghd7* locus of *Zhenshan 97* is deleted (Xue *et al.*, 2008). This indicates that the genetic constitution of a certain variety causes stronger impacts on the activity of *Ghd7* (Okada *et al.*, 2017).

Weng *et al.*, (2014) shows that when the *Zhonghua 11* (*ZH 11*) which has a weak allele of *Ghd7* (Xue *et al.*, 2008) is introduced with the constructs of *Ghd7* over-expression (OX-*Ghd7* ZH11), many of the transformants exhibited late flowering but the plant height and number of spickelets were not considerably increased. Also, when the artificial miRNA

(amiRNA; Ami-*Ghd7*) was introduced, many of the transformants exhibited hasten flowering and a decrease in other traits. When comparing the results with those of the *HJ* 19 transformants, it indicates that the genetic background influences the multiple arrays of traits controlled by *Ghd7*. This idea would be necessary in the development of yield-related traits (Okada *et al.*, 2017) in rice and to select the best genotypes in improving the yield.

2.4.5.3.2 Environmental signals

Cold treatments stimulated the build-up of *Ghd7* mRNA in rice (Weng *et al.*, 2014). *Ghd7* mRNA is much profuse at low temperature under long day conditions with compared to that of the short day conditions and at normal temperatures. Under SD conditions, the level of expressed *Ghd7* mRNA was not changed at low temperatures (23°C) relative to the normal temperature conditions (28°C) (Song *et al.*, 2012). Conversely, *Ghd7* was suppressed by drought, abscisic acid, jasmonic acid and increased temperature conditions. In addition, while biotic and abiotic stresses also regulate the *Ghd7* expression, 1-aminocyclopropane-1-carboxylic acid (ACC) and salicylic acid (SA) treatments are vaguely responsible for the expression of *Ghd7* (Weng *et al.*, 2014). These facts collectively reveal that the *Ghd7* expression is regulated by environmental signals other than the genetic make-up of a particular plant variety.

2.4.5.4 Effect of phytochromes on *Ghd7* regulation

The expression of *Ghd7* is mainly controlled by light signaling which is mediated by phytochromes and the timing of gates (Ogiso *et al.*, 2010). The integration of red light signals in the photoperiodic flowering pathway of rice is mediated by the involvement of phytochromes which in turn regulate *Ghd7* expression (Osugi *et al.*, 2011). Under LD conditions the red light signal stimulates the *Ghd7* expression in the morning through the activity of phytochromes while under SD conditions, this is mediated at the midnight with breaks (Itoh *et al.*, 2010). The flowering of rice is regulated by three types of phytochromes including PhyA, PhyB and PhyC (Takano *et al.*, 2005). The mRNA of *Ghd7* is regulated by PhyA/PhyA homodimer (PhyA alone) and PhyB/PhyC heterodimer signals (Osugi *et al.*, 2011) and PhyB and PhyC are responsible for the repression of flowering in rice (Takano *et al.*, 2005).

PhyB is stable under light (Hirschfeld *et al.*, 1998) and the signaling process through phyB is mediated by red light in rice (McCormac *et al.*, 1993). Shadow signal dependent plant

reactions and the regulation of branching in rice is determined by phytochrome B (PHYB)-TB1 pathway (Kebrom *et al.*, 2006) and in regulation of branching, *Ghd7* essentially act upstream of OsTB1 (Weng *et al.*, 2014). Osugi *et al.*, (2011) reveals that PhyB regulates the activity of *Ghd7* and the PhyB mutant in *ZH11* has been studied to evaluate the significance of PhyB in *Ghd7* pathway in flowering time and development of branches in rice. The results revealed that during the LD conditions, a low level of *Ghd7* was present in PhyB mutant and this result supports the idea that the PhyB controls the *Ghd7* expression (Weng *et al.*, 2014). PhyB controls the *Ghd7* expression either by repressing *Ghd7* or increasing the repressor activity of *Ghd7* (Osugi *et al.*, 2011) and the *Ghd7* acts downstream of phytochrome B in the flowering regulation pathway (Weng *et al.*, 2014). Previous studies also reveal that the timing of the light sensitive phase is not changed by phytochromes (Osugi *et al.*, 2011). This indicates that the phytochromes are required for the proper regulation of *Ghd7* expression.

2.4.5.5 Haplotype variation of *Ghd7* and their geographical distribution

Various types of *Ghd7* functional alleles have varied phenotypic effects on plant height, heading date and number of spikelets per panicle. This will results in diverse phenotypes specifically in above three traits in rice (Lu *et al.*, 2012). *Ghd7*-1 and *Ghd7*-3 are known to be functional alleles which cause late flowering and subsequently giving rise to large panicles and enhanced yield under LD conditions particularly in the regions with long growing seasons. This is accomplished by enabling the rice plant to maximally use the sunlight and temperature. *Ghd7*-3 allele differs from both the *Ghd7*-1 and *Ghd7*-2 by three amino acids which is present in Teqing cultivar and causes moreover similar phenotypic effects in rice plant as *Ghd7*-1 (Xue *et al.*, 2008). It has been found that *Ghd7*-1 (strong allele) is present in indica varieties and *Ghd7*-2 (weak allele) which differs by four amino acids from that of the former is present in japonica varieties (Xue *et al.*, 2008; Lu *et al.*, 2012). Therefore, it is desirable to have the *Ghd7*-1 allele with a fully functional potential in japonica varieties to enhance the yield and *Ghd7*-05SNP markers will be useful to incorporate the allele (Kim *et al.*, 2016).

Certain mutations raised in *Ghd7* locus result in *Ghd7-2* with decreased functions and *Ghd7-0* which is nonfunctional due to deletion of *Ghd7* locus make the plant adaptable in the areas with short growing seasons and temperate regions. Premature termination in the coding region of *Ghd7* gives rise to a mutation resulting *Ghd7-0* a which is nonfunctional and found in regions with short growing seasons and cool summers (Xue *et al.*, 2008). In another study

which has been conducted with 104 cultivars, 12 haplotypes (H0-H11) fall into both indica and japonica groups were identified based on 76 SNPs and 6 indels present in 3932bp region of *Ghd7*. During this study four novel types of proteins correspond to H4, H6, H9 and H11 haplotypes respectively were identified (Lu *et al.*, 2012). Kim *et al.*, (2016) shows the results obtained from analyzing the *Ghd7* sequence of another 6 genotypes using WGS data and the results indicate that ST6 variety has the *Ghd7*-6 allele which is the same as the DNA sequence of fully functional *Ghd7*-1 allele present in *Minghui 63*.

Ten haplotypes of *Ghd7* have been detected among 320 cultivars and 21 haplotypes among 45 wild rice cultivars were recognized which collectively represent the indica and japonica clades based on the results of another study. In addition to *Minghui 63* cultivar representing haplotype1 (H1) and *Nipponbare* cultivar representing haplotype4 (H4), the detected haplotypes of wild rice accessions were found to be resulted neither from frame-shift mutations nor amino acid changes resulting in a premature stop codon. These results reveal that non-functional alleles of *Ghd7* enable the rice plants to be adjusted to the regions with higher latitudes while the alleles found in wild rice make them more adaptable into lower latitude regions enhancing the yield of rice (Zhang *et al.*, 2015).

However, the phenotypic effects caused by different *Ghd7* alleles are counteracted by the effects of the interaction of the *Ghd7* with other related genes in controlling the heading date. This reveals that the indica *Ghd7* allele which is correlated to earlier flowering may have desirable effects on grain filling and thus can be incorporated into japonese high yielding rice (Yonemaru *et al.*, 2014). An array of variations caused by *Ghd7* significantly contributes to the adaptation of rice into different ecological regions and can be easily assigned for genetic improvement studies enhancing the rice yield worldwide (Lu *et al.*, 2012). This is because this variation of alleles is highly considered in breeding programs and those regulating heading date exhibits that the adaptability of rice is mainly controlled by photoperiod sensitivity (Fujino and Iwata, 2011).
3. MATERIALS AND METHODS

3.1 Morphometric analysis

3.1.1 Sample collection

A total of 12 rice genotypes under 3 main yield categories including high, moderate and low yielding were selected. High yielding genotypes are At 362, Bg 300, Bg 366, Bg 90-2 and moderate yielding varieties are At 307, Bg 310, Bg 352, Bw 367. Bg 250, Bw 272-6b, *Pachchaperumal, Suwadhal* were selected as low yielding genotypes (Table 3.1). Four genotypes per each category were obtained from Rice Research and Development Institute (RRDI) Bathalagoda, Sri Lanka.

3.1.2 Seed germination

Rice seeds were soaked in water for 24 hours and were transferred to a wet tissue paper until they were germinated.

3.1.3 Planting and sample collection for DNA extraction

Soil needed for the experiment was collected from a paddy field in *Gampola* area. Collected soil was placed up to about three quarter of each pot and water was added subsequently to a level of about one inch above the soil surface to make paddy field conditions in the pot. Urea, MOP (Muriate of Potash) and TSP (Tripple Super Phosphate) fertilizer were added to the soil in pots before cultivation of rice seedlings. At the very beginning, 10 seeds per each pot and collectively four pots were maintained per each genotype under the greenhouse conditions on the basis of completely randomized design (CRD). After the seedlings were well grown, the healthiest and well grown four plants were selected and the rest of the plants were discarded. The trial was conducted in both *Yala* and *Maha* seasons of the year 2017. Leaf samples were collected from one of the randomly selected plants per each genotype at the age of three weeks and were crushed in liquid nitrogen to get a fine powder and stored at -80 °C until processed for DNA extraction.

Name of the	Type of cultivar*	Natural distribution	Important characteristics
At362	NI	LL	High yielding, red pericarp, moderately resistant to brown plant hopper, blast and bacterial blight, highest
			tolerance for phosphorous deficiency, moderately Fe tolerant (Priyantha <i>et al.</i> , 2013)
Bg300	NI	LL	High yielding, early maturity, high adaptability, resistant to gall midge(Biotype1), brown plant hopper, bacterial
			blight, blast, moderately Fe tolerant (Priyantha et al., 2013; DOA, 2017; RRDI, 2017)
Bg366	NI		High yielding, white pericarp, non-glutinous endosperm, intermediate bold grain type, resistant /moderate
			resistant to blast, resistant to bacterial leaf blight, moderate resistant to gall midge and brown plant hopper
			(Bentota, 2009)
Bg90-2	NI		High yielding (RRDI, 2017)
At307	NI		Moderate yielding, white pericarp, resistant to brown plant hoper, gall midge, and blast, moderately Fe tolerant
			(Priyantha <i>et al.</i> , 2013)
Bg310	NI		Moderate yielding, white pericarp, intermediate bold grain type, resistant or moderate resistant to gall midge and
			brown plant hopper, blast, salt tolerant (DOA, 2015; Hemachandra and Amarasinghe, 2016)
Bg352	NI	LL/UP	Moderate yielding, white pericarp, intermediate bold type grain, earliness, wide adaptability, moderately tolerant
			for phosphorous deficiency, resistant to blast, gall midge (Biotype1), moderate resistant to bacterial leaf blight
			and brown plant hopper, sensitive to Fe toxicity, moderate sensitive to thrips (RRDI, 2017; DOA, 2017)
Bw367	NI	LL	Moderate yielding, white pericarp, highly Fe tolerant (Priyantha <i>et al.</i> , 2013)
Bg250	NI	LL	Low yielding, white pericarp, ultra-short in maturity, resistant/moderate resistant to brown plant hopper and blast,
			highly tolerant to salt, field level of Fe tolerance (Priyantha et al., 2013)
Bw272-6b	NI	LL	Low yielding, red pericarp, resistant to blast, resistant to lodging, highly Fe susceptible (DOA, 2017; Priyantha
			<i>et al.</i> , 2013; RRDI, 2017)
Pachchaperumal	Т	LL	Low yielding, red pericarp, medium grain shape, help control diabetes and cardiovascular diseases, nutritional
			value(IUCN, 2016)
Suwadhal	Т	UL	Low yielding, white pericarp, exquisite taste, fragrant aroma, promote fair and glowing skin, improve the function
			of excretory system, improve vocal clarity, help control diabetes (IUCN, 2016; DOA, 2017)

Table 3.1 Important characteristics and natural distribution of selected 12 rice genotypes

* NI indicates newly improved genotypes
* T indicates the traditional genotypes cultivating in Sri Lanka

3.1.4 Morphological data collection and analysis of data

Vegetative growth parameters including plant height, culm length, number of tillers, leaf blade length and leaf blade width were measured in 16 plants per each genotype at the age of 3,6,9,12,15 in *Yala* and *Maha* seasons since from the establishment in pots. Then the flowering measurements including heading date (days for the first panicle to emerge), flag leaf length, flag leaf width were taken. Harvesting parameters including days to harvest from the initial establishment, yield per plant, seed number per plant, seed length, seed width, seed weight, 100 seed weight and 100 endosperm weight per genotype, length, width of endosperms per each genotype were measured in *Yala* and *Maha* seasons. The length and width measurements of seeds were measured using a vernier caliper. The photographs of plants at flowering, harvesting stages and the panicles, seeds and endosperms were taken and scanned by a canon scanner (CanoScan LIDE 120). The data were subjected to normality test followed by General Linear Model (GLM) procedure and LS means/pdiff mean separation procedures using statistical package SAS 9.1.3 (SAS institute, NC, Cary, USA). Data transformation details using Anderson-Darling normality are given in the Table 3.2.

