CONSTRUCTION OF A PEDIGREE-DATABASE FOR RICE VARIETAL INFORMATION AND MARKER ASSISTED SELECTION OF PHOSPHOROUS EFFICIENT AND BROWN PLANTHOPPER RESISTANT RICE CULTIVARS IN SRI LANKA

By

P.G.R.G. Rathnayake

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
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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 and Dr. U.A.K.S. Udawela, Rice Research and Development Institute, Bathalagoda. It describes the results of my own independent research project 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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Signature: …… Date: …….

Department Stamp

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The varietal improvement using breeding is the foundation to maintain a sustainable rice production in Sri Lanka. The decision-making in breeding is a significant step in programming rice breeding. If an organized breeding database for the local rice germplasm can be constructed, the efficient, pragmatic, and successful breeding decisions could be made easily. In the present study, the pedigree history, phenotypic, and molecular marker data for all improved rice varieties released by Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka, were assembled to Pedimap; a pedigree visualization tool and a platform to construct as breeding database. The Pedimap visualizations reveal the phenotypic and genetic relationships of the improved Sri Lankan rice varieties. The Pedimap visualizations, incorporating FlexQTL-calculated identity-by-descent (IBD) probabilities, showed the allelic representation through the pedigree. The usability of breeding databases and the way of making breeding decisions under different circumstances were demonstrated by using several pedigree-visualization models. The breeding decisions such as parental identification, progeny identification, tracing founders and progeny, compatible marker detection in marker-assisted breeding (MAB), and allelic frequency calculations made at the Pedimap, can be used to plan novel, efficient and successful breeding programs. The development of phosphorus deficiency tolerant (PDT) and brown planthopper (BPH) resistant rice varieties can be highlighted as a significant breeding priority in domestic rice breeding. Therefore, we screened for PDT ones among 27 local rice varieties using PDT indicators, and the assessment of *Pup1* haplotypes using 17 *Pup1* linked DNA markers and sequence polymorphism of the two marker loci (*K29* and *RM28102*). According to the results, the landrace *Madathawalu* and H-4 exhibited the highest trait values for yield, shoot dry weight, and phosphorus utilization efficiency in both *Yala* and *Maha* seasons, while sharing common *Pup1* haplotype. The employability of haplotype variant linked-BPH resistant genes was assessed by using three BPH resistant (*Murungakayan302*, PtB33, *Sulai*), a moderate tolerant (Bg300) and sensitive (Bw367) local rice varieties. It was observed that screening haplotype variants is the most successful approach in MAB for the BPH resistant rice cultivars, as only the marker *C3-14* shows band-length polymorphism in 2.5 % agarose gel electrophoresis.

Keywords: Breeding database, breeding decision-making, BPH, Pedimap, *Pup1* QTL, rice breeding in Sri Lanka

DEDICATION

To

"all the fantastic research personalities,

those who need this,

and, love this to inspire...."

ACKNOWLEDGEMENTS

This research was conducted as a partial fulfillment of the requirement of the Degree of Bachelor of Science Honors in Molecular Biology and Biotechnology. We basically aimed to formulate an organized breeding database for local rice breeding programs; because the classical breeding approaches are tedious, laborious, lengthy, and less efficient. All primary data had to be collected, and DNA markers for marker-assisted breeding (MAB) got to be evaluated within the study. The latter part of the study was an extension of the previous research, where the identification of the *Pup1*-linked marker haplotypes in local rice varieties and the recognizing of the applicability of brown planthopper (BPH) resistant genes-linked DNA marker haplotype in MAB were already undertaken in parts. As molecular breeding researchers, we focused our primary attention to assemble the rice breeding database, using Pedimap. Because no one has practiced this kind of approach in Sri Lanka, the established database is novel to the large-scale plant breeding programming. The latter section of the study was based on the DNA marker haplotype and phylogenetic analysis. So that, the molecular genetic studies published in similar areas, the scientists and funding agencies who worked in and facilitated the studies, are gratefully reminded. Moreover, it is a great honor to be part of this prestigious scientific community having the ultimate aim of establishing a comprehensive rice breeding database.

A part of the research was facilitated by the funding from the National Research Council (NRC), Sri Lanka (Grant No.: NRC 11-87) and the research grant from University of Peradeniya, Sri Lanka (URG/2016/59S). I would like to immensely thank NRC and University of Peradeniya for their financial support to make this study a success. The research project was carried out at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka. During the research period of 12 months, so many people have contributed extensively. First and foremost, I would like to thank all of them, including the research supervisors, staff members, research assistants, supportive staff members, technical officers, and the non-academic staff members of the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka. Besides them, staff at Rice Research and Development Institute (RRDI) and their regional centers are also needed to be gratefully recalled, as I cannot mention them all by names in this limited space.

Executing a large project within eight months is truly challenging and life-changing experience for me. Without having unique supervision, support, and guidance from an expert, this would not have been a reality. I am so lucky to have such an excellent researcher with a strong personality as my project supervisor, Prof. S.D.S.S. Sooriyapathirana (Professor in Molecular Biology and Biotechnology, Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka). I am very grateful for his patient assistance, motivational enthusiasm, and extensive knowledge in the fields of Molecular Biology and Biotechnology. He was the pioneer behind the success of the research, as he guided me to plan a proper research, method of time management, correct arrangements, and laboratory ethics. His continues guidance and supervision made a work-friendly and unstressed environment. His vast understanding of the student's mind and humanity present within him, led to ends up with praiseworthy research findings. My highest respect goes to my supervisor for the opportunities that he has given me to enhance the knowledge and experiences as an infant researcher. His endless dedication, promising attitudes, and excellent planning always encourage me to overcome all the obstacles and achieve success at the end of the study. It was a blessing for me to work with him, with his helping hands, and without his guidance and constant feedback, this task would not have been successful. Acknowledging with these simple words will not enough to express his gigantic support throughout this project. However, I am pleased to be a student of such a unique research personality.

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I admiringly acknowledge Dr. Roeland Voorrips, who is the inventor of the Pedimap, at Wageningen University and Research Center Nijmegen Area, the Netherlands, for his support and consent to use Pedimap, as a rice breeding database in this study. I would like

to commemorate his responsiveness and flexibility to provide the license to the Pedimap. I also would like to thank Dr. Eric van de Weg at Wageningen University and Research Center, Nijmegen Area, the Netherlands, for his support, guidance, and instructions while providing the course materials and tutorials to study the Fl**e**xQTL™ for QTL analysis. Also, his grace and acquiescence to use FlexQTL™ were highly valuable to proceed with these analyses without any obstacles.

Also, I would like to appreciate the academic staff at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Prof. R.G.S.C. Rajapakse (Head of the Department), Prof. P. Samaraweera, and Dr. M.P.C.S. Dhanapala for their compliance in providing me working facilities to carry out all the experiments successfully. I offer my thanks to Mrs. A.G.M.L.K. Dayananda, Technical Officer of the Department, for the support given to me during the research period. I would like to memorize further, the help given by Mr. I.M.P. Abesiriwardane and Mr. H.G.G. Chandrarathna, Laboratory Assistants, for their help given to work at the Department laboratories.

The experience is highly necessary to adapt into a research laboratory environment. As well-experienced research assistants, Mr. Thilanka Ranaweera, Mr. Hashan Jayarathne, of the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya helped me a lot during my study. I extrapolate my gratitude for their enormous guidance, support, and assistance in conducting lab work and for making this study easier. I immensely appreciate the support received from the other teaching assistants along with Mr. Shehan Sahibdeen, who helped to complete my lab work on time under tight time schedules. My thankfulness also goes to the assistance I received from Mrs. Upendra Wijesundara (Teaching Assistant for Undergraduate Programs and Quality Assesments, Department of Molecular Biology and Biotechnology, University of Peradeniya, Peradeniya, Sri Lanka), to conduct my laboratory work based on DNA marker analysis. I am especially grateful to my colleagues for their constant support in the DNA extractions and conducting laboratory-related activities, and I would like to acknowledge them from the bottom of my heart. Ultimately, I like to offer my gratitude to my parents, who are always behind me and encourage me to achieve all special milestones in my life.

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LIST OF ABBREVIATIONS

bp	base pairs
BPH	Brown Plant Hopper
C	Cluster
cM	Centimorgan
DOA	Department of Agriculture
FLL	Flag Leaf Length
FLW	Flag Leaf Width
GBS	Genotyping By Sequencing
GEBV	Genomic Estimated Breeding Value
GLM	General Linear Model procedure
GP	Gene Pool
GS	Genome-wide selection
ha	Hectare
HSFS	Half-Sib Family Selection Methods
IBD	Identical-By-Descent
L	Landraces
LL	Low Land
MAB	Marker-Assisted Breeding
MABC	Marker-Assisted Backcross Breeding
MARS	Marker-Assisted Recurrent Selection
MCDM	Multi-Criteria Decision-Making
mt	metric
NI	Newly-Improved
NT	Number of Tillers
OI	Old-Improved
P	Phosphorus
PCR	Polymerase Chain Reaction
PD	Phosphorus Deficiency
PDT	Phosphorus Deficiency Tolerance
PLH	Plant Height
Pup1	Phosphorus Uptake 1
QTL	Quantitative Trait Loci

RAD	Restriction Site-Associated Genomic DNA
RRDI	Rice Research and Development Institute
SDW	The Shoot Dry Weight
sec	Seconds
SNP	Single Nucleotide Polymorphism
SPC	Shoot Phosphorus Concentration
SPU	Shoot Phosphorous Uptake
SSR	Simple Sequence Repeats
STMS	Sequence-Tagged Microsatellite Sites
STR	Short Tandem Repeats
STS	Sequence-Tagged Sites
Ta	Annealing Temperature
UL	Upland
UPGMA	Unweighted Pair Group Method with Arithmetic Means

CHAPTER 1
INTRODUCTION AND OBJECTIVES

Rice is the world's second most cultivating cereal crop (Van Nguyen and Ferrero, 2006), with an annual production of over 700 million tons (FAO, 2018). Due to the price volatility, lack of supply, geographic concentration, relatively low world stockholdings (Dawe, 2002; Jayne, 1993) and socioeconomic factors (Herath Banda *et al*., 1998), the demand for rice has been increasing rapidly. The local rice cultivation fails to fulfill domestic rice demand; therefore, the government has spent over USD 400 million in the last two years to import rice (Central Bank, Sri Lanka, 2017, 2018). The biotic and abiotic stresses (Seck *et al*., 2012) including droughts, soil nutrient deficiency (Walisinghe *et al*., 2010), pest attacks and plant diseases (Dhanapala, 2007) can be considered as significant factors causing the low rice production. In addition to that, problems in irrigation facilities (Davis *et al*., 2016), irregular rainfall patterns (Dharmarathna *et al*., 2014), soil salinity (RRDI, 2019) and land submergence (Mackil, 1996) also affects to the productivity of rice. Thus, overcoming these restraints is a challenging task to maintain local rice production at a satisfactory level.

The varietal improvement is the best potential strategy today to overcome the limitations (Duvick, 1984). Formerly due to the lack of capital, training, facilities, and defects in the socio-economical system, the practices including the application of fertilizers, use of new technologies (Walisinghe *et al*., 2010), intensive land selection, timely cultivation, post-harvest supervision, socio-economic development, and usage of chemical control methods only help to increase rice productivity with limitations (Dhanapala, 2007). To satisfy the changing market and consumer preference, and fulfill the breeding priorities (Iezzoni *et al*., 2009), the novel varieties with enhanced and defensive traits like fast-growing, high-quality, pest resistance, disease resistance, tolerance under significant abiotic stresses like heat and drought, and fast yielding varieties are needed (Khush, 1995; Peng *et al*., 2009). Initially, crop improvement was accomplished with tedious, lengthy, outdated, and subjective classical breeding techniques. However today, the knowledge of genetics helps to identify the genes responsible for desirable traits and marker-assisted breeding (MAB) (Jiang, 2013a) and to preserve valuable traits within the germplasm (Sasaki, 2011). The DNA markers are used to monitor the allelic segregation and confirm genetic stability in MAB with a high level of accuracy (Jiang 2013a,b). The hybridization is also involved in present breeding strategies (Xu, 2010).

The decision-making and planning in breeding is the most significant move to accelerate and increase the efficiency of varietal improvement by gene introgression from target parents to elite offspring (Iezzoni *et al*., 2009). Formulation of breeding decisions is a multi-step process, which consists of the identification of breeding priorities, the genetic architecture, pre-breeding methods, economic and technical feasibility, number of parents, number of selfing and breeding cycles, duration of breeding, the selection method and demand of the cross (Acquaah, 2012). First, market trends and consumer behaviors should be identified (Clark, 1999). The novelty and the uniqueness of the cross should be recognized as activation-breeding decision (Acquaah, 2012). The selection of parental lineages and the selection methods are the two most critical steps in an active breeding plan (Ragot *et al*., 2018). A prioritized order is needed in a multiple-trait introgression (Kariuki *et al*., 2017; Velasquez and Hesterc, 2013). The breeding strategies are selecting based on the technical and economic feasibility, level of the trait in the breeding germplasm, timing of response and the uniqueness of the product (Ragot *et al*., 2018). Besides the market trends, selected parents and their mating method, genetic architecture and the genetic variance of the trait, pre-breeding techniques, location of breeding, number of expected progenies and time duration also influence the design of a breeding program (Acquaah, 2012).

Breeding decision-making is entirely performed based on the phenotypic and genetic information, and breeding history. Nowadays the computer programs and databases are used to manipulate the pool of primitive data used for breeding programming. Assembling a breeding database boosts up the capacity of data sharing, mining, visualization, and retrieval (Yu *et al*., 2013). Pedimap is one of the breeding databases and pedigree visualization software (Voorrips, 2007), which is being practiced by many global plant geneticist and breeding programs. As shown in Voorrips *et al*., (2012), Pedimap can be used to record and utilize breeding history along with their phenotypic and genetic characteristics and recognize allelic representation. This software only presents the available phenotypic and genetic data through pedigrees, instead of performing statistical and quantitative calculations. All the primitive data, including parentage, qualitative and quantitative data, marker information, and the identical-by-descent (IBD) probabilities, can be incorporated with this database. It allows to access the large pool of genetic and phenotypic data quickly, and generate pedigrees as necessary in breeding-decision-making, which also can be utilized with MAB and marker-assisted selection.