Measurement type	Week	Parameter	Transformation	Transformation
			method in Maha	method in Yala
		PlH	Square root	Sin
		CL	Log	Log
	3 weeks	NT	Tan	Square
		LBL	Tan	Cos
		LBW	Square	Square root
		PlH	sin	Log
		CL	Log	Log
	6 weeks	NT	Log	Sin
		LBL	Sin	Sin
		LBW	-	Log
		PlH	Log	Log
Vagatativa		CL	Log	Log
vegetative	9 weeks	NT	Square root	Square root
		LBL	Sin	Sin
		LBW	Square	-
		PlH	Log	Cos
		CL	Sin	Sin
	12 weeks	NT	Square root	Sin
		LBL	-	Log
		LBW	Log	Log
		PlH	Square root	-
		CL	Log	-
	15 weeks	NT	Square root	Square root
		LBL	-	-
		LBW	-	-
Flowering		FLL	Log	Log
Tiowening		FLW	Square	-
		Yield	-	-
		SN	Log	Square root
		Seed weight	Square	Square
		SL	Log	Cube
Harvesting		Seed width	Log	Cube
		EL	Cube	Square root
		EW	Sin	Cube
		100 SW	Cube	-
		100 EW	-	-

Table 3.2 Data transformation methods employed for the data of different parameters in Maha and Yala

PlH (Plant height); CL (Culm length); NT (Number of tillers); LBL (Leaf blade length); LBW (Leaf blade width); FLL (Flag leaf length); FLW (Flag leaf width); EL (Endosperm length); EW (Endosperm width); 100 SW (100 Seed weight); 100 EW (100 endosperm weight)

3.2 Genetic analysis

3.2.1 DNA extraction and quantification

DNA was extracted from crushed immature leaf samples from each genotype using Promega DNA extraction kit and manual CTAB method; and was stored at -20 °C. The quality of DNA was determined by 1 % agarose gel electrophoresis.

3.2.2 Amplification of DNA by PCR

Extracted DNA was amplified using 12 DNA primer pairs (Table 3.3). PCR amplification was performed using 1.0 μ L genomic DNA for all the 12 rice genotypes. A total volume of 10 μ L of PCR reaction mixture contained the following. 5 μ L of Go Taq Green Master Mix, containing 2XGreen Go Taq® Reaction buffer (pH 8.5), 400 μ M dATP, 400 μ M dGTP, 400 μ M dTTP and 3 μ M MgCl₂ and in addition 0.5 μ L each forward and reverse primer, nuclease free water and template DNA. The amplification was performed in a thermal cycler (Takara, Otsu Shiga, Japan). Initial denaturation at 94 °C for 5 mins, followed by 35 cycles of 1minute denaturation at 94 °C, 1 min annealing at relevant primer annealing temperatures (Table 3.2), and 2 mins extension at 72 °C followed by a final extension at 72 °C for 10 mins. All the PCR conditions were the same for all the markers except the annealing temperatures for different markers.

3.2.3 Electrophoresis of amplified PCR products

Amplification was followed by gel electrophoresis and the amplified PCR products were resolved by 1.5% agarose gels in $50 \times TAE$ buffer [Tris-HCl (pH 8.0), EDTA, and Glacial acetic acid)] comprising of 2.0µl of ethidium bromide. The molecular weights of the amplified PCR products were compared using a 1kb DNA ladder [Blue/Orange 6x Loading Dye (G190A)] (Madison, USA).

3.2.4 Detection of DNA polymorphism of *Ghd7* locus

The composite gel image was constructed by combining all the gel images with DNA bands corresponding to 12 DNA primer pairs.

3.2.5 Allele scoring

Twelve DNA markers were examined for the genetic polymorphism. Markers with polymorphism were selected for the subsequent allele scoring, excluding the monomorphic markers. The DNA bands obtained from amplified products of polymorphic markers were scored for the presence and the absence of each marker allele with respect to the each rice genotype in a binary matrix. '1' was assigned for the presence of the band and '0' was assigned for the absence of the band.

3.2.6 Cluster analysis of 12 rice genotypes by dendrogram construction

The dendrogram was constructed based on the binary data of all the monomorphic and polymorphic marker alleles with respect to the rice genotype. 12 rice genotypes were grouped into clusters on the basis of the dendrogram using the algorithm of Complete linkage and Euclidean distance in Minitab 17 (Minitab Inc. USA).

3.2.7 Marker-trait association analysis

The data obtained from the binary matrix was subjected to single marker analysis. The correlation between each marker allele and the yield traits including yield, seed number, seed weight, seed length, seed width, endosperm length, endosperm width, 100 seed weight and 100 endosperm weight was analyzed by GLM procedure and LS means/ pdiff mean separation procedures using statistical package SAS 9.1.3.

3.2.8 Cross tabulation and Chi-square test analysis

The statistical association between the yield class and the marker allele was analyzed by Minitab 17 (Minitab Inc. USA) using binary data of the presence and absence of alleles.

Marker	Forward primer (5'→3')	Reverse primer (5'→3')	Ta °C	Reference
K20	TCAGGTGATGGGAATCATTG	TGTTCCAACCAAACAACCTG	55	Chin et al., (2010)
Seq1-2	GCAAGGGGATGTCTAAACGA	AATTTTTGACCGTCGGATTC	53	Lu et al. (2012)
Seq7-8	CATACGGATCCAGCCTCTGT	TTGCAATGATGCGTATTCAC	54	Lu <i>el al.</i> , (2012)
RM1135	AGCCAACCAAGCAAGATAGC	ACACACATGTAAGCCTCCCC	58	
RM5499	TGGAGTACGACGTGATCGTG	CAGAAACGGGAGGGGATC	57	Cromono OTI Detebase (2017)
RM5346	TGCCTCACGATGGTCGAG	CTTCGTCCACCCAATTTGAC	56	Gramene QTL Database, (2017)
RM5436	CAAAGGGGGGTGTCCTCTATG	GTTGCTCGTCCTACATGTGC	57	
G7rq	AGGTGCTACGAGAAGCAAATCC	GGGCCTCATCTCGGCATAG	59	
Hd3a	GCTCACTATCATCATCCAGCATG	CCTTGCTCAGCTATTTAATTGCATAA	56	
LHY	CAGAAAGGCCGACACCAAAC	GGTGTGTTGGAACCACATG	56	Xue et al., (2008)
PRR	CTGCTGAACCTCTGGACCCA	GGTTCCGATAACGCCAACTC	58	
GI	TGGAGAAAGGTTGTGGATGC	GATAGACGGCACTTCAGCAGAT	57	

Table 3.3 Ghd7 (OTL linked DNA markers used for the	haplotype detection

4. RESULTS

4.1 Morphometric parameters in Maha season

4.1.1 Vegetative measurements

4.1.1.1 Plant height

Mean PIH results of the 12 rice genotypes in *Maha* season are shown in Table 4.1. According to the results, there was no significant difference in mean PIH among the 12 rice genotypes at the 3 and 6 weeks age groups since from establishment (Table 4.1, P<0.05). At the age of 9 weeks, *Pachchaperumal* recorded the significantly highest mean PIH (129.24 cm) while it was the lowest for Bg 366, Bg 90-2, At 307 and Bw 367 (78.81 cm, 78.36 cm, 76.10 cm, 76.84 cm respectively). At the 12 weeks age, *Pachchaperumal* reached the significant highest mean PIH (136.8 cm) whereas the lowest PIH was recorded by Bg 366, At 307, Bw 367 and Bg 250 (85.39 cm, 82.15 cm, 86.30 cm, 84.61 cm respectively) (Table 4.1, *P*<0.05). At the age of 15 weeks, out of the four genotypes, still the significant highest mean PIH was recorded by *Pachchaperumal* (137.29 cm) whereas Bg 90-2 and Bw 367 showed the lowest mean PIHs (90.92 cm, 86.77 cm respectively) (Table 4.1, *P*<0.05).

4.1.1.2 Culm length and Number of tillers

Mean CL results in *Maha* season are shown in the (Table 4.1). At the age of 3 weeks, the significant highest mean CL was recorded by *Pachchaperumal* and *Suwadhal* (22.46 cm, 22.03 cm respectively), while there was no significant difference among the other genotypes (Table 4.1, P < 0.05). At the age of 6 weeks, the results were the same as those were at the 3 weeks age. Still the highest mean CL was recorded by *Pachchaperumal* and *Suwadhal* (29.78 cm, 29.59cm respectively). At the 9 weeks age, *Pachchaperumal* mean CL was significantly high (88.04 cm) with compared to the other genotypes and conversely it was the lowest for Bw 367 (28.69 cm) (Table 4.1, P < 0.05). At the age of 12 weeks, the highest CL results were recorded by *Pachchaperumal* (92.24 cm) and *Suwadhal* (83.17 cm). At 15 weeks age, *Pachchaperumal* recorded the significant highest mean CL (101.24 cm) while Bg 90-2 and Bw 367 recorded the lowest (59.61 cm, 59.37 cm respectively) (Table 4.1, P < 0.05). However, there was no significant difference in mean NT among the 12 rice genotypes at all age groups in *Maha* season (Table 4.1, P < 0.05).

		Mean	plant heig	ht (cm)			Mean	culm leng	th (cm)			Mean n	umber of	tillers	
Variety						Ag	e (weeks)	since esta	blishment	t					
	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
At362	41.41 ^a	72.38ª	85.57°	95.59 ^d	-	16.22 ^b	23.03 ^b	39.65 ^e	64.80 ^b	-	1.63ª	3.65 ^a	3.82ª	2.88ª	-
Bg300	45.76^{a}	72.21ª	83.33°	92.13 ^d	-	17.85 ^b	23.21 ^b	52.08 ^d	68.64 ^b	-	2.63 ^a	5.45 ^a	4.63 ^a	3.53 ^a	-
Bg366	47.61 ^a	68.28ª	78.81 ^d	85.39 ^e	-	17.40 ^b	21.45 ^b	37.53 ^e	59.38 ^b	-	2.31ª	4.58 ^a	4.09 ^a	3.11 ^a	-
Bg90-2	44.62 ^a	67.71ª	78.36 ^d	90.28 ^d	90.92 ^c	18.00 ^b	22.11 ^b	34.82 ^e	59.14 ^b	59.61°	2.19 ^a	4.75 ^a	4.30 ^a	3.33 ^a	2.81ª
At307	41.74 ^a	70.86 ^a	76.10 ^d	82.15 ^e	-	16.51 ^b	23.42 ^b	42.34 ^e	60.09 ^b	-	1.87^{a}	3.80 ^a	3.41 ^a	2.78 ^a	-
Bg310	46.30 ^a	73.88 ^a	89.21°	95.30 ^d	-	18.96 ^b	23.17 ^b	59.97°	67.13 ^b	-	2.19 ^a	3.89 ^a	3.33 ^a	2.40 ^a	-
Bg352	40.51 ^a	65.94ª	83.98°	93.05 ^d	-	16.01 ^b	20.66 ^b	53.56 ^d	66.46 ^b	-	2.06 ^a	4.79 ^a	4.40^{a}	2.91ª	-
Bw367	41.16 ^a	65.60 ^a	76.84 ^d	86.30 ^e	86.77 ^c	16.30 ^b	20.92 ^b	28.69^{f}	58.73 ^b	59.37°	1.87^{a}	4.52 ^a	4.54 ^a	3.85 ^a	3.06 ^a
Bg250	37.09 ^a	69.26 ^a	84.08 ^c	84.61 ^e	-	15.91 ^b	22.79 ^b	61.94 ^c	62.37 ^b	-	2.19 ^a	5.10 ^a	4.76 ^a	3.31 ^a	-
Bw272-6b	44.56 ^a	77.06 ^a	89.66 ^c	102.66 ^c	-	15.10 ^b	22.48 ^b	42.88 ^e	70.93 ^b	-	1.82 ^a	3.97ª	3.86 ^a	3.37ª	-
Pachchaperumal	59.25ª	83.36 ^a	129.24ª	136.80 ^a	137.29 ^a	22.46 ^a	29.78ª	88.04 ^a	92.24ª	101.24 ^a	1.82 ^a	3.54 ^a	3.77 ^a	2.72 ^a	2.35 ^a
Suwadhal	53.21ª	80.20 ^a	122.94 ^b	123.82 ^b	124.80 ^b	22.03 ^a	29.59 ^a	79.30 ^b	83.17 ^a	87.00 ^b	1.94 ^a	3.47 ^a	3.69 ^a	3.04 ^a	3.39 ^a

Table 4.1 The variation of mean plant height, mean culm length and mean number of tillers of 12 rice genotypes in Maha season



Figure 4.1 Morphological appearances of rice genotypes at the flowering stage in *Maha* **season under greenhouse conditions.** The topmost four genotypes including At 362, Bg 300, Bg 366 and Bg 90-2 are high yielding genotypes. Moderate yielding genotypes are At 307, Bg 310, Bg 352 and Bw 367. Low yielding four genotypes including Bg 250, Bw 272-6b, *Pachchaperumal* and *Suwadhal* are displayed at the bottom of the Figure. At the flowering stage, the highest PIH was recorded by *Pachchaperumal* and Bg 366, At 307 and Bw 367 recorded the lowest PIH.

4.1.1.3 Leaf blade length (LBL) and leaf blade width (LBW)

The mean LBL of the 12 rice genotypes in *Maha* season are compared in Table 4.2. According to the results, *Pachchaperumal* recorded the significant highest mean LBL (39.26 cm) and *Suwadhal* recorded the next highest mean LBL (33.66 cm) at the age of 3 weeks whereas there was no significant difference among the other genotypes at the age of 3 weeks (Table 4.2, P<0.05). At the age of 6 weeks, the significant highest mean LBL was recorded by both *Pachchaperumal* (58.04 cm) and *Suwadhal* (58.34 cm). However there was no significant difference among the rest of the genotypes as it was in the previous stage (Table 4.2, P<0.05). At the age of 9 weeks, still the significant highest mean LBL was obtained for *Suwadhal* (59.58 cm) and *Pachchaperumal* (59.13 cm) in contrast to the other genotypes. At the age of 12 weeks, there was no significant difference among all the genotypes (Table 4.2, P<0.05).

When considering LBW data, there was no significant difference at the age of 3 weeks among the genotypes (Table 4.2, P < 0.05). By contrast, at the age of 6 weeks, *Pachchaperumal* recorded the significant lowest mean LBW (0.92 cm) while no significant difference was shown among the rest of the genotypes (Table 4.2, P < 0.05). At the age of 9 weeks, the significant highest mean LBW was recorded by both Bg 352 (1.38 cm) and Bg 250 (1.35 cm) whereas *Pachchaperumal* recorded the lowest (0.96 cm). There was no significant difference in the results among the 12 rice genotypes at the age of 12 weeks (Table 4.2, P < 0.05).

		Ν	Iean leaf blad	de length (cm))		I	Mean leaf bla	de width (cm)	1
Variety				А	ge (weeks)	since establis	hment			
	3	6	9	12	15	3	6	9	12	15
At362	24.77°	48.20 ^b	48.99 ^c	50.60 ^a	-	0.70^{a}	1.22 ^a	1.22 ^b	1.29 ^a	-
Bg300	27.83°	47.58 ^b	48.55 ^c	51.10 ^a	-	0.72 ^a	1.16 ^a	1.16 ^b	1.22 ^a	-
Bg366	28.80 ^c	45.84 ^b	46.81°	47.28 ^a	-	0.74 ^a	1.14 ^a	1.13 ^b	1.20 ^a	-
Bg90-2	28.31 ^c	44.27 ^b	45.50 ^c	45.50 ^a	-	0.79 ^a	1.20 ^a	1.22 ^b	1.26^{a}	-
At307	26.40 ^c	43.67 ^b	46.06 ^c	42.79 ^a	-	0.83ª	1.29 ^a	1.29 ^b	1.32 ^a	-
Bg310	30.21°	49.69 ^b	50.33°	49.30 ^a	-	0.77 ^a	1.29 ^a	1.27 ^b	1.20 ^a	-
Bg352	25.13°	43.42 ^b	43.96°	43.58 ^a	-	0.77 ^a	1.41 ^a	1.38 ^a	1.42 ^a	-
Bw367	25.33°	42.77 ^b	43.91°	43.50 ^a	-	0.73 ^a	1.23 ^a	1.23 ^b	1.25 ^a	-
Bg250	24.41°	44.12 ^b	45.20 ^c	39.25ª	-	0.72 ^a	1.35 ^a	1.35 ^a	1.35 ^a	-
Bw272-6b	28.99°	51.85 ^b	54.00 ^b	55.00 ^a	-	0.63 ^a	1.22 ^a	1.21 ^b	1.14 ^a	-
Pachchaperumal	39.26 ^a	58.04 ^a	59.13ª	48.00 ^a	-	0.66ª	0.92 ^b	0.96°	0.90 ^a	-
Suwadhal	33.66 ^b	58.34 ^a	59.58ª	51.40 ^a	-	0.73 ^a	1.27 ^a	1.24 ^b	1.00 ^a	-

 Table 4.2 The variation of mean leaf blade length and mean leaf blade width of 12 rice genotypes in Maha season

4.1.2 Flowering measurements

In *Maha* season, results of heading date (days to flowering), FLL and FLW are shown in Table 4.2. Bw 367 showed the slowest flowering (79 days) whereas Bg 250 and *Suwadhal* showed the fastest flowering (54 days) (Table 4.2, P<0.05). The significant highest FLL was recorded by *Suwadhal* and *Pachchaperumal* (35.35 cm, 34.06 cm respectively) and At 362 and Bg 90-2 recorded the next highest values (28.19 cm, 29.59 cm respectively). Whereas no significant difference was recorded among the other genotypes studied (Table 4.2, P<0.05). When considering FLW results, At 307, Bg 310, Bg 352, Bw 367 and Bg 250 recorded the significant highest mean values (1.7 cm, 1.61 cm, 1.65 cm, 1.7 cm, and 1.55 cm). Conversely that was the lowest for *Pachchaperumal* (1.1 cm) (Table 4.2, P<0.05). The morphological appearances of rice genotypes at the flowering stage are shown in Figure 4.1.

4.1.3 Harvesting measurements

In *Maha* season, all the harvesting measurements including number of days to harvest, SN, SL, seed width, seed weight, yield per plant, 100 SW, 100 EW, EL and EW are shown in Table 4.3 (P<0.05). When considering days to harvest, the highest number of days was recorded by Bg 90-2, Bw 367, Bg 250, *Pachchaperumal* and *Suwadhal* (121 days) while the rest of the other genotypes recorded comparatively less number of days to harvest (105 days) (Table 4.3, P<0.05). Mean SN was not significantly different among the 12 rice genotypes (Table 4.3, P<0.05). The significant highest SL was shown by At 362 (0.89 cm), Bg 90-2 (0.88 cm) and Bg 250 (0.88 cm). On the contrary, both the *Suwadhal* and Bw 272-6b showed the significant lowest mean SL (0.62 cm) (Table 4.3, P<0.05). When comparing the seed width data in *Maha* season, it shows that Bg 310 got the significant highest mean seed width (0.4 cm) (Table 4.3, P<0.05). According to the seed weight results, it can be clearly seen that Bg 310 and Bg 250 recorded the significant highest mean seed weight (0.4 g). By contrast, it was the lowest for Bg 90-2, Bw 367, Bw 272-6b and *Suwadhal* (0.29 g, 0.28 g, 0.29 g, 0.28 g respectively) (Table 4.3, P<0.05).

No significant difference was recorded among the genotypes for the overall mean yield with the exception of *Suwadhal* which showed comparatively a low mean yield (3.77 g) (Table 4.3, P<0.05). Bg 90-2 recorded the highest mean 100 SW (2.85 g) and *Suwadhal* recorded the lowest (1.5 g) in comparison with the other genotypes (Table 4.3, P<0.05). The highest mean 100 EW was recorded by Bg 310 (2.39 g) and that was the lowest in Bg 352 (1.16 g) (Table 4.3, P<0.05). According to the obtained results, Bg 90-2 recorded the significant

highest mean EL (0.65 cm). Conversely, Bw 367, Bw 272-6b and *Suwadhal* recorded the lowest EL (0.41 cm, 0.43 cm, 0.42 cm) (Table 4.3, P < 0.05). On the other hand, the EW of them was not much different. Bg 352 and *Pachchaperumal* recorded the significant highest mean EW (0.29 cm). By contrast, there was no significant difference among the rest of the other genotypes (Table 4.3, P < 0.05). The morphological appearances of rice genotypes at the harvesting stage are shown in Figure 4.2. The phenotypic variation of panicles is shown in Figure 4.3. The morphological variability of seeds and that of the endosperms are displayed in Figure 4.4.

				- F		N.	lean data(cr	n, g)					
Variety	Heading date	Flag leaf length	Flag leaf width	Days to harvest	Seed number	Seed length	Seed width	Seed weight	Yield per plant	100 seed weight	100 Endosperm weight	Endosperm length	Endosperm width
At362	71	28.19 ^b	1.44 ^b	105	231.95 ^a	0.89 ^a	0.28 ^c	0.33 ^d	5.01 ^a	2.56	2.22	0.57 ^c	0.25 ^b
Bg300	62	23.47°	1.44 ^b	105	252.23ª	0.81 ^b	0.30 ^b	0.19 ^g	5.83 ^a	2.48	2.00	0.58°	0.26 ^b
Bg366	62	24.59°	1.42 ^b	105	224.80 ^a	0.75°	0.27°	0.36 ^c	5.05 ^a	2.36	1.92	0.57°	0.26 ^b
Bg90-2	72	29.59 ^b	1.38 ^b	121	219.08 ^a	0.88^{a}	0.27 ^c	0.29^{f}	6.02 ^a	2.85	2.31	0.65 ^a	0.26 ^b
At307	66	22.54°	1.70 ^a	105	258.70 ^a	0.73°	0.31 ^b	0.19 ^g	5.22 ^a	2.14	1.75	0.51 ^d	0.27 ^b
Bg310	61	25.60°	1.61 ^a	105	179.89ª	0.80^{b}	0.40^{a}	0.40^{a}	4.92 ^a	2.73	2.39	0.59°	0.27 ^b
Bg352	63	24.61 ^c	1.65 ^a	105	198.84 ^a	0.77 ^c	0.31 ^b	0.31 ^e	4.89 ^a	2.62	1.16	0.56 ^c	0.29 ^a
Bw367	79	25.03°	1.70 ^a	121	348.02 ^a	0.58 ^e	0.28 ^c	0.28^{f}	5.46 ^a	1.60	1.27	0.41 ^e	0.26 ^b
Bg250	54	22.24 ^c	1.55 ^a	121	201.79 ^a	0.88^{a}	0.30 ^b	0.40^{a}	5.09 ^a	2.71	2.22	0.64 ^b	0.25 ^b
Bw272-6b	66	29.83°	1.34 ^b	105	304.37 ^a	0.62 ^d	0.27°	0.29^{f}	4.74 ^a	1.78	1.41	0.43 ^e	0.24 ^b
Pachchaperumal	55	34.06 ^a	1.10 ^c	121	190.81 ^a	0.77 ^c	0.30 ^b	0.38 ^b	4.94 ^a	2.70	2.08	0.55 ^c	0.29 ^a
Suwadhal	54	35.35ª	1.32 ^b	121	258.35 ^a	0.62 ^d	0.26 ^c	0.28^{f}	3.77 ^b	1.50	1.44	0.42 ^e	0.25 ^b

Table 4.3 The variation of mean data for yield related parameters for 12 rice genotypes in Maha season



Figure 4.2 Morphological appearances of rice genotypes at the harvesting stage in *Maha* **season under greenhouse conditions.** The highest PlH at the harvesting stage was recorded by *Pachchaperumal* while it was the lowest for Bg 366, At 307 and Bw 367 genotypes.



Figure 4.3 Morphological variability of dried panicles of 12 rice genotypes in *Maha* season under greenhouse conditions. Panicles of *Suwadhal*, *Pachchaperumal* were reddish-brown color and brownish color in appearance respectively. They can be distinguished from the rest of the genotypes which are pale-brown color in appearance.



Figure 4.4 Morphological variance of rice seeds (left) and endosperms (right) of 12 rice genotypes in *Maha* **season under greenhouse conditions.** Seeds of *Suwadhal* are golden brown color and small in size making it possible to distinguish from the other genotypes. Bw 367, At 307 and Bg 250 genotypes have relatively pale brown color seeds. Bg 90-2, Bg 250 and At 362 have long and slender seeds while Bw 367 has small round shaped seeds. The other genotypes are moreover similar in the appearance. Endosperms of At 362, Bw 272-6b and *Pachchaperumal* are reddish brown color and those of the At 362 and Bg 90-2 are long and slender. All the other genotypes have a whitish endosperm. Endosperms of Bw 367 are round and comparatively small. The rest of the other genotypes look alike.