The development of the phosphorus deficiency tolerant (PDT) and brown planthopper (BPH) resistant rice varieties are the significant concerns in Sri Lankan rice varietal improvement programs. Phosphorus (P) is a macronutrient and its availability are limited in the soil for the plants (Wang *et al*., 2013). Due to P deficiency, the government spends about 0.3 billion USD annually (1.5 % of GDP) on importing P fertilizer to Sri Lanka (Aluwihare *et al*., 2016). The overuse of P fertilizer in the soil ends up with accumulation of P within the water bodies causing severe environmental problems such as eutrophication (Bulluck and Ristaino, 2002; O'neil et *al*., 2012). The only sustainable solution to face the undeniable P fertilizer crisis is to produce PDT rice varieties through MAB. The genetics of the PDT has been studied in detail in Ni *et al*., (1998) and Wissuwa *et al*., (1998). The major quantitative trait loci (QTL) displaying the PDT in rice has been identified as *Phosphorus Uptake 1* (*Pup1*) (Ni *et al*., 1998; Wissuwa *et al*., 1998). *Pup1* was further validated as the major QTL with 80 % effect on the trait PDT and the fact verified by Chin *et al*., (2010) and Wissuwa *et al*., (2002). The (BPH) outbreak can be considered as one of the most devastating biotic stresses in rice farming (Dyck and Thomas, 1979) and destroyed thousands of acres of rice fields in the world including Sri Lanka during last few decades, along with 2,800 ha in Ampara in 1974 (Dyck and Thomas 1979; Fernando *et al*., 1979; Khush *et al*. 1985). This outbreak ends up with an outstanding reduction of the grain yield (Nagadhara *et al*., 2003). The application of insecticides promotes resistance toward chemical control methods (Gallagher *et al*., 1994). So that the sole option to overcome this problem is of cultivating the BPH resistant rice varieties. The formulation of innovative breeding programs plays a massive role in the development of existing rice cultivation.

In Sri Lanka, RRDI has been conducting rice breeding programs, however, no one has ever constructed organized rice breeding databases to make proper breeding decisions as the existing local breeding programs are lengthy, tedious and inefficient. Therefore, the main aim of this study was to build an informative breeding database using Pedimap to plan new crosses and recognize the inheritance of DNA markers in MAB. The other aim was to screen a set of Sri Lankan rice landraces and improved rice varieties for PDT, identify their maker haplotypes by sequencing two key co-dominant sequence-tagged sites (STS), within *Pup1* locus and, the evaluation of the relevance of BPH resistance specific haplotype variants in marker loci with comparison to the band polymorphism in agarose gel electrophoresis using a set of BPH tolerant and sensitive rice cultivars to use as an effective screening method in MAB.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Rice as a staple food crop cultivation in Sri Lanka

Rice is the most important crop occupying 34 % (0.77 million ha) of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in rice cultivation island-wide (RRDI, 2019). Rice production in Sri Lanka is conducted under tropical climatic conditions within two seasons per year as *Maha* season from October to March, and the *Yala* season from April to September (Dharmarathna *et al*., 2014). The rice growing lands are divided under various climatic conditions to wet zone, dry zone, and intermediate zone, flood-prone areas, and rainfed lands (Swain *et al*., 2005). Among the 870,000 ha of the average annual extent of rice farmlands in Sri Lanka, 560,000 ha of land is cultivated in *Maha* season, and 310,000 ha of land is cultivated in *Yala* season. The present supply of 2.7 million tons of local annual rice production can satisfy around 95 % of the domestic rice demand. The per capita consumption of rice varies around 100 kg per year, which depends on the price of rice and other substitutes. Local rice production can provide the 45 % of calorie and 40 % of protein average requirement (Central Bank, Sri Lanka, 2017). The identified increment of the local rice demand is 1.1 % per year. Therefore, to meet total rice demand locally, Sri Lankan rice production should raise at the rate of 2.9 % per year (RRDI, 2019). With the influence of many barriers to sustain the rice cultivation, the new yield improvement techniques such as improved variety introduction, nourishing farmlands, application of new technology to land preparation and maintenance are needed with the support of scientific innovations.

2.1.1 Crisis in rice cultivation in Sri Lanka

The low performance of Sri Lanka rice production is mainly due to the biotic and abiotic stresses. In 2017, local rice production, which, was fallen by 46.1 % to 2.4 million metric tons, was the lowest production in the last decade. That was due to the critical influence of unfavorable weather conditions that had been endured from 2016 in Sri Lanka. The annual rice production of 1.7 million metric tons within both *Yala* and *Maha* seasons are only sufficient to fulfill the domestic demand for eight months. The remaining of 800,000 metric tons had to be imported in 2017. Due to the fact that local rice production is always decreasing, the trend to import rice is increasing annually (Central Bank, Sri Lanka, 2017). Not only drought, some other abiotic stresses such as variations in the climate, irregular

patterns in annual rainfall, salinity, temperature extremes, submergence, nutrient deficiencies (Walisinghe *et al*., 2010), mineral toxicities, and problems in irrigation facilities are also causing the low productivity (Davis *et al*., 2016; Farmer, 1979). Under the biotic stresses such as; weed infestation (Herath Banda *et al*., 1998), microbial infections including fungi, bacteria, viruses, nematodes, and attacks by the pest, mites, and insects including brown planthoppers heavily affect the local rice farming. The socio-Economic and institutional pressures are also influencing and reduce the productivity (Herath Banda *et al*., 1998). To conquer these problems, lots of novel approaches based on new technology, including the use of fertilizers, the introduction of improved rice varieties, and enhancement of irrigation facilities and government subsidies are needed.

2.1.2 Abiotic stresses in rice cultivation

Abiotic stresses are the adverse impact of non-living factors influencing on living organisms, which exceed the regular rate of encountering variations in a specific ecological zone (D. Vinebrooke *et al*., 2004). Temperature variations, salinity, drought, submergence, nutrient deficiencies, and heavy metals (Fageria *et al*., 2008) mainly affect the development of the plant, crop productivity and substantial crop losses worldwide (Davis *et al*., 2016; Huang *et al*., 2013; Tuteja and Gill, 2016). Sri Lanka has experienced a temperature increase of 0.016 C° per year in the last few decades (IPCC, 2007). The rice cultivation has shifted to more water stressed conditions in *Maha* season, due to the impacts of climatic changes that alter the rainfall patterns (Dharmarathna *et al*., 2014). It is recognized that 3 % of the total farmlands are affected by salinity (RRDI, 2019). The submergence ravages half of the local farmlands within the rainfed low land ecosystem (Mackill, 1996). The P is an important nutrient to the rice, and P deficiency directly affects the yield. High usage of the P fertilizers is also leading to many environmental, health, and economic crises in the country (Aluwihare *et al*., 2015). As a solution, the novel molecular techniques are focusing on identifying and improving phosphorus-deficiency tolerant crop varieties (Cordell *et al*., 2009; Rose *et al*., 2011).

2.1.2.1 Temperature

The crop growth requires optimum growing conditions in order to perform well. Generally, the optimum temperature for the normal development of rice plants ranges from 27 °C to 32 °C (Yin *et al.,*1996). The threshold temperatures limit for grain formation and ripening is laid at 34 °C and 29 °C, respectively (Morita *et al.*, 2004; Yoshida, 1978). The yield

reduction and grain filling can be recognized beyond the threshold levels. Due to the influence of global warming, rice biomass production has been diminishing, and grain sterility is becoming a critical issue. (Yoshida, 1978). A high level of night temperature is more destructive than the day-time temperatures. Booting and flowering stages are highly temperature-sensitive and can cause sterility under higher temperature levels (Satake and Yoshida, 1978; Farrell *et al.,* 2006). Humidity at higher temperatures enhances the spikelet sterility as well (Yan *et al.,* 2010). The tropical countries are highly influenced by temperature fluctuations. The highly affected countries include China, India, Bangladesh, Thailand, Indonesia, and Vietnam, which are the leading rice producers in the world (Seck *et al.,*2012).

Almost all the growing stages of the rice plant are temperature sensitive. Under severe temperature levels, specific responses are shown. More extended pollen viability, larger anthers, elongated basal dehiscence, and presence of large basal pores are some of the significant features identified, which express under the cold conditions at the flowing stage whereas, high level of temperature is fatal to most of the physiological processes along with stomatal opening, photosynthesis, growth, and grain yield. The reproductive stage of the plant is highly sensitive to temperature variations and, it is closely related to the amount of yield (Prasad *et al.,*2008; Jagadish *et al.,*2009). Because of this closer relationship, most of the temperature tolerant rice varietal improvement practices are mainly focusing on the reproductive stage. Identification of the expression of heat shock proteins (HSPs), which could be able to protect structural proteins, enzymes, and membranes at extreme temperature levels, is also helpful in temperature-dependent rice varietal development and breeding programs.

2.1.2.2 Drought

Over half of the world's rice production is rain-fed (McLean *et al,*. 2002), and the chance of befalling natural disasters such as drought and flood has become increased partly due to the alteration of global climate patterns (Tao *et al.,* 2003). Among the current climatic challenges to rice cultivation, one of the significant production constraints in rain-fed rice farmlands is drought. Especially in Asia, approximately 42 million hectares of shallow rain-fed lowland rice and upland rice cultivation (Huke and Huke, 1997), are subjected to sporadic or frequent drought stresses. Rice is sensitive to drought stress during the reproductive growth stage and, even under moderate stress, a drastic reduction in grain yield can be seen (Hsiao, 1982;

O'Toole, 1982). Germination, floret production, shoot and root proliferation, and photosynthesis are obstructed severely under the drought conditions. Not only that, grain filling, plant water and nutritional relations, and cell respiration are also affected by drought (Harris *et al.*,2002; Kaya *et al.*,2006). In Sri Lanka, the domestic rice production had been declined by 46 % to 2.4 million metric tons during 2017 due to the impact of adverse weather conditions, mainly with drought. That was ended up with the lowest local paddy production over the last decade (Central Bank, Sri Lanka, 2017).

Various morphological, biochemical, and physiological responses are induced in drought-tolerant plants to survive successfully under stress. The scientists in China have dissected the genetic basis and mapped the genes (QTLs) in crosses between drought-tolerant germplasm and elite cultivars (Yue *et al.,* 2006). The leaf water content, drought score, spikelet sterility, grain yield, and delay in flowering are identified as the drought-tolerant traits in rice plants (Jongdee *et al.,* 2006). These phenotypic traits and their QTL associations can be employed in molecular breeding approaches to develop new varieties with drought tolerance traits. As reported in Capell *et al.*, (2004), the modulation of the polyamine biosynthetic pathway in rice plants by transforming the *Datura stramonium*'s *adc* gene is also practiced in transgenic drought-tolerant rice variety production. It promotes spermidine and spermine synthesis, and a higher level of spermidine helps to protect the plant from the adverse conditions. The marker-assisted backcross breeding approach and QTL introgression in several other popular varieties also test with constructing drought-tolerant rice germplasm (Kumar *et al.,* 2014).

2.1.2.3 Salinity

Salinity can be considered as another severe abiotic stress, which includes all the problems due to salts. The salt stress is mainly because of the abundance of sodium chloride from natural accumulation or irrigation (Flowers and Flowers, 2005). The cations like Na^{+}, Ca^{2+}, Mg^{2+}, and anions such as SO_4^{2-}, Cl^{-}, HCO^{3-}, with residual amounts of K^{+}, CO_3^{2-}, and NO_3^{-} affect the occurrence of salinity (USDA-ARS, 2008). The soil with a pH in between 7-8.5 is pleasing to accumulate these ions in more significant amounts (Mengel *et al.,* 2007). A higher chance for salinity is mainly identified, especially in the farmlands in coastal areas worldwide (Reddy *et al.,* 2017), due to the higher sea levels that bring saline water further inland and expose more rice-growing areas to be salty. According to FAO (2008), more than 8×10^8 hm^2 lands throughout the world are affected by salinity. This stressful condition

reduces the growth of rice plants considerably (Roy *et al.*,2014). High levels of salts in soil affect plants by forming osmotic stress or ionic stress (Ghosh *et al.*, 2016). High salt levels in plants influence stomatal closure, seed germination, seedling growth, and the amount of yield (Zeng and Shannon, 2000). It raises the internal temperature in leaves and increases the inhibition of shoot elongation (Rajendran *et al.*, 2009).

The salt-tolerant traits are significantly worthy of salt-tolerant varietal selection. The traits like leaf size, shoot growth, shoot and root length, shoot dry weight, shoot fresh weight, number of tillers per plant, flowering stage, spikelet number, percent of sterile florets, and productivity are some of the significant traits, that can be used in varietal selection. (Munns and Tester, 2008; Hakim *et al.*,2010). The selected genotypes with a high degree of salinity-tolerance can be used as the parents in the marker-assisted selection or genetic engineering by introducing salt-tolerant genes (Reddy *et al.*,2017). The various breeding approaches, phenomics based system / biological approaches and transgenic methods are few of the most common salt-tolerant varietal improvement practices (Reddy *et al.*, 2017)

2.1.2.4 Soil fertility

Typically, a plant needs a balance, and a versatile nutrient profile to maintain its normal physiological functions. The elements, Na, P and K, play a massive role in the nutrient profile and the plant growth and developmental process (Tahir *et al.*, 2019). Along with N, P and K, Ca, Mg, S, Fe, Zn, Mn, B, Cu, and Al are also identified as the vital macro and micronutrients, which are essential to maintain a fertile rice soil. Instead of elemental deficiencies, Al, Cu, Be, Mn, iron, and P toxicity also can be affected by sustainable rice production (Fageria and Moreira, 2011). The application of N and P enhances rice yields, while K has a less effect on yield. The soil N levels exhibit slight fluctuation with time, but the soil P levels are significantly decreased concerning subsequent cropping seasons. However, the application of NPK fertilizers is vital to maintain soil NPK levels consistently in the commercial cultivation fields (Shen *et al.*, 2004). After two or three cropping seasons, the demand for the P and K is increased, and the external application of the fertilizers are needed. The use of the N, P, and K showed significant improvement in the gain of production (De Datta *et al.*, 1988). Due to the high rate of annual depletion for NPK from rice fields, the African countries experience a loss of USD 4 billion yearly (Sanchez, 2002). In Sri Lanka, over 0.6 million metric tons of fertilizers are imported annually, and among them, about 59 % is urea. The remaining included other fertilizers such as sulfate of ammonia,

triple superphosphate, and muriate of potash (Wijewardena, 2005). Instead, of spending millions of capitals on purchasing fertilizers annually, alternate crop cultivation methods, alternative P fertilizer utilizing methods (Fukuda *et al.,* 2012), and introduction of the nutrient's deficiency tolerant rice varieties (Wissuwa *et al.,* 1998) can be used as new options to surmount this dilemma.