4.2 Morphometric parameters in Yala season

4.2.1 Vegetative measurements

4.2.1.1 Plant height

The PIH variability among 12 rice genotypes at all the age groups in *Yala* season is given in the Table 4.4 (P<0.05). At the age of 3 weeks in *Yala* season, *Pachchaperumal* recorded the significant highest mean PIH (23.46 cm) while Bw 272-6b recorded the lowest (16.58 cm) (Table 4.4, P<0.05). At the age of 6, 9 and 12 weeks, *Suwadhal* recorded the significant highest mean PIH (123.45 cm, 150.51 cm, and 151.77 cm respectively) (Table 4.4, P<0.05).

4.2.1.2 Culm length and number of tillers

At the age of 3 weeks, *Pachchaperumal* recorded the significant highest mean CL (0.14 cm) whereas it was the lowest for Bw 272-6b (0.08 cm) (Table 4.4, P<0.05). By contrast, at the age of 6 weeks *Suwadhal* recorded the significant highest mean CL (42.27 cm) while both the *Pachchaperumal* and *Suwadhal* showed the significant highest CL at the age of 9 weeks (89.85 cm, 82.56 cm respectively) (Table 4.4, P<0.05). At the age of 12 weeks, *Suwadhal* recorded the significant highest mean CL (82.12 cm). On the contrary, Bg 366 and Bg 90-2 recorded the lowest (60.19 cm and 62.97 cm respectively) (Table 4.4, P<0.05). There was no significant difference in mean NT among all the 12 rice genotypes at all age groups in *Yala* season (Table 4.4, P<0.05).

Variety		Mean	plant heig	nt (cm)		Mean culm length (cm)						Mean number of tillers			
						Ag	e (weeks)	since esta	ablishmen	t					
	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
At 362	20.02 ^c	87.25 ^d	102.63 ^c	107.92ª	-	19.99°	30.18 ^c	42.53 ^d	71.41 ^b	-	2.46 ^a	3.81 ^a	3.32ª	3.31ª	-
Bg 300	19.67°	84.26 ^d	94.33°	98.64 ^a	-	19.64 ^c	28.67°	61.86 ^b	66.75 ^b	-	3.25 ^a	3.37 ^a	2.90 ^a	3.07 ^a	-
Bg 366	18.20 ^c	85.62 ^d	94.09 ^c	99.75 ^a	-	18.13 ^c	27.49 ^c	52.57°	60.19 ^c	-	2.78 ^a	3.06 ^a	3.33ª	3.12 ^a	-
Bg 90-2	18.61 ^c	85.99 ^d	93.36 ^c	104.18^{a}	104.88	18.58 ^c	27.94 ^c	39.50 ^d	62.97 ^c	64.19	2.84 ^a	3.62 ^a	2.90 ^a	2.75 ^a	2.58
At 307	18.52 ^c	87.14 ^d	89.04 ^c	94.58 ^a	-	18.48 ^c	29.60 ^c	61.29 ^b	66.48 ^b	-	2.50 ^a	3.37 ^a	2.76 ^a	2.50 ^a	-
Bg 310	20.33 ^c	80.58 ^d	95.77°	102.75 ^a	-	20.16 ^c	27.51°	61.55 ^b	67.96 ^b	-	2.55 ^a	2.92ª	2.89 ^a	2.82 ^a	-
Bg 352	18.99 ^c	83.61 ^d	107.26 ^c	113.58 ^a	-	18.96 ^c	27.72 ^c	64.79 ^b	72.31 ^b	-	2.67 ^a	3.50 ^a	3.16 ^a	3.27 ^a	-
Bw 367	18.97°	80.11 ^d	87.55°	98.91ª	-	18.04 ^c	28.02 ^c	39.65 ^d	69.16 ^b	-	2.69 ^a	3.19 ^a	2.98ª	2.93ª	-
Bg 250	19.03 ^c	83.87 ^d	86.39°	-	-	18.96 ^c	29.17°	54.31°	-	-	3.16 ^a	4.00 ^a	3.92ª	-	-
Bw 272-6b	16.58 ^d	95.59°	106.96 ^c	118.03 ^a	-	16.43 ^d	28.39 ^c	49.16 ^c	72.59 ^b	-	2.29 ^a	3.06 ^a	3.26 ^a	3.37 ^a	-
Pachchaperumal	23.46 ^a	110.38 ^b	132.59 ^b	133.92 ^b	-	23.37 ^a	35.04 ^b	89.85 ^a	77.62 ^b	-	2.52 ^a	3.19 ^a	2.84 ^a	2.93 ^a	-
Suwadhal	21.67 ^b	123.45 ^a	150.51ª	151.77°	-	21.58 ^b	42.27 ^a	82.56 ^a	82.12 ^a	-	2.59 ^a	3.73 ^a	2.27 ^a	2.29 ^a	-

Table 4.4 Variation of mean plant height, mean culm length and mean number of tillers of 12 rice genotypes in Yala season



Figure 4.5 Morphological appearances of rice genotypes at the flowering stage in *Yala* season under greenhouse conditions. The highest PlH was recorded by *Suwadhal*. *Pachchaperumal* recorded the next highest PH. The other rice genotypes recorded comparatively similar plant heights.

4.2.1.3 Leaf blade length and leaf blade width

At the age of 3 weeks *Pachchaperumal* recorded the significant highest mean LBL (47.78 cm). LBL of *Suwadhal* was also comparatively higher than that of the other genotypes (43.61 cm). There was no significant difference among the rest of the genotypes (Table 4.5, P<0.05). At the age of 6 weeks both the *Pachchaperumal* (66.07 cm) and *Suwadhal* (65.39 cm) recorded the significant highest mean LBL in contrast to the others (Table 4.5, P<0.05). However, *Suwadhal* was found to have the significantly highest mean LBL at the age of 9 weeks and 12 weeks (78.91 cm, 81.57 cm) (Table 4.5, P<0.05). When considering the LBW data, it was observed that only the *Pachchaperumal* had the significant lowest mean LBW (0.943 cm) whereas no significant difference was recorded among the rest of the genotypes at the age of 3 weeks (Table 4.5, P<0.05). On the contrary, at the age of 6 and 9 weeks, *Pachchaperumal* recorded the significant lowest LBW (1.07 cm, 1.1 cm respectively) whereas there was no significant difference was observed at the age of 12 weeks among the 12 genotypes (Table 4.5, P<0.05).

		Μ	lean leaf blac	le length (cm)			Ν	Mean leaf blade width (cm)			
Variety				Age	(weeks) sin	nce establishn	nent				
	3	6	9	12	15	3	6	9	12	15	
At362	35.12 ^a	50.69 ^b	57.53°	60.95 ^b	-	0.97 ^a	1.25 ^a	1.30 ^a	1.42 ^a	-	
Bg300	33.60 ^a	50.68 ^b	56.80°	57.66 ^b	-	0.96 ^a	1.38 ^a	1.36 ^a	1.23 ^a	-	
Bg366	29.91 ^a	54.25 ^b	56.97°	62.61 ^b	-	0.95 ^a	1.28 ^a	1.30 ^a	1.16^{a}	-	
Bg90-2	37.01 ^a	50.45 ^b	55.56°	58.05 ^b	-	0.98 ^a	1.36 ^a	1.36 ^a	1.24 ^a	-	
At307	34.35 ^a	50.28 ^b	56.47°	58.22 ^b	-	0.97^{a}	1.41 ^a	1.41 ^a	1.31 ^a	-	
Bg310	33.25 ^a	48.82 ^b	52.47°	54.34 ^b	-	0.96 ^a	1.16 ^a	1.20 ^b	1.15 ^a	-	
Bg352	31.05 ^a	50.42 ^b	55.81°	57.30 ^b	-	0.98^{a}	1.35 ^a	1.39 ^a	1.38 ^a	-	
Bw367	32.83ª	47.54 ^b	52.06 ^c	51.14 ^b	-	0.97^{a}	1.32 ^a	1.34 ^a	1.25 ^a	-	
Bg250	36.51 ^a	50.45 ^b	53.62°	-	-	0.98 ^a	1.44 ^a	1.42 ^a	-	-	
Bw272-6b	35.31 ^a	56.27 ^b	66.09 ^b	62.68 ^b	-	0.92 ^b	1.20 ^a	1.29 ^a	1.19 ^a	-	
Pachchaperumal	37.78°	66.07 ^a	69.79 ^b	-	-	0.94 ^a	1.07 ^b	1.10 ^c	-	-	
Suwadhal	43.61 ^b	65.39 ^a	78.91ª	81.57 ^a	-	0.95 ^a	1.40^{a}	1.46 ^a	1.41 ^a	-	

Table 4.5 Variation of mean leaf blade length and mean leaf blade width of 12 rice genotypes in Yala season

4.2.2 Flowering measurements

In *Yala* season, results of heading date, FLL and FLW measurements are shown in Table 4.6. According to the results, the slowest flowering was observed by At 362 (78 days) while the fastest flowering was observed by Bg 250 (52 days) (Table 4.6, P<0.05). The significant highest mean FLL was recorded by *Suwadhal* (54.11 cm) while there was no significant difference among the rest (Table 4.6, P<0.05). By contrast, the significant highest mean FLW was recorded by At 307 (1.9 cm), whereas no significant differences were recorded by the rest of the genotypes (Table 4.6, P<0.05). The morphological appearances of rice genotypes at flowering stage are shown in Figure 4.5.

4.2.3 Harvesting measurements

In *Yala* season, all the seed and endosperm related measurements are given in the Table 4.6. Mean SN was not significantly different among the 12 rice genotypes (Table 4.6, P<0.05). By contrast, there was a significant difference of the SL among the genotypes. The highest SL was recorded by Bg 90-2 (0.92 cm) while the lowest SL was recorded by *Suwadhal* (0.57 cm) (Table 4.6, P<0.05). It also shows that the two genotypes including At 307 and *Pachchaperumal* have the significant highest seed width (0.30 cm). On the contrary, *Suwadhal* recorded the lowest seed width (0.23 cm) (Table 4.6, P<0.05). The results obtained for seed weight measurement shows that At 362 has the highest seed weight (0.24 g) while it is the lowest for Bw 367 and Bg 250 (0.01 g and 0.06 g respectively) (Table 4.6, P<0.05).

Yield per plant was not significantly different among the 12 rice genotypes (Table 4.6, P<0.05). The highest 100 SW was recorded by Bg 90-2 (3.06 g) while it was the lowest for *Suwadhal* (1.30 g) (Table 4.6, P<0.05). When considering the endosperm measurements, significantly highest 100 EW was recorded by Bg 90-2 (2.42 g) while *Suwadhal* recorded the lowest (1.1 g). Bg 90-2 recorded the significantly highest EL (0.67 cm) in comparison with the other genotypes. Conversely, Bw 367, Bw 272-6b and *Suwadhal* recorded the significantly lowest EL (0.42 cm, 0.19cm and 0.40 cm respectively) (Table 4.6, P<0.05). The highest EW was recorded by *Pachchaperumal* (0.29 cm) while there was no significant difference among the rest of the genotypes (Table 4.6, P<0.05). The morphological appearances of rice genotypes at harvesting stage are shown in Figure 4.6. The phenotypic variation of panicles is shown in Figure 4.7. The variability of the external appearance of rice seeds and that of the endosperms are displayed in the Figure 4.8.