2.1.3 Biotic stresses in rice cultivation

Fungi, bacteria, viruses, nematodes, and insects are the most common biotic influents in Sri Lankan crop cultivation. On the other hand, diseases such as rice sheath blight, rice blast, brown spot, rice thrips, gall midge, leaf folders, and attacks by BPH, yellow stem borer, rice bugs, and false smut are of significant concern in Sri Lanka (Davis *et al*., 2016; Wang *et al*., 2005). Among them, BPH outbreak which is very famous and dangerous, destroyed thousands of acres of rice fields in the world including Sri Lanka during last few decades (Dyck and Thomas, 1979; Khush *et al*., 1985). Over the last decade, BPH attack has been identified in a large portion of rice fields all over the country, especially in Ampara district. During 1974, 16,200 hectares was ravaged, and 2,800 ha of rice cultivation was lost (Fernando *et al*., 1979) BPH attacks infrequently induce hopper burns as well. The genetically improved varieties are used to overcome these types of biotic stresses, instead of using chemical control methods. A survey initiated in 1996 unveiled that the trend is to cultivate newly bred rice varieties for increased pest resistance, alternately of using traditional varieties (Nugaliyadde *et al*., 2000).

2.1.3.1 Microbial effects

Microbes in the soil play a vital role in the plant growing processes. While utilizing plants as hosts, a plenty of interactions are maintaining between the plant and the soil microbial community. These interactions may help to promote plant growth, improve drought-tolerance, assist in environmental remediation, and even promote the defensive responses against pathogens (Jones *et al.,* 2019). Fungi, bacteria, viruses, nematodes, and protozoans are the dominant microbial groups present in the soil. Many studies have revealed that the soil microbes in the rice fields are helping to enhance the growth and yield of the rice by producing growth regulating substances, increasing soluble phosphate uptake, degrading cellulose, N-fixation, and siderophore production. Some microbial species also intermediate cell signaling and regulation in the rhizosphere (Doni *et al.,* 2013). However, some of the other microorganisms have resulted in a devastating effect on paddy cultivation.

Pathogenic diseases are the most harmful impact of the microbial implications, and this is a dominant bottleneck effect against sustainable rice production. The key identified pathogenic groups present in rice fields consist of fungi, bacteria, viruses, and nematodes. Fungi infections cause rice blast, sheath blight, brown spot, leaf scald, stem and sheath rot, and false smut. The dominant bacterial diseases are blight and leaf streak disease. The ragged stunt, grassy stunt, and tungro are the most common viral infections in rice cultivation, while nematodes causes white tip and root-knot diseases (Elazegui and Islam, 2003).

Bacterial leaf blight: This disease is considered to be one of the most significant microbial attacks in rice plants worldwide. This disease can be seen in both tropical and temperate countries (Saha *et al.,* 2015). The causative agent of the bacterial blight is *Xanthomonas oryzae pv. oryzae* (Xoo). The impact of this disease was responsible for a loss of 6-60 % of the yield (Ou 1985; Singh *et al.,* 1977). Wilting of seedlings or wilting and leaf yellowing are the significant symptoms of this infection.

Initially, at the seedling stage, the infected leaves are rolled up and appear in grayish-green color. When the disease progresses, the leaves turn into yellow to straw-color and get wilted, ending with the death of the plant (Rice Knowledge Bank, 2019; Elazegui and Islam, 2003). Higher nitrogen fertilization, range of temperature between 25-34 C°, and relative humidity above 70 % are the most favorable physical conditions to spread this epidemic. The yield loss is the significant effect of this infection, whereas bacterial blight does not affect yield if the disease has undergone at the booting stage. However, the quality of the grains gets reduced, and a high proportion of broken kernels can be obtained (Rice Knowledge Bank, 2019).

Rice blast disease: This is another globally spread, acute microbial infection, which has caused to reduce the annual rice production by 10 – 30 % (Skamnioti and Gurr, 2009). This massive loss was created by the fungi called *Magnaporthe oryzae*. This fungus is only capable of affecting above-ground parts of a rice plant, including parts of panicles, node, collar, neck, leaf, and sometimes leaf sheath (Gnanamanickam and Mew, 1992). Frequent and prolonged periods of a rain shower, low soil moisture, and cold temperature in the daytime are the most favorable conditions to persist this mold. The more significant temperature gradient between day time and night resulted in dew formation, and that will be highly favorable to develop blast disease (Kato, 1976). The cold temperature with dew

stimulates the fungus sporulation and conidiophore formation. The developing appressorium of *Magnaporthe oryzae* attaches to the plant and penetrates the plant tissue. It creates a lesion on upper vegetative regions of the rice plant further. Symptoms can be either lesions or spots. The shape, color, and size of the symptoms vary depending on varietal resistance, environmental conditions, and the age of the wounds. The attacked young leaves visualized as purple spots, and the infection can kill seedlings or plants up to the tillering stage (Gnanamanickam and Mew, 1992; Kato, 2001). Infection at the neck node produces triangular purplish lesions, followed by lesion elongation over the entire neck. Collar, panicles, and nodes are also affected in the same way and ended up with lethal abrasions, which are particularly devastating, causing up to 80 % yield losses in severe epidemics (Singh *et al.,* 2011).

As reported by Kato, 2001, several measures can be taken to control this overwhelming infection. Some of the most pragmatic approaches that can be taken against blast including burning infectious tissues, limiting the pathogen dissemination, using healthy seeds for the seed establishment, practicing proper fertilizer management, using of cultural systems, launching of the forecasting method, using chemical control, and using resistance rice varieties. The fungal ability to evolve and develop resistance against the improved rice cultivars, the varietal improvement, only shows a partial success. Therefore, chemical pesticide and fertilizer applications are still using to fight against the epidemic (Khalil *et al.,* 2014). However, due to the health concerns and environmental toxicity from fungicides, developing blast resistance rice varieties with multiple resistance genes is highly required.

2.1.3.2 Weeds

Weeds are undesirable and unwanted plants in the commercial crop cultivations. Weeds utilize nutrients, land, water, sunlight, and other resources competitively, and create adverse effects on crop cultivation. These plants are a nuisance for all sorts of crop cultivation and land management process, and they affect rice cultivation significantly. While competing for the resources available, weeds facilitate the pest and pathogenic microbes to disseminate as an alternative host. This effect leads to reducing the yield and quality of rice by 10 – 45 % (Agritech, 2019). The impact of the weedy plant became less when the rice establishment is done by transplanting (Ho, 1996). In the rice establishment by direct seeding, the impact of the weedy plants is at a higher level, and most of the direct-seeded rice fields are affected by more weeds (Karim *et al.,* 2004). The direct-seeded fields provide aerobic conditions for

weeds, as they are not flooded during the initial growth stages of the crop. These conditions may be favorable for weed growth at a high level along with competitive grassy weeds (Moody and De Datta 1982).

Species in family *Poaceae* are the most common class of weeds. *Cyperaceae* is the second-largest weedy family, and *Alismataceae, Asteraceae, Fabaceae, Lythraceae,* and *Scrophulariaceae* are also typical weedy grass families (Smith Jr, 1983). *Echinochloa crus-Galli* is the most troublesome weed in rice cultivation, which owns the highest distribution within the globe (Holm *et al.*, 1977). *E. colona* is the second most harmful weedy grass, and other important weedy species include *Cyperus difformis, C. rotundus, C. iria, Eleusine indica, Fimbristylis littoralis, Ischaemum rugosum, Monochoria vaginalis, and Sphenochlea zeylanicsa.* The loss of rice yield due to these weeds has ranged between 5 and 72 % (Kuan *et al.*, 1990). Therefore, efficient weed control and management process are needed. Herbicide-based weed management is the most popular method that the world has practiced. However, with the nasty outcomes of the chemical control method, the scientists try to identify human and eco-friendly alternative for weed management. Crop rotation with allelopathic crops and rice cultivars, growing competitive rice cultivars, weed-smothering with green manure, cultivation under controlled conditions, and the introduction of herbicide tolerance varieties via breeding are the most realistic approaches to tackle these problems (Labrada, 2003).

2.1.3.3 Insects and pest attacks

Insect and pest attacks can be undergone on any part of the rice plant at any growing stage. More than 100 insect species are sustainable to attack, and 20 of them can become responsible for themassive economic damages (Pathak and Khan, 1994). Rather than infesting all the parts of the plant, they are liable to spread a few viral transmitting diseases as well. According to the Cramer's estimation, the highest yield reduction of 31.5 % due to insects and pest attacks had recorded, mainly in tropical Asian countries and 21 % of the decrease from the North and Central Africa (Cramer, 1967). After that, a series of experiments coordinated by IRRI and conducted in farmer fields in six Asian countries and identified almost all the insects and pest attacks, which affect the commercial rice production (Heinrichs, 1994)

The root feeders, stem borers, leafhoppers and planthoppers, defoliators, and grain sucking

insects were identified as the most dangerous group of pests and insects in rice cultivation. Not only the vegetative parts of the plant, but the storage of the grains is also affected by these species (Heinrichs, 1994). With the introducing of new varieties, the pathogenesis behaviors of the insects and pests had been completely altered, and the threatening of the stem bore get reduced. However, leafhoppers, brown planthopper, rice leaf folders, white-backed planthopper, and some other bugs becoming more treacherous to the modern rice cultivars (Pathak and Khan, 1994). Chemical control methods and other classical pest controlling measures did not show enough potential to settle this problem. Therefore, the development of the pest and insect resistance rice varieties has become more crucial to maintain global rice production at a sustainable level.

Brown Planthoppers: During recent decades, brown planthopper (BPH) (*Nilaparvata lugens*) outbreak became a sporadic disaster as a significant pest in rice cultivation in the tropical counties (Rombach *et al.,* 1986). The light infestations of BPH cause a reduction of the plant height and crop vigor, a smaller number of productive tillers per plant, and defects in the number of filled grains per panicle. Heavy infestations resulted in the hopper burns, which leads to the complete drying and death of the crop (Ou and Rivera, 1969). Not only that, BPH is serving as a vector to transmit a grassy stunt virus and *Nephotettix virescens* virus disease, which may seriously harm the rice crop (Laksminarayana and Khush, 1977). BPH, is belonged to order Hemiptera, who are sucking insects having the capability to remove plant sap from the xylem and phloem vessels of the plant. This impact may cause severe damage to perform regular metabolic activities within the plant cells. Then the damaged plants get dried and appeared with a brownish color, called hopper burns. These symptoms can be spreading out rapidly and end up with the complete yield destruction. One of the most important strategies to control BPH is varietal improvement with BPH resistance ability (Kabis and Khush, 1988). As mentioned in Laksminarayana and Khush (1977) and Athwal *et al.,* (1971), the BPH resistance genes have been identified as *bph1*, *bph2*, *bph3*, and *bph4*. These genes can be introgressed into the elite cultivars using MAB, and that approach accommodated to control BPH epidemic up to a satisfactory level.

2.1.4 Strategies to overcome stresses

The stress controlling approaches are necessary to maintain the maximum gain of the rice cultivation. The enhancement of the soil fertility by applying fertilizers, using new technology (Walisinghe *et al*., 2010), intensive selection of proper lands, timely cultivation

plan based on the rainfall and the climate, and usage of chemical control methods to overcome biotic stress are some of the most common approaches that can be used to face these obstacles. The crop management under standard establishment, weed management, control of insects, and disease, post-harvest supervision, rice-based integrated farming and development of farmer organizations are also helping to surmount these problems (Dhanapala, 2007).

Fertilizers are applied to the soil as nutrients. Phosphorus, which is one of the main constituents in soil, is highly important for the soil fertility, at insufficient levels causes low harvest. Therefore, 41,000 tons of triple superphosphate is annually imported to Sri Lanka, and 80 % of them are used for rice cultivation (Wijewardena, 2005). Sri Lanka spends 0.3 billion US dollars annually to import P containing fertilizers (Aluwihare *et al*., 2016), and it is a heavy burden to the national economy. The introduction of the improved P efficient varieties has become a successful replacement than applying more fertilizer within the last few years. Variety selection helps to identify the most suitable variety with desirable traits under different biotic and abiotic conditions (Dhanapala, 2007). Introduction of the improved varieties with useful traits can be done by using the identification of the genes related to stress tolerance capability with the use of molecular techniques like microarray analysis and then, transform identified genes into novel varieties (Fowler and Thomashow, 2002; Rabbani *et al*., 2003)

The initial step to introduce PDT rice varieties is the recognition of the genes related to high P uptake, and utilization by the use of molecular linkage maps and QTL studies (Wissuwa *et al*., 1998). A major QTL related for PDT is designated as *Pup1*, which is positioned on the rice chromosome 12. Other minor effect QTLs are also located on chromosome 2 and 6 and 10. (Ni *et al*., 1998; Wissuwa *et al*., 1998). Studies were conducted to identify the PDT and sensitive cultivars within rice germplasm and integrate them into P deficiency sensitive varieties to enhance the yield under low P conditions (Aluwihare *et al*., 2017).

BPH attack is the most devastating biotic stress in local rice cultivation. BPH tolerant varieties are the most successful solution today to overcome this situation. (Pathak and Khush, 1979). BPH resistant varieties are initially developed in 1967 (Pathak *et al*., 1969). Among them, 20 resistant genotypes out of 985 native varieties of Sri Lanka are identified (Fernando *et al*., 1979). In these studies, 21 major genes related to BPH resistance were

identified, and 13 among them are dominant (Jena and Kim, 2010). The identified genes are designated as *bph1* (Khush, 1977), *bph2* (Laksminarayana and Khush, 1977), *bph3* and *bph4*, which are widely using in rice breeding programs (Jairin *et al*., 2007). Further, with the molecular genetic investigations, some of new BPH resistant genes were invented and mapped. Then gene pyramiding is done with the help of marker assisted selection methods to attain a strong resistance (Li-Hong *et al*., 2006; Sharma *et al*., 2004).

2.2 Crop improvement

The genetic-based approaches to crop improvements were used for long time, without having proper molecular biological knowledge. Selection by the crop domestication was the initiate approach in crop improvement. The plants with desirable traits were selected in domestication, where the genetic variability tends to be lower than in wild type relatives, as a consequence of the founder effect. All the valuable genes were not preserved in domestication, so genes related to other important traits such as pest resistance and stress tolerance are left out from the gene pool (GP) with the selection and domestication (Zamir, 2001).

During 1970 - 1995, the market demand for rice could fill due to the introduction of high-yielding semi-dwarf rice varieties (Khush, 1995). Later on, with the rapid increment of the world human population with time (Lutz and Qiang, 2002) and other socio-economic factors including a decline in arable land, inadequate supply of water, global climatic changes, labor shortages and high expenses (Peng *et al*., 2009), the demand for food production got increased. If the farmers disable for adequate supply, the food shortage can occur (Khush, 1995). The enhanced production of rice is needed to solve this dilemma. Application of the machinery in land preparation, crop establishment, water and nutrient management, harvesting and post-harvesting, improvements in soil cultural conditions and in the timeliness of planting, cultivating and harvesting are identified as the key determinants to improve the rice production. Use of herbicides and pesticides in controlling weeds and pest result in the higher yield. Improved crop management practices, such as nitrogen fertilization and adequate irrigation facilities, also affect the high yield of crop cultivation (Duvick, 1984; Peng *et al*., 2009).

The varietal improvement is one of the novel techniques which is used to increase rice production (Duvick, 1984). In here, the new genetic variations are incorporating to selected

variety without losing them from the germplasm. The selection based on the desirable variants, selection of expanded variation by controlled mating and selection based on the inheritance pattern of the traits within the genome and its recombination is the earlier method in conventional plant breeding (Breseghello and Coelho, 2013).

2.2.1 Rice breeding

From past to today, farmers have been changing the genetic makeup of the crops based on phenotype and stored them for the succeeding cultivation. Later on, plant breeders used the knowledge of genetics to select for specific desirable traits to develop improved varieties. A large amount of successful genetic variations from the old landraces can be saved within the novel varieties with the use of breeding (Sasaki, 2011). The traits like faster growth, higher quality yields, pest, insect and disease resistance (Khush, 1995), larger seeds, or sweeter fruits were improved in domesticated crops, which are relatives of the wild genotypes (Whitehead *et al*., 2017). By improving defensive traits, such as the utilization of nitrogen fertilizer, standability, major abiotic stresses, including heat and drought tolerance, and the yield can be enhanced (Peng *et al*., 2009). Today in a realistic world, all the breeding programs are involved with some amount of hybridization (Xu, 2010). The initial approach of producing improved plants was conventional breeding. With the findings in molecular biology, molecular techniques have also cooperated later with the conventional breeding, and the new discipline of plant breeding is formulated, called molecular breeding.

Releasing of new varieties leads to reduce hunger, malnutrition, poverty and increase the income of poor people. The improved varieties are proficient of increasing production efficiency and making food more accessible to all by reducing the market price, which will be leading to the transition from low productive subsistence agriculture to a high productivity agro-industrial economy (Just and Zilberman, 1988). These findings may completely alter the socio-economical behaviors of the society; including raising employment, increasing wage rates of the laborers and uplifting the livelihood of rural farmers who might be suffering from droughts and other abiotic and biotic stresses (De Janvry and Sadoulet, 2002; Irz *et al*., 2001). Hybridization, integrating germplasm, polyploidy breeding, transgenic breeding, and mutation breeding are the common approaches following in the conventional plant breeding process.

2.2.2 Conventional breeding

Conventional plant breeding, which is the process of identifying and selecting plants with desirable traits and combining those selected desirable traits into one individual, is based on phenotypic traits. The fundamental principles of conventional breeding are explicitly specified in Mendel's laws of genetics. The gene is the control unit of all trails, which are positioned in the chromosomes, and during breeding, the gene/allele combination on chromosomes is manipulating. The first massive scale conventional breeding strategy used in crop cultivation was recorded in 1930. The new and improved varieties of wheat, soybeans, rice, and grain sorghum were cultivated initially, and as the consequence of that, first hybrid maize yield was increased by 15 – 20 % and later on, it was raised to 50 % with the support of genetic alteration in hybridization (Duvick, 1984).

Combining the desirable characteristics from the superior plants to the novel plant offspring, and breeding them by artificial mating and cross-pollination was done in the past. The plant selection was made by mining a plant with desirable traits among thousands of descendants in the field manually. Depending on the plant species, the methods of cultivar development in conventional plant breeding diversifies. As listed in Table 2.1, the suitable breeding programs rely on the mating pattern of the plant and the expected result. In conventional breeding, seven types of cultivars are developing, including pure-line cultivar, open-pollinated cultivar, cross-pollinated cultivar, hybrid cultivars, clonal cultivars, apomictic cultivar, and multiline. Pure-line, pedigree, single seed descent, bulk, and backcross breeding methods can be applied to develop a single self-pollinated cultivar. If the target is to produce a mixture of plants; mass selection, composites or blends, and recurrent selection are the most suitable breeding methods (Borojevic, 1990). Mass Selection is only an inexpensive, simple, older, and rapid selection method which can be used for either cultivar development or cultivar purification. Pure-line selection ends up with narrow genetic-based identical alleles for all loci (Allard, 1960). Pedigree Selection, which is mainly based on breeding history and the $F_{2,}$ is a tedious and time-consuming breeding program. Then plants are reselected in subsequent generations until a desirable level of homozygosity is accomplished (Allard, 1960). Bulk population breeding is a late applying artificial selection method under the effect of natural selection pressure. Under natural selection, the desirable genotype can be lost. Self-pollinated cultivar development is highly suitable in improving individual plants, such as cross-pollinated cultivar development for advancing population.

Identification of the parental lines in hybrid cultivation is the most important, expensive, and time-consuming phase (Hallauer, 1967). The first generation of the hybridization is known as a commercial hybrid, which occupies a high level of heterozygosity since both alleles are fixed from different parental lines (Lamkey and Edwards, 1999). The single crossed hybrids were widely used in late nineties (Crow, 1998), and then multiple trait integration with the various parental association became popular, stable and more economically friendly approach (Peng *et al*., 2014). Production of the superior inbred lines by selfing is the initial step in hybridization which can be done by physical and chemical control of mating in earlier, and today it is done with the emasculation of male sterility genes (Poehlman and Sleper, 1995). After the superior inbred line was distinguished, breeders planted them separately under specific field design and produced superior inbreds (Kempe and Gils, 2011).

Table 2. 1 The steps and key features of conventional breeding methods.

	Type	Steps in breeding	Key features
Self-pollinated cultivars	Mass selection	i. Check for off types and discard them within the heterogeneous population ii. Purify in progeny rows and rogue out iii. Bulk harvest at last	• The oldest method of selection • Acquire limited genetic variability • Interpopulation improvement method by increasing genetic frequency • Can develop cross-pollinated species
	Pure line selection	i. Space plant the variable population ii. Select superior plant and selfing iii. Conduct repeated cycles of progeny rows iv. Conduct preliminary and advance yield trial v. Release the line with the highest yield	• Contain identical alleles for all loci • Isolate genetically different lines • All the traits within the line are not heritable
	Pedigree selection	i. Establish a base population by making a cross of selected parents. ii. Space plant progenies iii. Keep accurate records of selection for each generation	• Evaluate efficiency at the end of the process • Need superior genotypes selection and proper records of the selected plant's ancestry • Obtain a high degree of genetic purity in the progeny • Can develop cross-pollinated species
	Bulk population breeding	i. Cross $F_1 \rightarrow F_2$ ii. Close space the selected plants iii. Repeat the cycles of bulk harvesting iv. Plant the sample seeds. v. Select plants and conduct primary and advanced field trials, and then release superior lines	• Delay in artificial selection • Natural selection increase desirable genotype frequency for disease resistance, weed, herbicides, and pest resistance and stress tolerance
	Single-Seed Descent	i. Generate a large F_1 population ii. Advance single seed per plant up to F_5 iii. Harvest the F_6 superior seeds iv. Plant in progeny rows v. Conduct preliminary and advanced yield trails	• Delay in plant selection • Attain rapid homozygosity • Less space to early generations • Speed up breeding by randomly select seed from each of the desirable F2 plants for the next generation
	Back cross-breeding (can use with cross-pollinated species also)	i. Cross the recurrent parent with the donor parent ii. Cross F_1 with the recurrent parent again. (repeated crossing of F_1) iii. Self them, if needed to gene fixation	• Influence the selection pressure in selection • Female parent selection is essential, due to occurring cytoplasmic inheritance • Works the best with qualitative traits. • Replace a specific undesirable gene with a desirable alternative
	Multiline breeding and cultivar blends	i. Derive a series of backcross-derived isolines or near-isogenic lines ii. Generate hybrid and, repeat back-crosses of a hybrid with both homozygous recurrent and self them	• An expensive method to create mosaic • Have greater environmental buffering to resist biotic or abiotic factors and disease resistance • Increase heterogeneity of the cultivar • Show uniform phenotype with stable yield
	Composite	i. Select parental materials ii. Inter-mate the selected genotype iii. Evaluate and mix the parental material of superior crosses in an equal amount	• Similar to the multiline breeding • Able to work with a mixture of different genotype, which is not firmly related • Serve as a continuous source of new entries in the breeding
	Recurrent selection	i. Retain superior seeds from the superior plant ii. Plant the seeds and make all possible intercrosses iii. Composite intercross seed and individual plant progenies	• Include, pre-selection generation and inter-mating of the selected plant to produce the population for the next cycle of selection • A Cyclical improvement technique

Table 2.1 Continued.

	Type		Steps in Breeding	Key features
Cross-pollinated	Recurrent selection		i.Cross parents in all possible combinations ii.Evaluate the plants or families, and select the new set of parents iii.Inter-mate the selected parents to produce the population for the next cycle of selection	• Plant advancing by intermating with repeating cycles to improve cultivars • Improve the performance of a population concerning one or more traits of interest
Cross-pollinated	Interpopulation improvement methods	Individual plant selection method	i.Rouge out the undesirables in source population before flowering ii.Repeat cycles of preliminary yield trial with a check, and conduct advance yield trail	• Specify mass selection method for line selection
		Family selection method	i.Create a family structure. ii.Evaluate of families and select the superior ones by progeny testing. iii.Recombine selected families or plants within the population	• **Half-sib family selection methods** (HSFS): evaluation and recombination occur in one generation • **Ear to row selection:** a simplified version of HSFS • **Modified Half-sib selection** – Modify version of HSFS • **Full-sib family selection:** Involves selfing (S_1 or S_2 family selection). Select family based on testcross families to create a new base population for the next cycle of selection.
		Family selection method based on test cross	Evaluate the genotypes of the selected parents with tester parents • **HSFS with progeny test** • **HSFS with test** • **Interpopulation improvement methods** • **Half-sib reciprocal recurrent selection** • **Full-sib reciprocal recurrent selection**	
	Synthetic cultivar		i.Assemble the parents. ii.Assess the general combining ability iii.Produce synthetic cultivars by random mating	• Create from a cross-fertilized all combinations of parents • Intrapopulation development method • Decline inbreeding depression in vigor
	Hybrid cultivar		Cross the pairs of unrelated inbred lines	• Intrapopulation development method • Strength the cross expression by hybrid vigor • F_1 progeny is highly favorable with the favorable traits
	Clonal cultivar		• Identical copy • Asexual method of plant reproduction • Involve in vegetative propagation and tissue culture applications	
	Apomixis		• Commercial hybrid production methods can be simple • No more crosses • can be retained vigor • Produce seeds without fertilization • Diplospory, apospory, and adventitious embryony are three main types	

(Acquaah, 2012)

2.2.3 Weaknesses of the conventional breeding and need of the molecular breeding

Conventional breeding is successful in developing the plant-holding cultivars in green evolution and hybrid rice development in the 1970s. It is still an essential technique to generate new crop varieties and assure food security. However, it is a tediously long process and feels like little bit out-dated when compared with the novel molecular techniques. Subjective evaluation and observational selection are critical components in conventional breeding. Today with the addition of scientific knowledge on breeding, less subjectiveness and more science are needed in the practical and accurate evaluation, and effective and efficient selection process (Jiang, 2013a). As conventional breeding is based on the development of phenotypic traits, the availability of access to the plant genetic resources is limited. When crossing with a landrace by targeting specified allele, some of the undesirable alleles can be segregated and transferred into progeny. Therefore, the breeders have to break the association between these loci by using pre-breeding steps such as the production of pure lines by selfing, and it is essential to verify the segregation of loci. These types of allelic identification cannot be done with conventional breeding, and the advance identification methods based on DNA markers are needed (Sasaki, 2011).

Traditional breeding is thoroughly based on the phenotypic data of the parents, therefore little or nothing was identified about the segregation and genomic positions of the genes. This situation changed with the advent and propagation of molecular marker technologies, which made it feasible to track the transformation of chromosome segments from parental lines to the progeny (McNally *et al*., 2009). Gene pyramiding cannot be done with conventional breeding (Singh *et al*., 2001), as the multiple inheritances of the genes cannot be monitored. The most powerful phenomena such as genetic mapping, map-based gene discovery, QTLs, gene pyramiding, germplasm evaluation, and development, are highly beneficial than conventional methods of plant breeding (Breseghello and Coelho, 2013; Jiang, 2013a).

2.2.4 Molecular plant breeding

The in-depth use of molecular applications is needed to understand the genetic and physiology of the improved traits (Duvick, 1984). Usually, sequence alterations in the segment of the recombinant line can be used to identify parental inheritance. Visualization of the allelic segregation confirms the genetic combination and result of the cross. DNA markers including, simple sequence microsatellite markers or Simple Sequence Repeats

(SSR), Single Nucleotide Polymorphism (SNP)s and Genotyping by Sequencing (GBS) are frequently used to recognize the genetic control and metabolic process in a resulted progeny. By genotyping the molecular markers in progeny, the recombination frequencies can be estimated, and the high-resolution genetic map exploit genetic linkage between markers and important crop traits (Edwards *et al*., 1987; Paterson *et al*., 1988) can be constructed, which help to understand the genetic background of the crosses for planning, and monitoring proper breeding programs.

When identified DNA markers are used in breeding, it is called as MAB. This is based on genotype, so the environmental effect on traits can be omitted in the breeding. Molecular markers are cheap, faster, and accurate. By the way, molecular breeding cannot replace conventional breeding, and it only complements the use of it with the help of molecular techniques as the optimal strategy to trace the genomic segregation (Collard and Mackill, 2007; Jiang, 2013a; Ribaut *et al*., 2010)

2.2.4.1 Molecular/ DNA markers

Biological markers, also known as biomarkers, are measurable indicators which are used to determine some biological state and condition. Morphological markers, cytological markers, biochemical/ protein markers, and the DNA/ molecular markers are the major four types of biological markers. Among them, the DNA markers are widely used in highly accurate molecular level biological species identifications (Jiang, 2013b)

Different types of DNA markers are using today including RFLP, AFLP, RAPD, SSR, GBS and SNPs which are using with different polymorphism-detecting techniques and methods such as polymerase chain reaction (PCR) (Mullis, 1990), southern booting (Southern, 1975), DNA barcoding and sequencing techniques (Collard *et al*., 2005). High level of polymorphism, greater abundance within the genome and genome specificity, ability to distinguish heterozygotes from homozygotes, clear allelic identification, low cost, easy detection and automation, high availability in techniques such as duplex or multiplex PCR and independence from the phenotype are the properties of the DNA markers. These markers can determine allelic forms of genes or loci, and its transformation from one generation to next by tracking the target biological state including individuals, cells or tissues, genes, and alleles (Xu, 2010). At present molecular plant breeding, microsatellites or SSR, SNPs and GBS DNA markers are widely being used. Selection of correct markers according to the

breeding plan is essential (Semagn *et al*., 2006) since these techniques help in the selection of multiple desired characters simultaneously in all types of conventional breeding designs.