	Mean data (cm or g)												
Variety	Heading date	Flag leaf length	Flag leaf width	Days to harvest	Yield per plant	Seed number	Seed length	Seed width	Seed weight	100 seed weight	100 Endosperm weight	Endosperm length	Endosperm width
At362	78	32.59 ^b	1.33 ^b	135	7.31ª	278.64ª	0.89 ^b	0.26 ^d	0.24 ^a	2.63	2.17	0.42 ^b	0.23 ^b
Bg300	65	29.38 ^b	1.56 ^b	130	4.74^{a}	193.10 ^a	0.79 ^e	0.26 ^c	0.21 ^b	2.63	1.89	0.56^{d}	0.25 ^b
Bg366	65	39.71 ^b	1.57 ^b	117	4.91 ^a	213.49 ^a	0.56 ^g	0.26 ^c	0.17 ^d	2.28	1.71	0.55 ^d	0.23 ^b
Bg90-2	76	36.92 ^b	1.44 ^b	117	5.37 ^a	199.82ª	0.92ª	0.27°	0.23 ^b	3.06	2.42	0.67^{a}	0.24 ^b
At307	66	29.54 ^b	1.90 ^a	103	4.79 ^a	227.48 ^a	0.73 ^h	0.30 ^a	0.19 ^c	2.32	1.54	0.50 ^e	0.27 ^b
Bg310	62	31.54 ^b	1.48 ^b	117	4.69 ^a	115.28 ^a	0.83 ^d	0.28 ^b	0.16 ^d	2.64	2.14	0.60 ^c	0.26 ^b
Bg352	63	36.94 ^b	1.61 ^b	117	6.56ª	243.82ª	0.77^{f}	0.28 ^b	0.14 ^e	2.67	1.95	0.56 ^d	0.27 ^b
Bw367	74	26.97 ^b	1.65 ^b	135	5.84 ^a	350.63ª	0.59 ^j	0.27 ^c	0.01^{f}	1.71	1.28	0.42^{f}	0.25 ^b
Bg250	52	26.73 ^b	1.50 ^b	135	4.68^{a}	162.93ª	0.86 ^c	0.25 ^d	0.06^{f}	2.45	2.09	0.61°	0.22 ^b
Bw272-6b	68	41.67 ^b	1.44 ^b	117	6.21ª	358.17 ^a	0.64 ⁱ	0.26 ^c	0.13 ^e	1.64	1.28	0.19^{f}	0.24 ^b
Pachchaperumal	55	41.67 ^b	1.24 ^b	103	4.59 ^a	201.71 ^a	0.77^{f}	0.30 ^a	0.23 ^b	2.62	1.85	0.55 ^d	0.29 ^a
Suwadhal	61	54.11ª	1.33 ^b	117	3.56 ^a	242.69 ^a	0.57 ^k	0.23 ^e	0.11 ^e	1.30	1.10	0.40^{f}	0.22 ^b

Table 4.6 The variation of mean data of 12 rice genotypes after harvesting in Yala season



Figure 4.6 Morphological appearances of rice genotypes at the harvesting stage in *Yala* **season under greenhouse conditions.** The highest PIH at the harvesting stage was recorded by *Suwadhal* while it was the lowest for At 307.



Figure 4.7 Morphological variance of dried panicles of rice genotypes in *Yala* **season under greenhouse conditions.** The panicles of *Suwadhal*, *Pachchaperumal* were reddish-brown color and brownish color in appearance respectively. They can be distinguished from the rest of the genotypes which are pale-brown color in appearance.



Figure 4.8 Morphological variance of rice seeds (left) and endosperms (right) of 12 rice genotypes in *Yala* **season under greenhouse conditions.** Seeds of *Suwadhal* are golden brown color and small in size making it possible to distinguish from the other genotypes. Bw 367, At 307 and Bg 250 genotypes have relatively pale brown color seeds. Bg 90-2, Bg 250 and At 362 have long and slender seeds while Bw 367 has small round shaped seeds. The other genotypes are moreover similar in the appearance. Endosperms of At 362, Bw 272-6b and *Pachchaperumal* are reddish brown color and those of the At 362 and Bg 90-2 are long and slender. All the other genotypes have a whitish endosperm. Endosperms of Bw 367 are round and comparatively small. The rest of the other genotypes look alike.

4.3 Genetic analysis

4.3.1 Detection of polymorphism of Ghd7 locus of 12 rice genotypes

A total of 12 DNA markers for a total of 12 rice genotypes were detected with 20 alleles with an average of 1.67 alleles per locus (Figure4.9). Among the 12, six markers (*RM5346*, *RM5436*, *Seq1-2*, *Seq7-8*, *G7rq* and *GI*) were polymorphic while the rest of the other six markers (*RM5499*, *RM1135*, *Hd3a*, *LHY*, and *PRR*) were monomorphic. All the 12 markers gave successful amplifications with the exception of *GI* locus for which null alleles were detected in Bg 90-2, Bg 352 and Bw 367 rice genotypes. The number of alleles detected per locus was in the range between 1-3. Minimum number of alleles (one) was detected at six monomorphic loci. Two alleles were detected at four loci including *RM5436*, *RM5346*, *Seq7-8* and *G7rq*. The highest polymorphism was detected for *GI* marker and *Seq1-2*, which amplified a total of three alleles.

The PCR product size for different loci varied from 200bp – 900bp. For a particular locus, the difference of molecular bp size between the smallest and largest allele was in the range of 8 to 305 (Figure 4.9). The ten marker haplotypes of the *Ghd7* QTL in 12 rice genotypes are shown in the Figure 4.10. The ten haplotypes were identified using the haplotype of Bg 90-2 – the most high yielding genotype which was used as the reference haplotype. High yielding Bg 366, moderate yielding Bg 310 and low yielding Bw 272-6b were belonged to the same haplotype.



Figure 4.9 The DNA polymorphism of 12 markers linked to *Ghd7* **QTL in 12 rice genotypes.** The name of the marker is shown at the left side of the figure and the size of the band corresponding to each marker allele is displayed at the right side. The names of the 12 rice genotypes are given at the top of the Figure. The bottommost marker is *K20* marker which is universal for all rice genotypes. Only six markers including *RM5436*, *RM5346*, *Seq1-2*, *Seq7-8*, *G7rq* and *GI* out of the 12 markers were detected with polymorphism. The number of alleles detected per locus was in the range between 1-3. The size of the band for each allele was varied between 200bp and 900bp.



Figure 4.10 Ghd7 linked marker haplotypes of the 12 rice genotypes. The diagram shows the relative allelic arrangement of 11 DNA markers at Ghd7 locus on chromosome 7 with respect to the band size and their polymorphism among the 12 rice genotypes. K20 marker is excluded in this Figure as it is located in chromosome 12 and hence not linked to Ghd7. The haplotypes among the 12 rice genotypes were identified using the haplotype of Bg 90-2, the highest yielding genotype, which was used as the reference haplotype. Large rectangular shapes represent the region of the Ghd7 QTL on chromosome 7 of the 12 rice genotypes. Each small colored rectangular shape indicates the particular locus. The name and the size of the corresponding locus are displayed at the left side of the chromosome while the name of the genotype is given at the top of the each chromosome. The relevant yield class is shown at the bottom of the figure. All loci are shown in pink color in the reference haplotype Bg 90-2 (High yielding) except GI locus for which a null allele was detected. Null alleles were detected with GI marker for Bg 90-2, Bg 352 and Bw 367 and the state of non-amplification of alleles is indicated by a white color rectangular shape. All the alleles were given a particular color with respect to the band size of the relevant allele in the reference haplotype. Some of the markers amplified one allele while rest of the others amplified more than one allele. The state of three alleles and two alleles are displayed with three and two small rectangular shape boxes respectively. The markers are arranged according to the order, which they are positioned within the chromosome 7 of IR8 genome (NCBI, 2017; Gramene QTL database, 2017).

4.3.2 Cluster analysis of 12 rice genotypes

The cluster analysis conducted based on the band data are shown in Figure 4.11. The cluster analysis was used to construct the dendrogram of 12 rice genotypes, thereby categorizing them on a genetic basis. At 17% molecular similarity coefficient, all the 12 rice genotypes were clustered into three main groups. At 362, *Suwadhal* were independently grouped. Bg 300, Bg 366, Bg 310, Bw 272-6b, At 307 and Bg 250 were clustered together while Bg 90-2, Bw 367, Bg 352 and *Pachchaperumal* were grouped into a distinct cluster. These three main clusters were further subdivided into seven clusters at 80% molecular similarity coefficient. Based on the results, Bg 366, Bg 310 and Bw 272-6b were the three most similar genotypes with 100% similarity. At 362 and *Suwadhal* were 45.23% similar so as the Bg 90-2 and Bw 367. The cluster containing Bg 352 and *Pachchaperumal* had the least similarity of 29.30%. The two traditional genotypes including *Pachchaperumal* and *Suwadhal* were categorized into two diverse groups. Further it revealed that all the four rice genotypes in each high, moderate and low yielding class were clustered into four different groups. Also, Bg 300, At 307 and Bg 250 were remained unique.



Figure 4.11 Dendrogram constructed for 12 rice genotypes based on 20 alleles at 12 polymorphic and monomorphic marker loci linked to the *Ghd7* QTL region using the algorithms of Complete Linkage and Euclidean distance in Minitab 17 (Minitab Inc. USA). The level of the similarity is shown at the bottom of the Figure. Three main clusters could be identified at 17% molecular similarity coefficient. These three clusters can be further subdivided into seven clusters at 80% of molecular similarity coefficient.

4.3.3 Association between the DNA markers and the yield related traits

4.3.3.1 Association in Maha season

The results of marker-trait association in *Maha* season are given in Table4.7. According to the results obtained, nine alleles out of the 13 of total polymorphic alleles were significantly associated with yield (P<0.05). Among those, 226bp allele of *RM5346*, 900bp allele of *Seq7-8* and 208bp allele of *G7rq* were significantly associated with high yield when the respective allele was present (5.20 g, 5.56 g, and 5.22 g respectively). On the contrary, a significant high yield was observed in rice genotypes when the 215bp allele of *RM5346*, 625bp allele of *Seq1-2*, 850bp allele of *Seq7-8*, 200bp allele of *G7rq*, 720bp allele and 485bp allele of *GI* were not present (Table 4.7, P<0.05). A significant association between the SN and four marker alleles was detected. Out of them, 625bp allele of *Seq1-2* (256.73) and 850bp allele of *Seq7-8* (240.05) were significantly associated with high SN when the corresponding allele was present. Conversely, the 750bp allele of *Seq1-2* and 900bp allele of *Seq7-8* were significantly associated with high SN when the allele was absent (Table 4.7, P<0.05).

Association between the seed weight and marker allele clearly showed that 10 alleles were significantly associated with seed weight (Table 4.7, P<0.05). In the presence of 225bp allele of RM5436, 226bp allele of RM5346, 750bp allele of Seq1-2, 900bp allele of Seq7-8, 790bp, 720bp and 485bp alleles of GI, a significant association between the allele and the seed weight could be observed (0.4 g, 0.32 g, 0.34 g, 0.12 g, 0.34 g, 0.32 g, 0.32 g respectively) (Table 4.7, P<0.05). Whereas the high seed weight was significantly associated with the absence of 234bp allele of RM5436, 215bp allele of RM5346, and 850bp allele of Seq7-8 (Table 4.7, P<0.05). A significant high SL was observed in the presence of 225bp allele of RM5436 (0.88 cm), 226bp allele of RM5346 (0.76), 750bp allele of Seq1-2 (0.77 cm), 900bp allele of Seq7-8 (0.88 cm), and three alleles of GI (0.77 cm, 0.76 cm, 0.76 cm respectively). Conversely, the high SL was observed when the 234bp allele of RM5436, 215bp allele of RM5346, 625bp allele of Seq1-2 and 850bp allele of Seq7-8 were absent (Table 4.7, P<0.05).

In addition, 234bp allele of *RM5436*, 226bp allele of *RM5346*, 625bp allele of *Seq1-2*, 850bp allele of *Seq7-8*, 208bp allele of *G7rq* and three alleles of *GI* were significantly associated with high seed width when the respective allele was present (0.35 cm, 0.35 cm, 0.40 cm, 0.35 cm, 0.36 cm, 0.44 cm, 0.36 cm, 0.36 cm) (Table 4.7, *P*<0.05). On the contrary, 225bp allele of *RM5436*, 215bp allele of *RM5346*, 750bp allele of *Seq1-2*, 900bp allele of *Seq7-8*, 200bp allele of *G7rq* were significantly associated with high seed width when the allele was

absent (Table 4.7, P < 0.05). The association between EL and the marker allele revealed that 10 alleles out of 13 of total alleles were significantly associated with the trait. 225bp allele of RM5436 (0.63 cm), 226bp allele of RM5346 (0.56 cm), 900bp allele of Seq7-8 (0.65 cm), 208bp allele of G7rq and 485bp allele of G7rq (0.56 cm and 0.57 cm respectively) were significantly associated with high EL when the corresponding allele was present (Table 4.7, P < 0.05). 234bp allele of RM5436, 215bp allele of RM5346, 625bp allele of Seq1-2, 850bp allele of Seq7-8, 200bp allele of G7rq were significantly associated with high EL when the allele was absent (Table 4.7, P < 0.05).

According to the results of the association between EW and the marker allele, it showed that, in the presence of 226bp allele of RM5346, 750bp allele of Seq1-2, 850bp allele of Seq7-8, 208bp allele of G7rq, the EW was significantly high (0.26 cm, 0.29 cm, 0.26 cm, 0.26 cm respectively) (Table 4.7, P<0.05). Conversely, in the absence of 215bp allele of RM5346, 625bp allele of Seq1-2, 900bp allele of Seq7-8, 200bp allele of G7rq and three alleles of GI, the EW results were significantly high. It also showed that only the 625bp allele of Seq1-2 was significantly associated with 100 SW and 100 EW when the allele was absent in both the circumstances (2.66 g and 2.00 g respectively). None of the other polymorphic alleles were significantly associated with the above two traits (Table 4.7, P<0.05).