2.2.4.1.1. SSR markers

SSRs are also known as microsatellites, short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS). All of these PCR-based markers which contain short random repeats of 2 – 6 nucleotides, are highly abundant in animal and plant genome and they are genome-specific (Song *et al*., 2005). According to the recent findings, microsatellites are more abundant in transcribed regions (Morgante *et al*., 2002), and many of SSRs are spread all over the genome (Gupta *et al*., 1994). Hyper-variability, reproducibility, co-dominant nature (Koreth *et al*., 1996; Tautz, 1989), stability, locus-specificity, and random genome-wide distribution are the key features of SSR markers. (Akkaya *et al*., 1992; Powell *et al*., 1996; Röder *et al*., 1995) The variation of these repeats produces a high level of polymorphism (Song *et al.*, 2010).

As the DNA sequences flanking microsatellite regions are usually conserved, specific primers for these regions are designed for use in the PCR. By PCR and gel electrophoresis, the DNA sequences flanking in SSR sequence can be amplified and resolved. Depending on the resulted bands, the DNA polymorphism of the particular loci, where SSR sequence is located can be defined. PCR based SSR marker analysis is easy to handle and able to use as multiplex PCR (Coburn *et al*., 2002; Sharopova *et al*., 2002). The initial cost is low, and a small sample of DNA is sufficient for SSR analysis. High level of allelic variation is one of the essential attributes in SSR markers, which make them valuable genetic markers. The sequence information about the particular DNA region is initially necessary to design suitable primers in the SSR analysis (Song *et al*., 2010).

For QTL mapping, genetic linkage map construction, map-based cloning, MAB, germplasm analysis are the principal users of the SSR markers today. SSR markers in many crops are already identified, and marker data are publicly available, therefore breeders can use them in molecular breeding (Song *et al*., 2010). For the inheritance of closely related gene transformation, high-density SSR marker maps are needed. (McCouch *et al*., 2002).

2.2.4.1.2 SNP markers

An SNP is a single nucleotide base variation between two DNA sequences or individuals, which can be classified as nucleotide substitutions either as transitions or transversions. Single base insertions or deletions (indels) in the genome is also considered as SNP. These are the most abundant variation in the genome, which leads to phenotype (Risch and Merikangas, 1996). As single nucleotide is the smallest unit of inheritance, SNPs can be known as the smallest marker among the DNA markers. SNPs are highly abundant in animal and plant genomes including cording, non-cording regions of a gene or at the intergenic sequences in different frequencies. Typically, there can be a possibility of having an SNP for every 100-300 base pairs (bp) within the plant genome (Edwards *et al*., 2007; Xu, 2010). SNPs are most abundant, co-dominant markers, which are the simplest form for polymorphism with the high level of detection, hence they have become attractive and potential genetic markers in the genetic studies, high-resolution map construction and breeding (Nasu *et al*., 2002).

Detection of SNPs can be done quickly with RFLP or using the CAPS marker technique based on SNPs (Nasu *et al*., 2002). SNP genotyping assays, including primer extension, allele-specific hybridization, invasive cleavage, and oligonucleotide ligation are based on the molecular mechanisms (Sobrino *et al*., 2005), but today SNPs are widely detected by sequencing (Gupta *et al*., 2001; Xu, 2010). SNPs genotyping is used in genome-wide linkage analysis. They are also used to construct haplotypes and diploid haplotypes in association tests for use in clinical studies (Judson *et al*., 2000). In rice genome, frequency of most of the SNPs on all 12 chromosomes, their SNPs profiles and genetic distances were identified, which can help to plan plant breeding (Nasu *et al*., 2002). Among the hundreds of SNPs, the significant SNPs associated with the phenotypes are being identified statistically by conducting ANOVA tests and regression tests to identify SNP-based and haplotype-based markers for quantitative traits (Bader, 2001).

2.2.4.1.3 Genotyping by sequencing (GBS)

GBS, also known as Sequence-Based Genotyping, is the detection method for high throughput discovery of SNPs and simultaneous genotyping in multiple DNA samples. This method is technically straightforward, highly multiplexed, low cost (He *et al*., 2014), easy, quick, extremely specific, highly reproducible, reduced sample handling, no size fractionation, less number of PCR events are involving, no reference limitation and it enables

to access essential regions of the genome that are inaccessible in sequencing (Davey *et al*., 2011; Elshire *et al*., 2011).

The initial step in GBS is restriction enzyme-mediated complexity reduction. Then the genotyping of the resulted DNA fragments is done with Illumina platforms, and random markers across an entire genome are scored (Elshire *et al*., 2011). The digestion of multiple samples of genomic DNA to reduce the complexity is the initial step in GBS by choosing appropriate restriction enzymes. This digestion avoids the amplification of repetitive regions of genomes, therefore low copy regions can be targeted in PCR (Gore *et al*., 2007,2009) which simplifies the computational alignment process in sequencing. After digestion, the DNA fragments, barcodes, and common adapter pairs are plated with the T4 ligase and allow to ligate. The adaptors, then barcodes and finally common adaptor pair is ligated with the DNA fragment respectively. Then by heating, further activation of ligase can be inhibited, and with the use of size exclusion column, unreactive adaptors can be removed. Then the PCR can be conducted using appropriate primer pairs. The resulted PCR product, which is called restriction site-associated genomic DNA (RAD) are cleaned up, and the fragment sizes of the library are evaluated. High-density SNP discovery and genotyping from RAD is initially demonstrated by Baird *et al*., (2008). RAD are incorporates a multiplex sequencing strategy for constructing reduced representative libraries (Elshire *et al*., 2011). After the next generation sequencing of representative libraries, the complete sequences of the desired region can be obtained. It can provide high-density SNP coverage (Gore *et al*., 2009,2007) and it also can be used as the inexpensive barcoding system for increased efficiency (Elshire *et al*., 2011). With GBS approaches, the rapid discovery of sequence-based molecular markers is used to construct a high-density genetic map without a reference genome (He *et al*., 2014), and it can serve as a reference genome for anchoring and ordering physical maps and refining or correcting unordered sequence contigs (Poland and Rife, 2012). Co-dominant SNP genotyping, genetic diversity analysis, genetic mapping, QTL mapping, bulk segregate analysis, mapping to whole genome sequence, the discovery of (rare) SNP variants, genome-wide association Mapping, and genomic selection/ prediction are the typical applications of GBS.

As restriction sites are generally conserved across species, the GBS protocol can be applied for a population genetic mapping, breeding, germplasm characterization, trait mapping in diverse organisms and population genomics (Elshire *et al*., 2011). As genomic selection is a

novel approach in MAS, GBS can recognize complex and economically critical quantitative traits in GS, using genome-wide molecular markers at a lower cost (Meuwissen, and Goddard *et al*., 2001; Poland and Rife, 2012). In here, the whole genome is utilized with pedigree data so that the accuracy of breeding and genetic studies is increased without considering phenotypic data (Heffner *et al*., 2010). The breeders can predict the level of traits of new experimental lines at early stages, which can accelerate the rate of development of new crops in plant breeding (Jannink *et al*., 2010).

2.2.4.1.4 Quantitative trait loci (QTL)

A quantitative trait locus defines as the locus which associates with the quantitative trait variation for a particular phenotype of a population of organisms (Miles and Wayne, 2008). Quantitative trait variations depend on the contribution of all the loci, genetic architecture, and the various environmental conditions (Complex Trait Consortium, 2003). QTLs are often found on different chromosomes, and a single trait can depend on many QTLs.

With the use of molecular markers, QTLs are mapped concerning the traits. The markers and the phenotypic traits are statistically analysed and based on the result, the linkage map, relative gene locations, number of QTLs in a particular trait and chromosomal position can be determined (Price, 2006), which are essential for planning molecular technique-based breeding program (Breseghello and Coelho, 2013). Genotyping of QTLs by sequencing can reveal the genetic relations dealing with QTLs and traits. Then the nucleotide polymorphism and associated phenotypic values can be easily determined (Watanabe *et al*., 2009). Though the QTL mapping can distinguish two parents, when working with the broader genetic diversity, the association mapping approach is used by densely genotyping the different diverse lines and revealing the connection between traits and the loci. The accuracy of QTL mappings depends on population size, type, level of replication of phenotypic data, environmental effects, and genotyping errors (He *et al*., 2007). Even the small effect of the above factors makes the variation in phenotype; therefore, confirmation, validation, and additional marker testing steps may be required after QTL mapping and before MAS. Information in QTL mapping extensively used in marker-assisted selection, which is the foundation of the development of markers for MAS.

2.2.4.2 Markers assisted breeding (MAB)

Using DNA markers in plant breeding process is referred to as MAB. The efficiency and the

reliability of the DNA markers are higher than classical genetic markers, so DNA markers are predominantly used in MAB. It needs more complicated equipment and facilities than the conventional method. Appropriate marker system, marker trait association data, and reliable markers are essential. MAB is easy, a low-cost method which only needs a small amount of sample DNA for analysis. The result is repeatable and high levels of polymorphism can be detected as markers are codominant (Jiang, 2013a).

Construction of a high-density linkage map by using codominant SSR and SNPs markers is the initial and most crucial step in MAB (Jiang 2013b), which can be used to detect the zygosity. An efficient genetic map should have a sufficient number of evenly-spaced polymorphic markers of desired QTLs/genes at the precise positions (Babu *et al*., 2004). MAB can be carried out at the seedling stage without evaluating phenotypic traits, and it can apply to gene pyramiding with QTLs (Jiang, 2013a).

2.2.4.2.1 Markers assisted selection (MAS)

Marker-assisted selection is the method of the most promising for cultivar development based on DNA marker patterns, which allows selecting desirable individuals for the breeding plan. Rather than consideration of the traits, the use of trait-associated molecular markers is applied in this practice, which can help plant breeders to select more efficient progeny. Though the cost is high, MAS is a simple and reliable method, and within a short period, the assay can be completed by only using seedling materials as the sample. The judgment of the cross is not affected by environmental conditions, and in some cases, the heritability of the traits is controlled by the environmental variation and need to have reliable markers to better detection. The presence of recessive allele also can be recognized by MAS (Jiang, 2013a).

The findings of the QTL maps can be used in MAS. To build an efficient selection tool, fine mapping of the genotype within a large population using denser markers are necessary. Knowledge of allelic polymorphism, an inmate of the allele and genomic location enhances the agronomical value of MAS. Gene pyramiding is the most extensive application in MAS, which allows monitoring the introgression of a set of genes instead of one. When developing stress tolerance or pest resistant varieties, the single introduction of the gene is not potentially successful, as, after the several generations, pest get adopted, and the pest resistance is shown. Accumulation of several resistant genes can produce strong resistance against pathogens, since they would not be able to succeed all the gene effects simultaneously,

therefore, no way to adapt to the situation (Breseghello and Coelho, 2013).

2.2.4.2.2 Marker assisted backcross breeding (MABC)

Marker-assisted backcrossing is used for the introgression of traits and reduction of linkage drag. In here, molecular markers are used to track and confirm the transformation of the transgene and favourable parental alleles from the recurrent parent genome. Markers are used to select against the donor alleles (Johnson and Mumm, 1996). MABC can effectively select the target loci and accelerate the recovery of recurrent parents in plant breeding. MABC is capable to trace the introgression of one or more donor alleles to elite lines. Target trait scanning in parental identification, selecting backcross progeny with the target gene and tightly-linked flanking markers in order to minimize linkage drag and selecting backcross progeny with background markers are the three levels of selection in MABC.

2.2.4.2.3 Marker-assisted recurrent selection (MARS)

Marker-assisted Recurrent Selection (MARS) is a method of recurrent selection to the efficient transformation of multiple genes related to complex traits, where molecular markers are using for selection and identification (Ribaut *et al*., 2010). Based on phenotypes, this allows to perform genotypic selection and intercrossing among the selected individuals within the same crop season for one cycle of selection (Jiang, 2013b). The primary purpose of the MARS is to reduce linkage drag and optimize population sizes (Hospital and Charcosset, 1997; Visscher *et al*., 1996). Repeating the selection and intercrossing is made to accumulate more desirable genotype into the progeny in MABC, which allows the introgression of multi-genes or QTLs efficiently from different parents (Gazal *et al*., 2015). Gene pyramiding, multiple QTLs transformation, and forward breeding can perform in MARS by looking for traits including traits like high yield, pest resistance, and disease tolerance (Crosbie *et al*., 2008; Ragot *et al*., 2018; Ribaut *et al*., 2010). The parental lines with favorable traits should be selfing by control pollination to enhance the genetic strength before the MARS. De novo QTL mapping is beneficial in identification and transformation of QTLs in recurrent selection and breeding process (Gokidi *et al*., 2016). In this method, small effect QTLs can also be determined (Eathington *et al*., 2007).

2.2.4.2.4 Marker-assisted gene pyramiding

Gene pyramiding is the process of simultaneously linking multiple genes/QTLs together into a single genotype. In conventional breeding, the multiple gene introgression for the same

trait cannot be monitored and distinguished, since all the genes are incorporated with the same phenotype in different ways. With the use of selection based on DNA markers, the gene transformation can be monitored easily without waiting for phenotyping. The most popular application for pyramiding has been for combining multiple disease resistance genes in order to develop strong disease resistance (Singh *et al*., 2001). MAS can be used efficiently with gene pyramiding in multiple gene introgression, which seeds up to the breeding process (Hittalmani *et al*., 2000). The resulted progeny displays a high level of resistance than single gene transformation (Huang *et al*., 1997).

2.2.5 Genome-wide selection (GS)

GS is a new strategy and a revolutionized application for developing quantitative traits within large plant breeding populations (Desta and Ortiz, 2014). With the evaluation of high-density markers placed all across the genome, the genomic estimated breeding value (GEBV) is computed for each candidate. Unlike MAS, the GEBV is based on all markers, including both minor and major marker effects. Thus, GEBV enables to cover almost all the genetic variation for a particular phenotype (Newell and Jannink, 2014). The breeding planning is based on marker score, phenotypic data under different environmental conditions (Collard and Mackill, 2007), genotypic, and pedigree information, which increase the accuracy of prediction by concerning about the effect of minor genes as well (Heffner *et al*., 2009; Xu *et al*., 2012). The specific statistical model is used to combine data and formulate GEBV.

GS uses two types of datasets: a training set and a validation set. Marker effect estimated for the training set is calculated based on the reference population. The validation set contains progeny that have been genotyped and selected based on marker effects estimated in the training set. Instead of searching for an individual effect of specific QTL, the overall effect is evaluated in GS. Moreover, GS can accelerate the breeding cycles and as a result, the unit genetic gain of cost and time can be intensified, as a result, improved higher yielding, broadly adapted, and stable genotypes can be transferred much faster rate (Heffner *et al*., 2010). Alternatively, of phenotyping, high-density genetic map construction is cheaper today. MARS (Crossa *et al*., 2010) and gene pyramiding (Servin *et al*., 2004) are still essential methods in plant breeding which can be complemented with GS.

2.3 Breeding decision making

Breeding decisions are the decisions taken by breeders at different stages of the breeding process to improve or discard a selection unit including plant, animal, progeny, clone, experimental variety or breed and supervised by the breeding priorities, using specific decision-making tools such as classical selection methods or molecular based selection techniques (Ragot *et al*., 2018). The planning of the plant breeding process is highly complicated, risky, and need to have a good awareness of market trends, as the ultimate users of these products are the customers. So that the clear understanding of the customer behavior is compulsory to recognize market trends (Clark, 1999).

Today, rice production has a worldwide market segment, especially in Asian countries (Seck *et al*., 2012). Different type of product profiles (traits) are trending, so breeding plans should be suitable and smart to fit the trends. If the decision is faulty, all the effort, financiers and the time become fruitless. Therefore, the broader knowledge in genetics is essential for a successful breeding program. That knowledge can be used to take fundamental and critical breeding decisions to decide the suitable cultivar in breeding and determine mostly suited selection method, which are the two significant steps in plant breeding decision making (Acquaah, 2012).

2.3.1 Breeding priorities and central factors for the decision-making process

Breeding priorities are the set of attributes that have to be considered based on knowledge, experience, and germplasm, which signifies the market demand for the new product (Ragot *et al*., 2018). Once the breeding priorities are identified, the action is needed to translate those priorities into concrete breeding decision and action. The initial decision in the plant breeding program determines whether the breeding programs exist or not, as if it or closely related one exists, the plan no more needs to implement. If there is no variety with alike known breeding priorities, then the new breeding strategies can be executed to develop a new variety. It shows that breeding or breeding decisions are essentially of two types, promote or discard (Figure 2.1).

In an active breeding program, parental identification is the first step, which is based on the number of breeding decisions wishing to proceed. Not like past, multiple breeding priorities are used in a parental selection today, described by Multi-Criteria Decision-Making (MCDM) (Kariuki *et al*., 2017; Velasquez and Hesterc, 2013) which was introduced by V.

Belton and T.J. Stewart in the early 2000s. In MCDM, all the traits cannot integrate simultaneously, thereby according to breeding priorities, the order of parental crosses is defined. First, the list of desirable trait combination, which is expected to have in the progeny, is prepared and ranked them according to the leading priority and relative importance in breeding. According to the prioritized order, the appropriate breeding strategy can be incorporated with the leading priorities, while higher attention is needed for highly prioritized traits in breeding planning (Ragot *el at*., 2018).

When considering the rice cultivation, with the expanding demand for rice (Samanta *et al*., 2011; Velasquez and Hester, 2013), the breeders have to consider multiple breeding priorities in breeding design according to MCDM and plan the breeding program. In here the selection of the breeding strategies are based on the technical feasibility of the demand, the economic feasibility of the demand, level of the trait in the breeding germplasm, timing of response and the similar product profiles in the ongoing market (Ragot *et al*.,2018). The market trends, selected parents and their mating system, genetic architecture and the genetic variance of the trait, pre-breeding, location of breeding, number of expected progenies and time duration are also influenced in the breeding decision making the process.

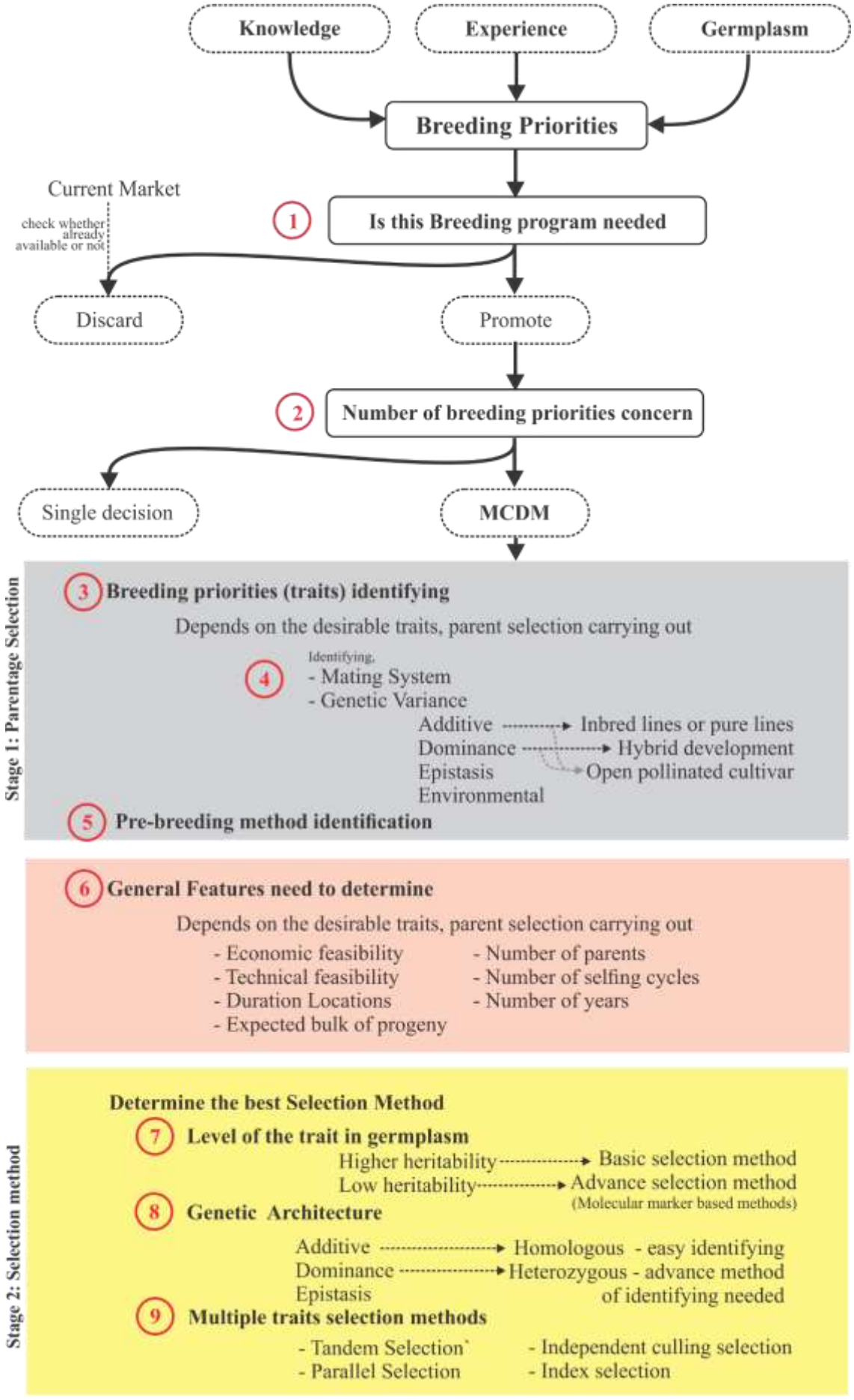


Figure 2.1 The summary of the decision-making process

2.3.1 Selection of the best parents

In the parental selection with the desirable traits, there are several significant facts to concern. Among them, the breeding or mating system of the species is the most important fact to consider, which can be temporally and artificially altered while the genetic control should be retained. The genetic control of the desirable traits is heavily influenced in the breeding (Acquaah, 2012). The breeding decision about the cultivar should be taken based on the genetic architecture of the trait, especially the nature and extent of genetic variance (Falconer, 1981). The genetic variance can be described in terms of additive variance, dominance variance, and the epistasis variance. Additive variance involves the inheritance of a particular allele which affects independently to the phenotype. Dominance variances are the phenotype deviation made by the alternative allelic interaction in a specific locus, which response to the control of the same trait. The phenotypic variation due to the interaction between different loci is described in epistasis genetic variation. Usually, inbred cultivars and pure lines are recommended to develop, when the additive variance and additive x additive interaction are predominated (Falconer, 1981). Hybrid cultivars are recommended to develop when the dominance variance and dominance x dominance interactions get predominance. Open-pollinated cultivars are suitable where a mixture of the above genetic architecture occurs (Fehr, 1987).

2.3.2 Trait selection method based on the genetic architecture

On the heritability and the genetic control of the desirable trait, the competence of the selection method depends. The heritability of the trait mainly manifests by the genetic variance under additive, dominance, epistasis, and environmental variance. The effect of the genetic variance mainly influences on identifying the most efficient selection method in the breeding program (Kearsey and Pooni, 1998).

The strong genetic accruement of additive genetic variance can be achieved by a repeated accumulation of favorable genes over selfing to retain the homozygous state of the genotype. The efficiency and effectiveness of the successful breeding program are hugely depended on the heritability of the gene and the selection method. The progeny with high heritable traits can be easily identified with the primary selection methods like mass selection; however, for the selection of low heritable traits need more effective, efficient and advanced methods of selection such as molecular techniques (Acquaah, 2012). Since comparatively more genetic variation is regularly being generated throughout continuous intermating, the additive

genetic variance can be utilized for a durable amount of time with the open pollinated species (Eberhart and Gardner, 1966).

Developing hybrid cultivars for the crop cultivation is the most common application when concerning dominance genetic variances. If the overdominance is predominant in traits, developing hybrids are the most successful method of breeding to gain short term genetic variation efficiently (Falconer, 1981).

Self-fertilizing species possess a high degree of homozygosity, by the way after subjected to crosses, the degree of homozygosity reduced and become less responsive to the classical selection methods (Briggs and Knowles, 1967). As describes above, genetic purity plays a vital role in selection criteria, as in most cases, homozygous genotypes can be selected easily than heterozygous. However, the use of molecular markers-based selection approaches such as MAS can be easily used to distinguish the novel allelic combinations from the parental types.

2.3.3 Selection of single traits or multiple traits (MCDM)

Plant breeders often interested on have more desirable traits than having only a single one. As an example, in the breeding design to improve a disease resistant rice variety, the breeders do not like to have only disease resistance trait, they also expect high yield and high amount of crop quality as well (Poehlman and Sleper, 1995). It seems that high yield is the leading identified market priority, so high or reasonable yield is a part of every breeding program (Acquaah, 2012). Not only positively correlated traits, but the negatively correlated can also be the breeding priority. If traits are negatively correlated, improving one diminishes the other. The chance of linkage can be reduced with the selection approaches such as recurrent selection, allows negatively linked trait retain to select in the breeding program (Paterniani and Vencovsky, 1977). Multiple traits development is not an easy task due to their complex, sometimes antagonistic relationships between traits. When dealing with multiple traits, progress will depend on individual trait attributes and relationships among these traits. A clear understanding of all about the traits, whether they are qualitative or quantitative, genetic location in the genome, gene interactions, and external factors influence the gene expressions are essential in dealing with multiple traits.

According to the scientific literature, many multi-trait selection approaches are available and, all of them can be categorized into four main groups as tandem selection, parallel selection, independent culling, and index selection. Tandem selection is the method of selecting traits one after the other in successive generations. Usually, positively correlated traits give good progress in breeding. If traits are negatively correlated, they can be influenced to erase the previous trait. In parallel selection, the traits are selected independently within the breeding populations and combine the selected traits later by crossing. Independent culling approach is another type of selection method, where traits are selected simultaneously and independently, based on culling threshold. The most successful novel method of selection is index selection, which is an artificial method of selection; the traits are selected simultaneously using a performance indicator. In here, each trait holds a specific value by considering the relative importance of the trait and, those values should highlight the breeding priorities as well. In this method, all the resulted progenies get a numerical value according to the performance indicator, and the best cultivar can be easily selected. Depends on the practical application in the breeding program, one of the above selection criteria or combination of the methods can be used in multiple trait selection steps in the latter part of the breeding plan (Ragot *et al*., 2018).

2.3.4 Pre-breeding

Pre-breeding and cultivar development are the two main phases in plant breeding (Acquaah, 2015). Pre-breeding is also described as germplasm enhancement, whose primary activity is the varietal improvement by introgression or transferring target genes from the wild type into breeding materials (Harlan, 1976; Zamir, 2001). This kind of genetic manipulations needs high tech facilities, money, time and advance knowledge in genetics, so typically breeders find it hard to handle this alone, and further assistance is needed from government and other authorities who have involved with germplasm enhancing experiments. The germplasm used in breeding can be categorized into three GPs, as GP1, GP2, GP3 and the latest one is 4 (Harlan and de Wet, 1971). GP1 consists of natural biological species, domesticated, and wild and weedy, which are capable to intercross without genetic consequences. Crosses involving GP2 and more, so GP3 species are problematic. The novel category, GP4, which consists of synthetic strains ethic, are unavailable in the natural environment (Harlan and de Wet, 1971). In the pre-breeding stage, the desirable genes from the wild type GP have to be transferred into the domesticated breeding material, and the factors influential in gene introgression should be properly planned (Acquaah, 2015).

2.3.5 Duration of plant breeding

The time needed to complete the plant breeding program usually extends from four to ten years or over ten years regardless of the type of breeding, neither conventional or molecular (Poehlman and Sleper, 1995). The lifecycle of the species plays a crucial role in determining the duration of plant breeding program. Generally, crop plants need more time than annuals. Rice is a seasonal crop, which is having 90 – 120 day of the life cycle. If the gene introgression from wild type origin to the domesticated gene pool, it spends longer time duration than the time occupied for simple hybridization between two inbred lines (Chahal and Gosal, 2002). Interested gene type; either qualitative or quantitative, also influences the duration of the breeding program, since qualitative traits can be easily bred than quantitative traits. An additional step of selfing would be needed to introduce recessive traits during each cycle in selection. The number of crosses, breeding procedure and involved steps are differed by method (Acquaah, 2012).