		Presence/					Trait				
Mailan		absence	Yield	Seed	Seed	Seed	Seed	Endosper	Endosper	100 seed	100
Marker	Allele (bp)	of the		number	weight	length	width	m length	m width	weight	endosperm
		allele			C	U U		U U		C	weight
RM5436	234	1	5.08 ^a	238.06ª	0.31 ^b	0.74 ^b	0.35ª	0.54 ^b	0.26 ^a	2.39ª	1.81ª
		0	5.09 ^a	201.81ª	0.40 ^a	0.88^{a}	0.30 ^b	0.64 ^a	0.25 ^a	2.71ª	2.22ª
RM5436	225	1	5.09 ^a	201.81 ^a	0.40^{a}	0.88^{a}	0.30 ^b	0.64^{a}	0.25 ^a	2.71 ^a	2.22 ^a
		0	5.08 ^a	238.06 ^a	0.31 ^b	0.74 ^b	0.35 ^a	0.54 ^b	0.26 ^a	2.39 ^a	1.81 ^a
RM5346	226	1	5.20 ^a	232.78 ^a	0.32 ^a	0.76 ^a	0.35 ^a	0.56ª	0.26 ^a	2.47 ^a	1.89 ^a
		0	3.77 ^b	258.36 ^a	0.28 ^b	0.62 ^b	0.26 ^b	0.42 ^b	0.25 ^b	1.50 ^a	1.44 ^a
RM5346	215	1	3.77 ^b	258.36 ^a	0.28 ^b	0.62 ^b	0.26 ^b	0.42 ^b	0.25 ^b	1.50^{a}	1.44 ^a
		0	5.20 ^a	232.78 ^a	0.32 ^a	0.76 ^a	0.35 ^a	0.56ª	0.26 ^a	2.47 ^a	1.89 ^a
Seq1-2	750	1	4.91 ^a	194.77 ^b	0.34 ^a	0.77^{a}	0.31 ^b	0.55 ^a	0.29 ^a	2.67 ^a	1.62 ^a
•		0	5.11 ^a	243.75 ^a	0.31 ^b	0.75 ^b	0.35 ^a	0.55ª	0.26 ^b	2.36 ^a	1.89 ^a
Seq1-2	625	1	4.86 ^b	256.73 ^a	0.31ª	0.68^{b}	0.40^{a}	0.50 ^b	0.26 ^b	2.11 ^b	1.70 ^b
-		0	5.30 ^a	214.76 ^b	0.32 ^a	0.83 ^a	0.29 ^b	0.59ª	0.27 ^a	2.66 ^a	2.00^{a}
Seq7-8	900	1	5.56 ^a	210.26 ^b	0.12 ^a	0.88^{a}	0.28 ^b	0.65 ^a	0.25 ^b	2.78^{a}	2.27^{a}
-		0	4.98 ^b	240.05 ^a	0.09 ^b	0.73 ^b	0.35 ^a	0.53 ^b	0.26 ^a	2.33ª	1.76 ^a
Seq7-8	850	1	4.98 ^b	240.05 ^a	0.09^{b}	0.73 ^b	0.35 ^a	0.53b	0.26 ^a	2.33 ^a	1.76 ^a
-		0	5.56 ^a	210.26 ^b	0.12 ^a	0.88^{a}	0.28 ^b	0.65 ^a	0.25 ^b	2.78^{a}	2.27^{a}
G7rq	200	1	4.39 ^b	244.79 ^a	0.30 ^a	0.75 ^a	0.27 ^b	0.50 ^b	0.25 ^b	2.16 ^a	1.83 ^a
		0	5.22 ^a	232.86 ^a	0.32 ^a	0.75 ^a	0.36 ^a	0.56ª	0.26 ^a	2.46^{a}	1.85 ^a
G7rq	208	1	5.22 ^a	232.86 ^a	0.32ª	0.75 ^a	0.36 ^a	0.56ª	0.26 ^a	2.46^{a}	1.85 ^a
		0	4.39 ^b	244.79 ^a	0.30 ^a	0.75 ^a	0.27 ^b	0.50 ^b	0.25 ^b	2.16 ^a	1.83 ^a
GI	790	1	5.13 ^a	228.75 ^a	0.34 ^a	0.77 ^a	0.44 ^a	0.57 ^a	0.26 ^b	2.46^{a}	1.99ª
		0	5.04 ^a	239.22 ^a	0.30 ^b	0.74 ^b	0.29 ^b	0.53 ^b	0.27 ^a	2.38^{a}	1.75 ^a
GI	720	1	4.95 ^b	230.72 ^a	0.32 ^a	0.76 ^a	0.36 ^a	0.55ª	0.26 ^b	2.40^{a}	1.94 ^a
		0	5.46 ^a	247.50 ^a	0.29 ^b	0.74 ^b	0.29 ^b	0.56ª	0.27 ^a	2.47 ^a	1.58 ^a
GI	485	1	4.95 ^b	230.72 ^a	0.32ª	0.76 ^a	0.36ª	0.55ª	0.26 ^b	2.40^{a}	1.94 ^a
		0	5.46 ^a	247.50 ^a	0.29 ^b	0.74 ^b	0.29 ^b	0.56^{a}	0.27 ^a	2.47 ^a	1.58 ^a

Table 4.7 Marker-trait association analysis for rice related traits with DNA markers linked to Ghd7 QTL in Maha season
4.3.3.2 Association in Yala season

In *Yala* season, the association between the marker allele and the yield traits are given in the Table 4.8. According to the obtained results, only four alleles were significantly associated with high yield. Out of those, only 226bp allele of *RM5346* was significantly associated with high yield (5.47 g) when the allele was present. On the contrary, 215bp allele of *RM5346*, 720bp and 485bp alleles of *GI* were significantly associated with high yield when the corresponding allele was not present (Table 4.8, P<0.05). By contrast, 6 six alleles were significantly associated with SN. Among those, 234bp allele of *RM5436*, 625bp allele of *Seq1-2* and 850bp allele of *Seq7-8* were significantly associated with high SN when the relevant allele was present (236.78, 249.47 and 240.91 respectively). However, the 225bp allele of *RM5436*, 900bp allele of *Seq7-8* and 790bp allele of *GI* were significantly associated with high SN when the allele was absent (Table 4.8, P<0.05).

According to the association of seed weight and the allelic association, it revealed that the high seed weight was significantly associated with the presence of 234bp allele of RM5436 (0.18 g), 226bp allele of RM5346 (0.18), 200bp allele of G7rq (0.33 g), 720bp and 485bp alleles of GI (0.18 g and 0.18 g respectively). Conversely, 225bp allele of RM5436, 215bp allele of RM5346, 625bp allele of Seq1-2, 208bp allele of G7rq and 790bp allele of GI were significantly associated with seed weight when the allele was absent (Table 4.8, P<0.05). The SL was significantly associated with 9 alleles. Accordingly, the 225bp allele of RM5436, 226bp allele of RM5346, 900bp allele of Seq7-8, 208bp allele of G7rq were significantly associated with 9 alleles. Accordingly, the 225bp allele of RM5436, 226bp allele of RM5346, 900bp allele of Seq7-8, 208bp allele of G7rq were significantly associated with 9 alleles. Accordingly, the 225bp allele of RM5436, 226bp allele of RM5346, 900bp allele of Seq7-8, 208bp allele of G7rq were significantly associated with 9 alleles. Accordingly, the 225bp allele of RM5436, 226bp allele of RM5346, 900bp allele of Seq7-8, 208bp allele of G7rq were significantly associated with 9 alleles. Accordingly, the 225bp allele of RM5436, 226bp allele of RM5346, 900bp allele of Seq7-8, 208bp allele of G7rq were significantly associated with high SL, when the particular marker allele was present (0.86 cm, 0.78 cm, 0.90 cm, 0.77 cm respectively). In contrast to that, the high SL was observed when the 234bp allele of RM5436, 215bp allele of RM5346, 625bp allele of Seq1-2, 850bp allele of Seq7-8, 200bp allele of G7rq were absent (Table 4.8, P<0.05).

The 234bp allele of *RM5436*, 226bp allele of *RM5346*, 750bp allele of *Seq1-2*, 850bp allele of *Seq7-8*, 208bp allele of *G7rq* were significantly associated with seed width when the allele was present (0.27 cm, 0.27 cm, 0.29 cm, 0.27 cm, 0.28 cm respectively). However, in the absence of 225bp allele of *RM5436*, 215bp allele of *RM5346*, 900bp allele of *Seq7-8*, 200bp allele of *G7rq*, 790bp allele of *GI*, the seed width was significantly high (Table 4.8, P<0.05). Seven alleles were significantly associated with EL. Accordingly, 225bp allele of *RM5436* (0.61 cm), 226bp allele of *RM5346* (0.56 cm), 900bp allele of *Seq7-8* (0.64 cm) were significantly associated with high EL when the corresponding allele was present. On the

contrary, 234bp allele of *RM5436*, 215bp allele of *RM5346*, 625bp allele of *Seq1-2* and 850bp allele of *Seq7-8* were significantly associated with EL when the allele was absent.

The association between marker allele and the EW was also analyzed. Correspondingly, the high EW was significantly associated with 234bp allele of *RM5436* (0.25 cm), 226bp allele of *RM5346* (0.25 cm), 750bp allele of *Seq1-2* (0.28 cm), 850bp allele of *Seq7-8* (0.25 cm), 208bp allele of *G7rq* (0.25 cm) when the allele was present (Table 4.8, P<0.05). Conversely, the 225bp allele of *RM5436*, 215bp allele of *RM5346*, 625bp allele of *Seq1-2*, 900bp allele of *Seq7-8*, 200bp allele of *G7rq*, 790bp allele of *GI* were significantly associated with high EW when the relevant allele was not present (Table 4.8, P<0.05). In addition, 625bp allele of *Seq1-2* was significantly associated with high 100 SW and 100 EWs when the allele was absent (2.69 g, 2.06 g respectively).

		Presence/					Trait				
Mailan		absence	Yield	Seed	Seed	Seed	Seed	Endosperm	Endosperm	100 seed	100
Marker	Allele (bp)	of the		number	weight	length	width	length	width	weight	endosperm
		allele			C	C				U	weight
RM5436	234	1	5.36 ^a	236.78ª	0.18 ^a	0.76 ^b	0.27 ^a	0.54 ^b	0.25ª	2.42 ^a	1.76 ^a
		0	4.68 ^a	162.93 ^b	0.06 ^b	0.86 ^a	0.25 ^b	0.61 ^a	0.22 ^b	2.45 ^a	2.09 ^a
RM5436	225	1	4.68 ^a	162.93 ^b	0.06^{b}	0.86^{a}	0.25 ^b	0.61ª	0.22 ^b	2.45 ^a	2.09 ^a
		0	5.36 ^a	236.78 ^a	0.18 ^a	0.76 ^b	0.27 ^a	0.54 ^b	0.25 ^a	2.42 ^a	1.76 ^a
RM5346	226	1	5.47 ^a	230.87ª	0.18^{a}	0.78^{a}	0.27 ^a	0.56ª	0.25 ^a	2.49 ^a	1.85 ^a
		0	3.56 ^b	242.69 ^a	0.11 ^b	0.57 ^b	0.23 ^b	0.40^{b}	0.22 ^b	1.30 ^a	1.10 ^a
RM5346	215	1	3.56 ^b	242.69 ^a	0.11 ^b	0.57 ^b	0.23 ^b	0.40^{b}	0.22 ^b	1.30 ^a	1.10 ^a
		0	5.47 ^a	230.87 ^a	0.18^{a}	0.78^{a}	0.27 ^a	0.56ª	0.25 ^a	2.49 ^a	1.85 ^a
Seq1-2	750	1	5.61 ^a	222.99 ^a	0.19 ^a	0.77 ^a	0.29 ^a	0.56 ^a	0.28 ^a	2.64 ^a	1.90 ^a
-		0	5.26 ^a	233.54ª	0.17 ^a	0.76 ^a	0.26 ^b	0.55ª	0.24 ^b	2.38 ^a	1.76 ^a
Seq1-2	625	1	5.04 ^a	249.47 ^a	0.14 ^b	0.69 ^b	0.27 ^a	0.49 ^b	0.24 ^a	2.09 ^b	1.51 ^b
		0	5.60 ^a	214.57 ^b	0.20^{a}	0.84 ^a	0.27 ^a	0.60 ^a	0.25 ^a	2.69 ^a	2.06 ^a
Seq7-8	900	1	5.09 ^a	184.34 ^b	0.18^{a}	0.90 ^a	0.26 ^b	0.64 ^a	0.23 ^b	2.79^{a}	2.26 ^a
		0	5.36 ^a	240.91ª	0.17 ^a	0.74 ^b	0.27 ^a	0.53 ^b	0.25 ^a	2.34 ^a	1.69 ^a
Seq7-8	850	1	5.36 ^a	240.91ª	0.17 ^a	0.74 ^b	0.27 ^a	0.53 ^b	0.25 ^a	2.34 ^a	1.69 ^a
		0	5.09 ^a	184.34 ^b	0.18 ^a	0.90 ^a	0.26 ^b	0.64 ^a	0.23 ^b	2.79 ^a	2.26 ^a
G7rq	200	1	5.56 ^a	261.56 ^a	0.33ª	0.76 ^a	0.24 ^b	0.54ª	0.22 ^b	2.17 ^a	1.64 ^a
		0	5.27 ^a	225.94ª	0.17 ^b	0.77^{a}	0.28 ^a	0.55ª	0.25 ^a	2.47 ^a	1.82 ^a
G7rq	208	1	5.27 ^a	225.94 ^a	0.17 ^b	0.77 ^a	0.28^{a}	0.55ª	0.25 ^a	2.47 ^a	1.82 ^a
_		0	5.56 ^a	261.56 ^a	0.33ª	0.76 ^a	0.24 ^b	0.54 ^a	0.22 ^b	2.17 ^a	1.64 ^a
GI	790	1	5.09 ^a	209.71 ^b	0.16^{b}	0.77^{a}	0.26 ^b	0.56 ^a	0.24 ^b	2.38 ^a	1.82 ^a
		0	5.47 ^a	247.20 ^a	0.18^{a}	0.76^{a}	0.27 ^a	0.55ª	0.25 ^a	2.46^{a}	1.76 ^a
GI	720	1	5.11 ^b	222.38 ^a	0.18 ^a	0.76^{a}	0.27 ^a	0.55ª	0.24 ^a	2.36 ^a	1.75 ^a
		0	5.91ª	259.68ª	0.15 ^b	0.78^{a}	0.27 ^a	0.56ª	0.25 ^a	2.60 ^a	1.88 ^a
GI	485	1	5.11 ^b	222.38 ^a	0.18 ^a	0.76^{a}	0.27 ^a	0.55ª	0.24 ^a	2.36 ^a	1.75 ^a
		0	5.91ª	259.68 ^a	0.15 ^b	0.78^{a}	0.27ª	0.56 ^a	0.25 ^a	2.60 ^a	1.88^{a}