2.3.6 Location of plant breeding

The yield of the rice cultivation depends on several biotic and abiotic factors such as soil condition, temperature, and rainfall. The effect of the environmental factors can affect the visual output of traits and identification of the phenotypic characters of the new varieties, so when planning breeding program for abiotic or biotic stress resistance, it may need ideal environmental conditions for optimal selection for rapid genetic gains. The location of the breeding program is also crucial in the duration of cultivar development since the effect of the seasonal variations also influence the life cycles of the crop plants. The breeding program may be possible to conduct inside the greenhouse to overcome the natural environmental distribution. However, while crossing for stress tolerance and pest or herbicides resistance verities, the varieties should be tested under the natural environmental conditions as well (Acquaah, 2015).

2.3.7 Plant breeding decisions based on numbers

Optimal breeding decisions need correct numerical values based on the statistical foundation (Acquaah, 2012). Understanding the nature of the gene is crucial, as the gene is related to a qualitative or quantitative trait, and if it is QTL, the number of loci involving is also needed to know. To initiate or to advance the breeding program to fulfill breeding priorities, the number of parents in the cross and crossing order is also essential (Acquaah, 2015; Brown and Caligari, 2008). The breeders must have to decide the number of selfing cycles needed

to introduce a new trait, number of recurrent cycles or backcrossings needed to adopt a parent (Borojevic, 1990). Ultimately, the number of years needed to conduct field trials, and the number of locations needed to be tested is also needed to decide (Duvick, 1984). Furthermore, to obtain highly uniform progeny as the final cycle of breeding to release, breeders may require additional cycles of selfing (Brown and Caligari, 2008).

2.4 Breeding database

The cultivar selection is the first step in the breeding decision-making process (Acquaah, 2012). In today's rice cultivation, many essential breeding priorities were identified such as, yield (Peng *et al*., 1999), pest resistance, herbicide resistant, disease resistance, plant height (PLH) and maturation time, pericarp color, amylose content, gelatinization temperature, brown rice recovery, grain shape, milling recovery, and head rice recovery (RRDI, 2018). When selecting most appropriate parents for a cross, all of the essential breeding priorities should be considered according to the objectives of the breeding program. The main objective of most of the breeders is to transform desirable trait related chromosomes to harvest elite individuals in the breeding process. (Voorrips *et al*., 2012)

Breeding history plays a significant role in the breeding decision-making process. Data about each trait (breeding priorities) and parentage of the crosses are central components in breeding history, which are evaluated and recorded by different institutions, including the Department of Agriculture (DOA) in Sri Lanka, RRDI in Bathalagoda and their substations, private sector crop breeders and some data are freely available in public databases such as NCBI. Thousands of log records about the crosses done by the research centers were written in field notebooks and stored. Those records contain a pool of data which are initially necessary for formulating a proper breeding plan. They are primarily categorized as the genetic and phenotypic data, which are not correctly ordered, and valuable bulk of information are scattered within many field recordings, so it is hard to use them worthily (Voorrips *et al*., 2012)

A database is an organized form of data collection, which can be accessed electronically and manipulated with the use of computer knowledge (Hogan, 2018). Today, the support of information technology and data analysis is highly used in recording breeding history easily, efficiently, and effectively on collecting data, data validation, monitoring activities, and decision making. Maintaining database to record historical breeding information, their

qualitative and quantities evaluation and genetic diversity boost the parental selection step in the decision-making process of plant breeders. The breeding database supplies the enhanced tools for more easy data sharing, data mining, visualization, and data retrieval in research activities (Yu *et al*., 2013), which helps to easy transformation of available genetic information into breeding planning (Voorrips *et al*., 2012).

A substantial quantity of breeding databases and pedigree diagramming software programs are available in today based on phenotypic and molecular maker data such as PEDRAW (Curtis, 1990), CoPE (Brun-Samarcq *et al*., 1999), Pelican (Dudbridge *et al*., 2004), HaploPainter (Thiele and Nürnberg, 2004), PediGrach (Garbe and Da, 2008), Progeny (http://www.progenygenetics.com/), R package Kinship (http://www.cran.r-project.org/), PediTree (Van Berloo and Hutten, 2005) and E-Brida (http://www.e-brida.nl/). Among them, most of the programs are commercially available and based on statistical interpretation on breeding information, while Pedimap is based on the virtualization of genetic information in pedigrees, which can easily understand by the breeders. It does not perform statistical or quantitative genetic calculations; instead, it presents available genotypic and phenotypic data in a way that enables the understanding of the data. Furthermore, Pedimap can support different ploidy levels too.

2.4.1 Pedimap: A pedigree-based breeding database

Pedimap is the genetic and phenotypic data visualization software for related individuals linked pedigree. It Is free available (http://www.wur.nl/), user-friendly, flexible software as breeders without holding in-depth knowledge in computer science, can use it comfortably. The database capable of storing breeding records with the detailed evaluation of the breeding priorities and pattern of inheritance of valuable traits. Pedimap can accommodate all types of inheritance, including selfing, cloning, and repeated backcrossing, and polyploidy as well. Pedigree visualization is an easy, and understandable graphical representation method of inheritance patterns (Cheng *et al*., 2008), which is one of the critical components in Pedimap. As it can visualize the genetic inheritance patterns such as the flow of phenotypes and related marker alleles of the individuals clearly, it is easy to predict errors, potentials, suitable parental lines in the breeding decision-making process and an idea about the suitable method of selection in breeding. Visual representation of the genetic data along with phenotypic data in Pedimap simplifies the search of large data sets by specifying the subpopulations

according to the molecular association, which helps to improve the breeding decision-making process (Voorrips *et al*., 2012).

2.4.1.1 Data types in Pedimap

Parentage, phenotypic, and genotypic data are the three core elements of Pedimap input. The database can represent all possible forms of parentage in plant pedigrees, including biparental crosses and selfings, sib-mating, backcross relationships, vegetative propagation, mutants, and doubled haploids regardless of the population size. Acceptance of the polyploidy is one of the significant features in this software. Under the phenotypic data category, all type of phenotypic data can be accommodated, along with continuous quantitative data, discrete quantitative data, and qualitative data. Marker scores, Identity-by-Descent (IBD) probabilities, and marker linkage map positions permitting the visualization of haplotypes through lineages are the genetic data categories in Pedimap input file (Voorrips *et al*., 2012).

The data about the distinct plant breeding features, including polyploidy, hermaphroditic individuals, and mutants are also capable of Pedimap input, is one of the specific features in this software. Information about all the maker types can be added such as putative alleles, and well identified null alleles. Calculated IDB probabilities also can be presented in the database. To study the basic structure of the pedigree without having a complete set of the genetic and phenotypic data set is also conceivable (Voorrips *et al*., 2012).

Pedimap, as a novel approach in plant breeding, having an ability to maintain data quickly from a text file and data conversion between other genetic visualization and analysis software such as FlexQTL™ (Bink *et al*., 2008; http://www.flexqtl.nl/) and GenomeStudio™ (Illumina Inc., San Diego, CA). FlexQTL is used to QTL analysis and IBD probability calculations in multiple pedigreed populations (Bink *et al*., 2008). These data are used as raw input to Pedimap. The first data file is created in Microsoft Excel and imported as a text file. Large scale SNP data analyze from GenomeStudio software (http://www.illumina.com/) can be imported into Pedimap after converting it into appropriated text data file with the help of conversion script available in Pedimap official website.

2.4.1.2 Features in Pedimap

Pedimap is a user-friendly, secure handling software. It can hold single, multiple or combination of data related with the desirable phenotypes including linkage map data which are generated through DNA markers, and the flexible handling of pedigrees in different breeding approaches including selfing, doubled haploid production, repeated backcrossing, and clonal propagation with the same parent. The most prominent feature in here is the capability to construct customized visualization of pedigree trees. The interface of the software is set up based on the subpopulation concept. Total population and subpopulation can be distinctly defined and chosen. Each subpopulation supports multiple views or different graphical presentations. Pedimap incorporated the ability to define extra features such as color, qualitative or qualitative values, and text formats to the pedigrees according to the easiness of presenting. The legend for the phenotypic traits can be shown separately. Different views of the pedigree diagrams are blended with the software. DNA marker scores and IBD probabilities are also can be included in the database as the additional features. All the information concluded in the database can be used for germplasm selection in the breeding planning process (Voorrips *et al*., 2012).

2.4.1.2.1 Pedigree structure

Pedigrees are a simple representation of the parents and offspring of a particular individual. With the addition of multiple generations, including sib mating and backcrosses with ancestors, and so on, the pedigree becomes complex. As pedigrees become large and sophisticated with the addition of more individuals, it is harder to examine. However, in Pedimap, it allows selecting necessary individuals and levels of generations manually, as a simplified version of the intricate pedigree illustration. Inheritance patterns in many organisms are relatively easy to determine since any cross can illustrate as the simple graphical presentation in Pedimap (Bennett *et al*., 1995; Keith *et al*., 2008). The subpopulation concept is mainly considered in Pedimap to design pedigree relationships. From the total population, various subpopulations can be derived and present in different graphical presentations. These subpopulations can be assembled manually, but tools are available to automatically include or exclude further individuals based on their pedigree relations with another individual. (Voorrips *et al*., 2012).

Visual presentation of Pedigrees is intuitive, more comfortable to discover relations and support in selecting suitable germplasm in breeding programs. Different color codes can be

used in qualitative data representation, whereas, a color gradient can be used to present quantitative trait variations in the phenotypic data section (Voorrips *et al*., 2012).

2.4.1.2.2 Genetic marker data

Following the pedigree and phenotypic data, genetic marker data can be added to the database. The basic profile of each DNA marker contains information about the linkage group, a linkage map position, and locus names. By identifying the possible alleles for particular loci, and the outcome of the breeding can be estimated. The ploidy levels of each locus can be specified in this data input, which allows working with polyploidy. Rather than well-known alleles, unidentified alleles and missing alleles also can be added by using symbols and null value respectively. Assigning specific colors to the alleles also possible, which is useful to understand and investigate the inheritance quickly (Voorrips *et al*., 2012).

2.4.1.2.3 Identity by descent (IBD) probabilities

The concept of gene identity by descent (IBD) is an important concept which describes the genetically mediated correlations or similarities among relatives (Thompson, 2013). IBD probabilities calculate the chance of having particular allele in a selected progeny or variety from common ancestry. These probability values of the founder alleles can visualize in Pedimap, which are essential in QTL mapping (Meuwissen and Goddard, 2001). The calculated IBD probability values must be fed into Pedimap as separate input file since Pedimap does not calculate these values itself. A package that can perform these calculations and produces output files compatible with Pedimap is FlexQTL (Bink *et al*., 2008). The inheritance of a distinct haplotype through the pedigree can be visualized in Pedimap with these calculations. It can also be illustrated when the founder chromosomes are progressively recombined through the different generations (Van Dijk *et al*., 2012).

CHAPTER 3

ORGANIZATION OF THE PHENOTYPIC AND GENETIC INFORMATION OF RICE BREEDING GERMPLASM IN SRI LANKA USING PEDIMAP TO FACILITATE THE DECISION-MAKING PROCESS IN VARIETAL IMPROVEMENT

3.1 ABSTRACT

The development of improved rice cultivars with desirable traits is essential. The decision-making is a crucial step in rice breeding programs. The breeders can make efficient and pragmatic decisions if an organized database is available for the material of the rice breeding germplasm. The staple food in Sri Lanka is rice, and there is a great demand for improved varieties with high yield and other promising traits. In the present study, the available data of all the rice varieties released by Rice Research and Development Institute, Sri Lanka, and the related landraces and genotypes were arranged as a database using Pedimap, a pedigree visualization tool and a database platform. Pedimap can store pedigree relationships, phenotypic, and molecular data. The Identical by Descent (IBD) probabilities were calculated using FlexQTL software and included in the Pedimap database. The parentage selection based on the variations of phenotypic traits, selecting marker alleles for molecular breeding, and tracing founders of genetic effects can be swiftly conducted using Pedimap. However, the power of harnessing the value of Pedimap for making breeding decisions relies on the availability of data for the traits, markers, and genomic sequences. Thus, it is imperative to characterize the breeding germplasms using standard phenomic and genomic characterization procedures before organizing as Pedimap databases. Thereby, the worldwide breeding programs can benefit from each other to produce improved varieties to meet global challenges.

Keywords: Breeding database, breeding decisions, marker assisted breeding, pedigree visualization, planning crosses

3.2 INTRODUCTION

Rice is one of the major crops in the world, with an annual production over 700 million metric tons (FAO, 2018). Half of the world population consumes rice as staple food (Muthayya *et al.*, 2014). Currently, the demand for rice is rapidly increasing due to the growth of the human population (Ali, 2006). However, the current rice production cannot meet the increasing demand causing severe food security issues. The biotic and abiotic stresses also exert a negative influence on rice production (Seck *et al.*, 2012). The rice farming is also a way of living for many people, especially in numerous Asian countries (Segal and Le Nguyet, 2019). At present, 1.8 million Sri Lankan families engage in rice farming over 870,000 hectares (RRDI, 2019). The annual rice production in Sri Lanka is approximately 2.3 million metric tons (MT), which is insufficient to fulfill the domestic rice demand of 3.0 million MT (Central Bank, Sri Lanka, 2017). Hence, the government has to spend about USD 400 million to import rice (Central Bank, Sri Lanka, 2017, 2018).

The rice production is mainly affected by drought and irregular rainfall patterns caused by climate change (Dharmarathna *et al.*, 2014; IPCC, 2007; Mackil, 1996), adverse soil conditions such as salinity (RRDI, 2019; Walisinghe *et al.*, 2010), and pest and disease attacks (Dhanapala, 2007). The biotic and abiotic stresses in rice farming can be controlled using numerous agronomic practices such as irrigation, drainage, fertilization, and the application of pesticides. However, the rate of success of the controlling methods is limited (Dhanapala, 2007) due to the unpredictable nature of climate change, soil degradation, variations in pest dynamics, and development of resistance (Shetty, 2004). Therefore, breeding is considered as the most successful strategy to produce high yielding and stress resilient rice varieties (Duvick, 1984). The improved rice genotypes can also contain the traits for higher consumer preference and organic rice production (Calingacion *et al*., 2014). Initially, the rice varietal improvement was conducted with classical breeding techniques, which are tedious, lengthy, and impracticable in cases such as breeding for pest resistance. However, the marker-assisted breeding (MAB) (Jiang, 2013b) is employed in modern breeding programs to introgress valuable traits from landraces and traditional varieties (Sasaki, 2011) and pyramid the desirable haplotypes of Quantitative Trait Loci (QTL) to the improved rice varieties (Collard and Mackill, 2007; Tanksley and Nelson, 1996; Thomson *et al.*, 2009).