Table 4.8 Marker-trait association analysis for rice related traits with DNA markers linked to Ghd7 QTL in Yala season

Means denoted by the same letters within the column are not significantly different at P < 0.05

4.3.4 Association between the marker allele and the yield class

The association between the polymorphic marker allele and the yield class (high, moderate and low yielding), analyzed by cross tabulation and chi-square test analysis is given in the Table 4.9 (P<0.05). According to the results obtained, none of the marker allele was significantly associated with the yield class. The Cramer's V-square revealed that there was a much less strong association between the marker allele with that of the yield class (Cramer's V-square was closer to 0) for all the associations.

Marker	Allele (bp)	Pearson chi-square	p-value	Cramer's V-square
RM5436	234	2.18	- "	0.18
RM5436	225	2.18	- "	0.18
RM5346	226	2.18	- "	0.18
RM5346	215	2.18	_ ''	0.18
Seq1-2	750	1.20	- "	0.10
Seq1-2	625	2.00	0.37	0.17
Seq7-8	900	1.20	_ ''	0.10
Seq7-8	850	1.20	- "	0.10
G7rq	200	1.20	_ ''	0.10
G7rq	208	1.20	- "	0.10
GI	790	0.69	0.71	0.06
GI	720	2.67	0.26	0.22
GI	485	2.67	0.26	0.22

Table 4.9 Association between the yield class and the marker allele by Chi-square test analysis

"Only one individual in one genotypic class

5. DISCUSSION

This study was basically conducted with the intention of improving the rice yield in Sri Lanka. Similar studies to improve the rice yield by various strategies have been conducted worldwide (Aggarwal *et al.*, 1997; Dingkuhn *et al.*, 2015). A total of 12 rice genotypes were selected in which ten of them are newly improved and two of them are traditional genotypes (Table 3.1). The yield potential of them has been assigned previously. Based on that, At 362, Bg 300, Bg 366, At 307 are high yielding, At 307, Bg 310, Bg 352, Bw 367 are moderate yielding and Bg 250, Bw 272-6b, *Pachchaperumal* and *Suwadhal* are low yielding genotypes (RRDI, Bathalagoda).

5.1 Phenotypic screening of rice genotypes for yield related agronomical traits

First objective of this study was to screen the 12 rice genotypes for various yield-related agro-morphological parameters such as yield per plant, SN, SL, seed width, seed weight, EL, EW, 100 SW, 100 EW, days to flowering, days to harvest, FLL, FLW, PlH, CL, NT, LBL and LBW. Different rice genotypes can be categorized based on the phenotypic evaluation of yield and yield-related characters and this phenotypic evaluation would be useful in enhancing the crop yield (Sanni *et al.*, 2008; Do Nascimento *et al.*, 2011; Moukoumbi *et al.*, 2011; Roy *et al.*, 2012). Rice genotypes were planted under greenhouse conditions in both the *Yala* and *Maha* seasons to study the effect of seasonal changes on crop yield. According to the obtained results, the morphological parameters except the yield and SN, NT and LBW varied considerably among the genotypes in both the seasons differentially. Similar studies have been conducted in Sri Lanka (Weerakoon *et al.*, 2011; De Costa *et al.*, 2003; De Costa *et al.*, 2006) as well as in worldwide (Welch *et al.*, 2010; Matthews *et al.*, 1997; Jeng *et al.*, 2006; Zhang *et al.*, 2010; Sarker *et al.*, 2012).

The results of the present study indicate a reduction in rice yield in *Yala* season in most of the genotypes in comparison to the *Maha* season. This may be due to the detrimental effects of brown plant hopper (BPH), thrips and fungi on the entire cultivation during *Yala* season. The entire plant population was badly affected by BPH subsequently hampering the plant growth. It was coupled with a significant yield loss. Due to the sucking effect of BPH, the rice plants turned into a yellowish-brown color appearance. Etofenprox insecticide was used against BPH and the plants recovered during 2-3 weeks. The plant population was next severely affected by thrips causing a burning symptom at the very topmost portion of the

leaves which extended towards the center of the leaves ultimately causing them to die. The leaves also appeared with brown colored small eye-like spots. This may be due to a fungal effect. Actara insecticide was applied against thrips. Two weeks after the application of Actara, carbin carbondesim was applied as a fungicide. This enabled the entire population to recover from the situation.

The increased susceptibility of the plants to pests might be attributed to increased temperature and humidity conditions in the greenhouse. The similar effects of increased temperature on a rise in BPH population have been reported in other studies (Pandi *et al.*, 2016; Bao-Kun *et al.*, 2014). Further as denoted by Baker *et al.*, (1992), the increased temperature results in increased tillering subsequently giving rise to a dense population. This might be coupled with increased number of pests (Pandi *et al.*, 2016). Similar studies on the effect of temperature on rice yield have been reported (Oh-e *et al.*, 2007; Barker and Allen, 1993). In addition to the temperature, the solar radiation (IRRI, 1983) and inflorescence architecture (Huang *et al.*, 2009) also influences rice yield. The present study reveals the differences in 100 EW and 100 SWs with respect to the genotype. Although the season has no effect on the highest and lowest 100 SW recorded, it affects the 100 EW. The variation of the latter across the genotype is determined by the amount of starch produced in the endosperm and the size of the hull. The grain weight is also determined by the temperature (IRRI, 1983). Similar studies related to the grain traits have been reported (Roy *et al.*, 2012).

NT in different genotypes does not vary significantly. However, this result is inconsistent with a previous study (Ahmad *et al.*, 2016) where a variation in tillers has been reported. In *Maha* season, the NT produced was found to be high with compared to that in the *Yala* season. This may be due to the negative effects of pests on crop growth and development. It was observed that, in both *Yala* and *Maha* seasons, the NT produced was decreased after six weeks, except certain genotypes. This result does not comply with the observation made by Fageria and Knupp, (2013). According to that, the tillering is increased with increasing the age of the plant. The reduced tillering was may be due to the increased competition among the plants for the existing nutrients and solar energy. The increased NT may decrease the resistance of plants to lodging (Sheehy *et al.*, 2001). However, no significant difference in tillering among genotypes was observed in this study so as the yield.

Another important character; indirectly associated with plant yield is the PIH (Weng *et al.*, 2014; Xue *et al.*, 2008). It is a major determinant of the architecture of the plant which in turn affects the yield (Ahmad *et al.*, 2016). According to the results of the present study, the two traditional genotypes (*Pachchaperumal* and *Suwadhal*) recorded the highest PIH in both the seasons. The height of those which were newly improved seemed to be lower than that of the traditional genotypes. This is because the newly improved genotypes are developed with a better resistance against lodging thereby improving their yield (IRRI, 1983). However the results of this study were not compatible with the above statement since there was no significant difference in yield among the genotypes. The incompatibility of the results would be due to the fact that the lodging may be a major determinant in the field but has a less impact in the greenhouse. Also, other climatic and environmental effects might be attributed to the ultimate yield regardless of the genotype. The results of the present study are corroborated with Saito *et al.*, (2006) where a similar relationship between the PIH and the yield has been recorded.

In addition, days for flowering and maturity also play important roles in determination of yield. The results indicate that both the time duration for flowering and harvesting was increased in *Yala* season in comparison to *Maha* season. The results may be due to increased temperature in the greenhouse. However, as IRRI, (1983) emphasized, longer the time that the crop is exposed to the field conditions, better the crop yield since it gains more sunlight. However the difference in days to flowering and harvesting in two seasons might be attributed to the temperature fluctuations in the greenhouse. Similar studies report significant variation of these two traits among other sets of rice genotypes (Ahmad *et al.*, 2016; Ashfaq *et al.*, 2012). Grain yield with respect to above mentioned agronomical traits including number of grains, 100 grain weight, PlH, NT, days to flowering and maturity also have previously been reported by other research (Tejaswini *et al.*, 2016; Lakshmi *et al.*, 2017; Kalyan *et al.*, 2017; Khan *et al.*, 2009; Rajeswari and Nadarajan, 2004). Further, as denoted by Ranawaka *et al.*, (2013), NT and PlH are not much desirable traits in selecting high yielding genotypes.

The morphology of flag leaf was also accessed in the present study. Based on the results, it shows that traditional genotypes have the significant highest FLL in both the seasons whereas the highest FLW was recorded by several newly improved genotypes. FLL plays an essential role in delivering carbohydrates to rice grains (Gladun and Karpov, 1993).

Previous studies have indicated a positive correlation between the FLL and panicle length which in turn having a correlation with the rice grain yield (Rahman *et al.*, 2013). The importance of FLL in regulating rice yield has also been revealed by other studies (Li *et al.*, 1998; Bing *et al.*, 2006). Increase of photosynthetic capability of flag leaf would be a major breakthrough in improving the rice yield (Rahman *et al.*, 2013). However, the present study reveals that the morphological variability itself would not be desirable in selecting the genotypes with high yield. This is due the fact that the yield of the plant is highly determined by environmental factors other than the genetic constitution of the plant. Further, most of the parameters used in this study are measures of yield which are evaluated after harvesting, suggesting the cumbersomeness of phenotypic evaluation.

5.2 Detection of Ghd7 polymorphism of 12 rice genotypes

The present study mainly focused on the detection of genetic polymorphism of *Ghd7* locus in 12 Sri Lankan rice genotypes. Since the phenotypic evaluation is not much accurate and it is dependent on the environment, DNA markers were used in this study to detect the *Ghd7* polymorphism across the 12 genotypes. The present study demonstrated a high diversity among the 12 rice genotypes on the basis of DNA marker analysis. Although the phenotypic evaluation and selecting the crops with most desirable characteristics is useful, its applicability is limited due to its less accuracy, high cost, space and time consuming nature (Evenson *et al.*, 2002; Smith and Smith, 1992). This study used 12 DNA markers to assess the genetic polymorphism. The development of DNA markers and their applicability in molecular breeding is a major breakthrough in improving an array of agronomical traits. Valuable characteristics such as relatively low cost, great informativeness, easiness of use, polymorphic nature, ability to select the plants at the seedling stage, saving of time and space, greater power of resolving, have led to the increased potential use of SSR markers preferentially over the other markers (Yang *et al.*, 1994; Wu and Tanksley, 1993; Evenson *et al.*, 2002; Dirlewanger *et al.*, 2004; Maroof *et al.*, 1994; Akagi *et al.*, 1996).

The DNA markers used in this study are distributed in chromosome 7 (Xue *et al.*, 2008). The DNA markers like SSR have been used in many approaches in previous studies such as developing resistant genotypes to submergence in rice (Neeraja *et al.*, 2007), selection of disease resistant rice plants (Basavaraj *et al.*, 2010) and genetic diversity studies (Yang *et al.*, 1994; Panaud *et al.*, 1996; Blair *et al.*, 2002; Cao *et al.*, 2006). From a total of 24 DNA markers, only 12 markers gave successful PCR amplifications. Five were excluded from this

study due to the unsuccessful amplifications. The rest of the markers were excluded due to the non-relevance to this particular study. Non-amplification of certain markers is consistent with the results of previous studies. Non amplification might be attributed to poor PCR conditions (Pemberton *et al.*, 1995) or non-amplified alleles (Callen *et al.*, 1993). In the circumstances where successful PCR amplification does not occur due to the PCR failure, it can be resolved with improving the PCR conditions.

Among the RM markers Both the *RM5499* and *RM5436* SSR markers have been discovered to co-segregate with the QTL- *Ghd7* (Xing *et al.*, 2014). A total of 20 alleles were detected across the 12 DNA marker loci with an average of 1.67 alleles per locus. This value is lower than that of the results reported by previous studies (Nagaraju *et al.*, 2002; Siwach *et al.*, 2004; Joshi and Behera, 2006; Herrera *et al.*, 2008) (3.8, 4.58, 2.6, 4.23 alleles respectively). Results in this study are much lower than previous studies (Thomson *et al.*, 2007; Brondani *et al.*, 2006) (7.8 and 13 alleles respectively). However, our result is less comparable with Singh *et al.*, (2004) (2.3). The number of alleles detected per locus (1-3) is quite comparable with (2-4) reported by Singh *et al.*, (2004). The variations of the results might be due to the differences in selected rice genotypes in different studies. The results of the present study reveal a genetic diversity across the 12 rice genotype based on the polymorphic DNA marker data further supporting the morphological variability.

5.3 Cluster analysis

The 12 rice genotypes were subjected into cluster analysis based on the molecular marker data. Previous studies have been conducted to categorize the Sri Lankan rice genotypes based on yield and other yield-related morphological traits (Ranawake *et al.*, 2014; Rathnathunga *et al.*, 2016; Worede *et al.*, 2014). The present study categorized the 12 rice genotypes into three major clusters based on 12 DNA markers. The cluster 1 included one of the newly improved (At 362) and traditional (*Suwadhal*) rice genotypes. The cluster 2 could be further subdivided into four clusters containing the newly improved genotypes. The third cluster could be further clustered into two groups containing both the newly improved and traditional genotypes. Interestingly the highest similarity (100%) was detected among Bg 366, Bg 310 and Bw 272-6b suggesting a close relationship among them. Further, 45.23% similarity in sub-cluster 1 and 6, infer a less strong association among those genotypes. Bg 352 and *Pachchaperumal* were grouped with a 29.30% similarity, deducing a weak relationship between those two genotypes.

The DNA markers used in this study to investigate the genetic diversity among diverse Sri Lankan rice genotypes have not been previously reported. However similar studies to evaluate the genetic variation among rice cultivars using different SSR markers have been reported by other studies (Pervaiz *et al.*, 2009; Ming *et al.*, 2010). The major cluster I could be differentiated from cluster II by *RM5346* and from cluster III by *RM5436*. *RM5346*, *GI* and *Seq7-8* were the markers that were used to differentiate between cluster I and III. Bg 300, At 307 and Bg 250 could not be grouped with any of the genotypes. This might be attributed to the unique allelic pattern that they are comprised of. The alleles of *GI*, *G7rq*, *Seq7-8* and *RM5436* SSR markers were conserved in At 362 and *Suwadhal* grouping them into sub-cluster 1. The highest genetic similarity within the sub-cluster 3 comprising of Bg 366, Bg 310 and Bw 272-6b might be attributed to the allelic pattern of *RM5436*, *RM5346*, *Seq7-8*, *G7rq* and *GI*. The genotypes in sub-cluster 6 and 7 shared the alleles of *RM5436*, *RM5346* and *G7rq* and the two clusters could be differentiated by *GI*, *Seq1-2* and *Seq7-8* markers.

However, the results of the cluster analysis are not in a good agreement with that of the morphological data from which a detectable variation in yield among the genotypes could not be observed. The characterization of the genotypes with the use of a cluster analysis based on morphological data was not tracked during this study. However, the fact that, the morphological characterization is often dependent on the environment, the characterization of genotypes based on the genetic constitution would increase the accuracy, precision and the efficiency of the study.

5.4 Marker-trait association analysis

The genetic polymorphism among the rice genotypes further gave insight into marker-trait association analysis. The association between all the yield traits and the polymorphic marker alleles revealed significant associations for some marker-trait combinations whereas no such significant association could be detected for the rest. The marker-trait association in turn facilitates the marker assisted selection, which is based on selection of superior genotypes with the use of molecular markers. Marker assisted selection is powerful and beneficial over the phenotypic selection due to its easiness in screening, ability to select the desirable genotypes early at the seedling stage, distinguishability between homozygotes and heterozygotes and saving of timing and space (Acquaah, 2012). The markers used in this

study are linked with *Ghd7* QTL which simultaneously regulates an array of traits such as days to heading, height of the plant and yield potential of rice (Xue *et al.*, 2008; Lu *et al.*, 2012; Weng *et al.*, 2014). Although this QTL has been cloned and sequenced in diverse indica and japonica rice genotypes (Xue *et al.*, 2008; Lu *et al.*, 2012), it has not yet been studied within the Sri Lankan rice germplasm. Therefore, the studied 12 DNA markers in this study would give insight into a marker validation, which is important in marker assisted selection in molecular breeding programs.

Selection of desirable rice genotypes for different agronomic traits with the use of molecular markers have been previously reported for rice (Zhou *et al.*, 2003; Ikeda *et al.*, 2001; Neeraja *et al.*, 2007; Talukdar *et al.*, 2017), wheat (Zhang *et al.*, 2004; Doebley *et al.*, 1990: Anderson *et al.*, 2001; Wang *et al.*, 2017), cotton (Kohel *et al.*, 2001; Akano *et al.*, 2002), common bean (Miklas *et al.*, 2000), potato (Li *et al.*, 2013), rosaceae fruit crops (Dirlewanger *et al.*, 2004) and pea (Liu *et al.*, 2017). However, since the grain yield is a complex agronomic trait, in which the expression is influenced by gene-by-environment interaction and epistasis interaction effects, the applicability of MAS is somewhat challenging (Acquaah, 2012). According to the present study, all the six DNA markers including *Seq7-8*, *Seq1-2*, *RM5436*, *RM5346*, *G7rq* and *GI* markers were significantly associated with nine yield related traits.

A total of 30 significant marker-trait associations could be detected in *Maha* season, while it was only 25 for the *Yala* season when the corresponding allele was present. These results are in contradiction to the previous studies (Borba *et al.*, 2010; Supari *et al.*, 2016; Vanniarajan *et al.*, 2012). Such discrepancies might be due to the differences in number and the variation of selected rice genotypes, yield related traits, selected markers and the environment in which the particular study was conducted. However, it was noted that the marker alleles which were significantly associated with the traits are highly varied between the seasons. Incompatibility of the results between the two seasons might be attributed to the seasonal variation on overall yield. This variation of association due to the season is in consonance with the previous studies (Kannan *et al.*, 2014).

The association analysis further revealed that the presence of marker alleles was coupled with higher trait values in most of the cases. Only the higher 100 SW and 100 EW were significantly associated with the 625bp allele of *Seq1-2* marker when the allele was absent. This is inconsistent with the results of the previous study (Chamikara *et al.*, 2015), where an

enhanced state of the trait was coupled with the absence of alleles. This might be attributed to a negative selection occurred in breeding. However, this would not be desirable for selection in breeding. In *Maha* season, *Seq7-8* marker was detected to be significantly associated with seven traits including yield, SL, EL, seed weight, width, SN and EW. In *Yala* season, both the *RM5436* and *RM5346* markers were significantly associated with six traits including SN, seed weight, seed width, EW, SL, EL and yield, Seed weight, SL, seed width, EL, EW respectively. Significant marker-trait associations were detected for *RM5346* (Yield, seed weight, SL, seed width, EL and EW) and *Seq7-8* (SL, EL, SN, seed width and EW) in both the seasons. This suggests that *RM5346* and *Seq7-8* markers could be more useful in marker assisted selection. However, interestingly it was noted that all of the six polymorphic marker loci were significantly associated with different rice yield traits.

Here in this study, some limitations must be taken into account. Only 12 DNA markers were detected with successful amplifications and the number of rice genotypes was limited to only 12. This study was basically conducted only under greenhouse conditions, but no field trials were carried out. However, since the selection of high yielding rice genotypes is done based on the markers, further studies could be employed with more markers to enhance the selection. This is useful due to the fact that, more the number of markers, better the chance to find the exact and precise marker-trait associations. To get the successful amplifications for the markers detected with PCR failures, the PCR conditions could be improved. To enhance the reliability of the study, it would be better to select a population with increased number of individuals. Further studies can be carried out with different yield-related characters used other than this study. It also would be further beneficial to conduct the morphological evaluation in field conditions along with greenhouse conditions.

6. CONCLUSIONS AND FUTURE DIRECTIONS

Yield is a complex agronomic trait which is governed by both the genetic and environmental factors. This study was basically focused on improving the rice yield in Sri Lanka with three main objectives. The first objective was to phenotypically screen the 12 rice genotypes with respect to a total of 17 traits including vegetative, flowering and harvesting measurements across the two seasons. Yield being a multifactorial trait, most of the morphological traits studied in the present study found to be significantly different among the genotypes though few of them did not seem to be significantly different. Vegetative measurements including

NT, LBW and yield measurements were not useful in differentiating the genotypes. The rest of the other measurements were significantly different among the genotypes. This suggests the significantly high diversity of 12 rice genotypes with respect to morphology. Phenotypic screening, being one of the means of evaluation of the yield, the results of the present study thus reflect the less efficacy of phenotypic screening on determination of yield potential.

The second objective was to detect the genetic polymorphism of *Ghd7* locus among the 12 rice genotypes which would in turn facilitate the identification of marker haplotypes of *Ghd7* locus. A total of 20 alleles out of the 12 DNA markers were detected with an average of 1.67 alleles per locus. Six DNA markers were polymorphic and six were monomorphic. The minimum number of alleles detected per locus was one while the maximum number was the three. The dendrogram constructed for 12 rice genotypes based on all the 12 DNA markers categorized the genotypes into three main clusters. Marker haplotype analysis is one of the important ways in deducing genetic similarity between genotypes. The results of the present study thus reflects ten marker haplotypes among the 12 rice genotypes using the haplotype of Bg 90-2, the highest yielding genotype, which was used as the reference haplotype. Bg 366, Bg 310 and Bw 272-6b belong to one haplotype, while the rest of the other nine genotypes represent nine individual haplotypes. Thus, the results of the present study reflect a genetic polymorphism of *Ghd7* locus across the 12 rice genotypes.

The third objective was to reflect the association between the six polymorphic DNA marker alleles (*RM5436*, *RM5346*, *Seq7-8*, *Seq1-2*, *G7rq*, *GI*) with yield traits (yield, SN, seed weight, SL, seed width, EL, EW, 100 SW, 100 EW and yield class). A total of 30 and 25 marker-trait associations could be detected in *Maha* and *Yala* seasons respectively. The fact that all of the six polymorphic marker loci were significantly associated with different rice yield traits reflects their applicability in MAS. In *Maha* season, *Seq7-8* marker was detected to be significantly associated with seven traits including yield, SL, EL, seed weight, width, SN and EW. In *Yala* season, both the *RM5436* and *RM5346* markers were significantly associated with six traits including SN, seed weight, seed width, EW, SL , EL and yield, Seed weight, SL, seed width, EL, EW respectively. In both the seasons, the association of the *Seq7-8* and *RM5346* markers with more than four yield traits thus reflects their usefulness in MAS in future breeding programs. The association between the marker alleles and the yield class was found to be not significant thus reflecting a less strong relationship between those two parameters.

The results of the marker-trait association analysis would be useful in marker-assisted backcrossing and marker-assisted pyramiding. This would facilitate the selection of superior rice genotypes with enhanced yield, in Sri Lanka. The results of the DNA markers used in this study could be further used to detect the genetic diversity among a diverse collection of newly improved and traditional genotypes grown in Sri Lanka other than the rice genotypes assessed in this study. It would facilitate the detection of genetic similarity and divergence between rice accessions. The DNA markers used in this study could be used for the selection of parents with a diverse germplasm for subsequent breeding programs. The comparison of *Ghd7* linked marker haplotypes would enable the identification of rice accessions belonging to different rice yield categories. The haplotype data on the region of chromosome 7 would be useful for the development of novel varieties with specific combinations of alleles with the help of MAS.

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