The decision-making process in a breeding program is crucial for successful outcomes. The formulation of decisions before breeding is a multi-step process that consists of the identification of breeding priorities, determination of the genetics of target traits, and employment of pre-breeding methods if required. The economic and technical feasibility, number of parents for crosses, number of selfing and outcrossing cycles, length of the breeding program/cycles, and identification of the selection methods must also be assessed (Acquaah, 2012). In the decision-making process, initially, the market trends based on consumer and other stakeholder preferences must be recognized (Clark, 1999). Subsequently, the novelty and the uniqueness of the breeding objective must be assessed (Acquaah, 2012) before the execution of the breeding program.

The selection of suitable varieties/genotypes as parents and the determination of the selection methods are the two most critical aspects in planning breeding programs (Ragot *et al.*, 2018). The parental selection depends on the number of prioritized traits for breeding. When the multiple characteristics are to be introgressed, the breeders require a prioritized order of parents for stepwise crossing and selection (Kariuki *et al.*, 2017; Velasquez and Hester, 2013). The decision-making process in breeding is entirely performed based on the available information on phenotypes, genotypes, and pedigree. Although the data for decision-making for breeding are indispensable, the haphazardly collected information would provide less value to the breeders. In conventional breeding programs, most of the data are recorded in field notebooks and stored in the breeding stations, while very few information is available as computerized databases. If an organized database containing all the essential information for the rice varieties released and the parental genotypes used in breeding, the decisions can be made more swiftly.

The construction of a database with all the necessary information from varieties and their parents promotes the capacity of data sharing, mining, visualization, and retrieval (Yu *et al.*, 2013). Pedimap is a pedigree visualization software that can also be used as a platform to formulate as a database. Pedimap is used by many contemporary plant genetics and breeding programs worldwide. As stated in Voorrips *et al.*, (2012), Pedimap can be used to record and utilize breeding history. Pedimap illustrates the available phenotypic and genetic data through pedigrees. All the information, including parentage, qualitative and quantitative data, marker alleles/genotypes, and the calculated identical-by-descent (IBD) probabilities, can be presented in Pedimap. Currently, breeders prefer to use databases like Pedimap since

it allows them to access the large pool of genetic and phenotypic data quickly and generate pedigrees that are essential in making breeding-decisions.

In Sri Lanka, Rice Research and Development Institute (RRDI) is the sole organization conducting the rice breeding programs the national needs. Therefore, in the present study, we report an attempt to organize the information of the released varieties and the parental genotypes of RRDI breeding programs as a Pedimap database which is a valuable step to take accurate breeding decisions and speed up the process of releasing novel varieties.

3.3 METHODOLOGY

3.3.1 Plant material

The breeder seeds of 90 rice cultivars were obtained from the Rice Research and Development Institute (RRDI) Bathalagoda, Sri Lanka (Table S3.1). The seeds were germinated and established under greenhouse conditions at University of Peradeniya, Sri Lanka.

3.3.2 DNA extraction and PCR

Immature leaf samples of rice cultivars (Table S3.1) were collected and subjected to DNA isolation using Dneasy® plant mini kit (Qiagen, Solna, Sweden). The isolated DNA of each cultivar was subjected to PCR amplification with the markers developed for the *Pup1, Ghd7 and BPH* QTL (Table 3.1). The PCR conditions were provided in the Thermal Cycler (Takara, Japan) as follows; initial denaturation at 94 °C for 5 min, then 35 cycles of 94 °C for 30 sec for denaturation, primer annealing temperature (Ta) (Table3.1) for 90 sec, and 72 °C for 2 min, finally extension at 72 °C for 10 min. The PCR products were size separated using ethidium bromide-stained 2.5 % agarose gel electrophoresis.

Table 3.1 The details of the selected DNA markers will use in genetic analysis.

Marker	Forward and reverse primer sequence (5' → 3')	Ta /C°	Reference
K29	CCATAGTAGCACAAGAAACCGACA GCTTCAATGAGCCCAGATTACGAA	55	Chin *et al.* (2010)
RM 463	GGCAAAATTAGACGGCACG GAATATGCATTTTGTTTGGAG	55	Hu *et al*. (2015)
Seq7-8	CATACGGATCCAGCCTCTGT TTGCAATGATGCGTATTCAC	54	Lu *et al*. (2012)

3.3.3 Data curation

The data were collected from RRDI, Sri Lanka and classified under three main categories, namely pedigree history, phenotypic data, and molecular data on rice varieties/landraces/genotypes (herein after collectively referred to as cultivars). The male and female parents and the order of crosses were taken under pedigree history. The average yield of the rice plants, the maturity period in different growing seasons [*Yala* and *Maha* seasons of Sri Lanka as explained in Aluwihare *et al.*, 2016], plant height, basal leaf sheath color and additional color patterns, recommended type of the land, level of phosphorus deficiency tolerance, amount of brown rice recovery, amount of milling recovery, amount of head rice recovery, amylose content, gelatinization temperature, weight of 1000 grains, shape of the grain, pericarp color, weight of a kg, color of the buff coat and resistance/susceptibility to pests and diseases; brown planthopper (BPH), bacterial leaf blight and rice blast disease were recorded under phenotypic data (Table S3.1). The available DNA marker alleles, and marker positions in the linkage map, allelic scores were noted under molecular data (Aluwihare *et al.*, 2015; Jayarathne *et al*., 2019; Nakandala *et al*, 2019; Rathnayake *et al*., 2019) (Table S3.2).

3.3.4 Pedimap procedure

A Pedimap input data file is created in MS Excel (2019), and the data file is exported as a tab-delimited text (.txt) file (Table S3.3). The input file contains four main subdivisions; header, pedigree, marker data, and IBD probability section (Figure 3.1). The header consists of five essential elements and one additional element. The name of the population and symbols for unavailable or missing data, null homozygous alleles, and confirmed null alleles are entered to the pedigree section, as shown in Figure 3.1A. The name of the cultivar must be a string with text or numerical values without spaces.

Next to the header, the pedigree section is entered, as shown in Figure 3.1B. The first column denotes the name of the variety or landrace, and second and third columns are reserved for maternity and paternity information, respectively. From the fourth column onwards, any desirable quantitative or qualitative trait values can be entered. The numerical values or texts were compatible in this section. All the collected phenotypic data are introduced, as shown in Figure 3.1B. The third section of the input data file is for marker information. The linkage group of the DNA marker and the marker positions in the linkage map are entered, as shown in Figure 3.1C. If there are more than one linkage group, all the linkage group maps should

be defined successively before entering the allelic scores. The detailed data sheet for each DNA marker can be inserted after revealing the map positions. The respective number of columns, according to the ploidy level, should be incorporated to enter allelic scores. The fourth section is for IBD probability values (Figure 3.1D). The IBD probabilities cannot be calculated within Pedimap, but can be calculated using FlexQTL (Bink *et al.*, 2008), which is a software for QTL analysis. Also, FlexQTL can generate a Pedimap input data file while running the QTL analysis.

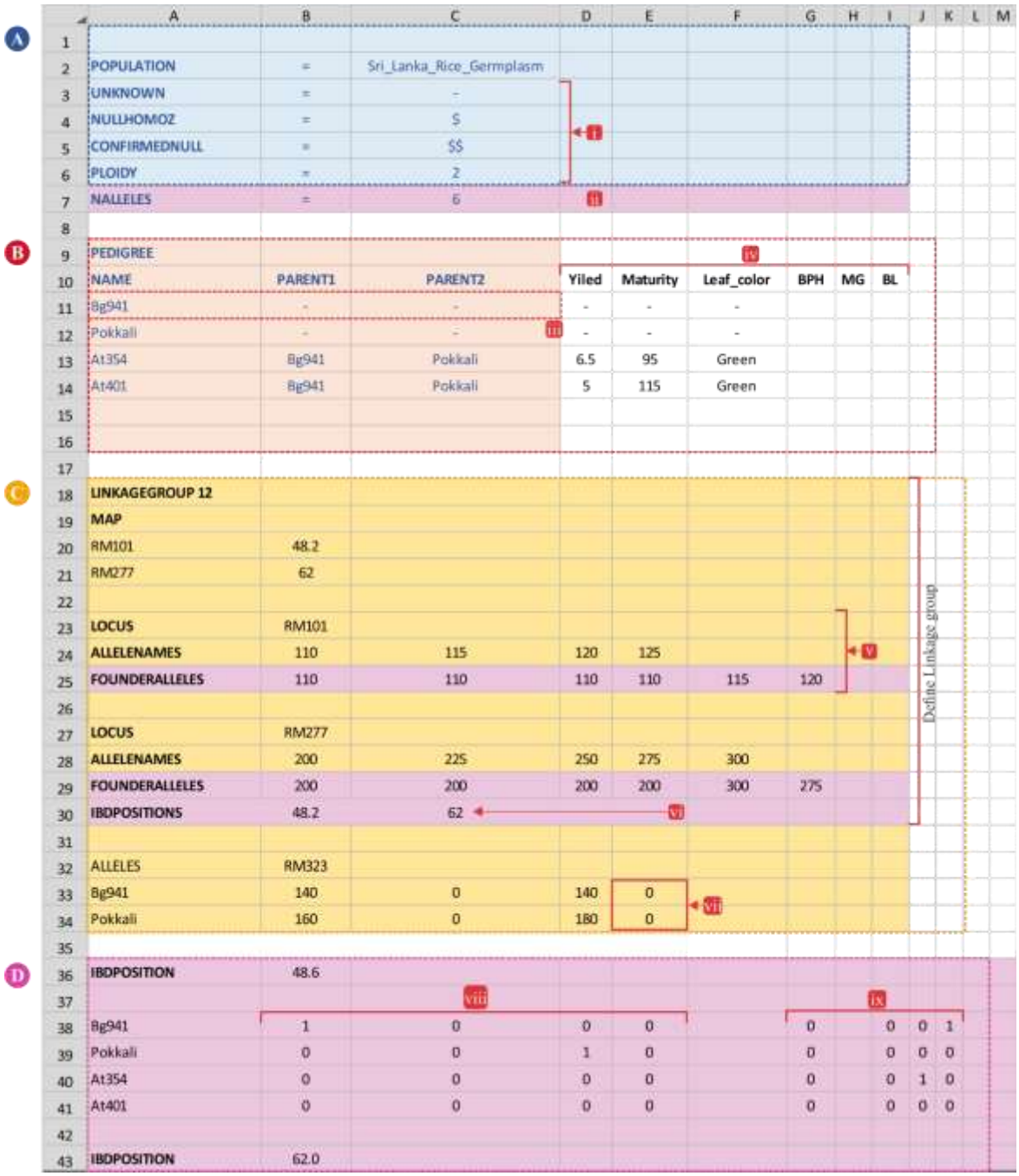


Figure 3.1 The input data file structure of the Pedimap database; The input file was created as an MS Excel worksheet, contains four main sections. A: Header, B: Pedigree, C: Marker data, D: IBD probabilities. A: In the header section, essential elements are highlighted in blue color, which contains primary data and abbreviations for the components in the database. (i) abbreviations for missing data (i.e., unknown), null alleles, confirmed null alleles and an integer to represent the ploidy level are inserted respectively. (ii) "NALLELE" section is only necessary if the IBD probabilities are used. A number of total founder alleles are mentioned here. Each text must be entered without spaces, and the abbreviations should not be used with other texts. B: Pedigree section contains the pedigree data of all the individuals. The essentials are highlighted in orange. Qualitative and quantitative data are added into transections as additional data. (iii) Initial parents must be entered, and missing values were accepted if it is properly mentioned. (iv) Qualitative and Quantitative data about the QTL are entered in these columns. The average yield, period of maturity and the basal leaf color are entered here as phenotypic data. C: Marker data section contains linkage map information for all available markers and marker scores. (v) Each linkage group should be cited separately in ascending order. After defining the linkage group, list of alleles (markers) with the recombination frequency in cM is entered. Then each locus is defined with details. Allele score for all observed allele types is entered and, all founder allelic states are entered which is necessary to work with IBD probabilities. (vi) At the end of each linkage group, IBD probabilities for each locus is cited, and multiple positions are separated by spaces. After defining all linkage

groups, allele information for all the mentioned alleles must be entered according to the above order in the linkage group section. (vii) The additional column can be used to assign specific colors for each locus as needed. All the essential elements in this section are highlighted in yellow. D: IBD probabilities for each allele is entered separately. Elements in the IBD probability section is highlighted in pink. IBD probability for each allelic combination is separately calculated and entered. (viii) Maternal probability and (ix) paternal probability are entered and separated by a column. All the IBD probabilities are calculated using FlexQTL. The qualitative and quantitative information in Sections B, C, and D, are not compulsory items in the input data file. The final file must be saved as a tab-delimited text (.txt) file.

3.3.5 Demonstration of the usability of Pedimap

We used the examples 1 and 2 given in Table 3.2 to show how parental cultivars can be selected for crossing based on diverse breeding objectives and the prioritized traits. The example 3 in Table 3.2 was used to select parents, indicate the DNA marker allelic representation for MAB, identical by descent calculations, and planning crosses to deduce related details necessary for decision-making for breeding.

Table 3.2 The examples used to demonstrate the use of Pedimap in making breeding decisions

Example	Trait*					
	Priority[#] 1	**Priority 2**	**Priority 3**	**Priority 4**	**Priority 5**	**Priority 6**
1	White pericarp	Yield ≥3.5 mt/ha	Resistance or moderate resistance to brown planthopper (BPH)	Maturity period ≤125 days	Grain shape[$]	-
2	High, high-intermediate, and intermediate amylose content	Yield ≥3.5 mt/ha	Maturity period ≤125 days	Resistance or moderate resistance to blast	-	-
3	Phosphorous deficiency tolerance	Yield ≥5.0 mt/ha	Maturity period 90-105 days	Resistance or moderate resistance to BPH	Resistance or moderate resistance to blast	High, high-intermediate, and intermediate amylose content

*The trait classes and records are from RRDI records (RRDI, 2018)
[#]The traits are given in the order of priority in making breeding decisions
[$]Variable grain shapes for the intended varieties to be released

3.4 RESULTS AND DISCUSSION

3.4.1 DNA marker analysis

The co-dominant DNA markers *K29* and *Seq7-8* only showed the significant band polymorphism in the 2 % agarose gel electrophoresis, while *RM463* shows monomorphic band pattern for all 90 rice varieties (Figure 3.2).

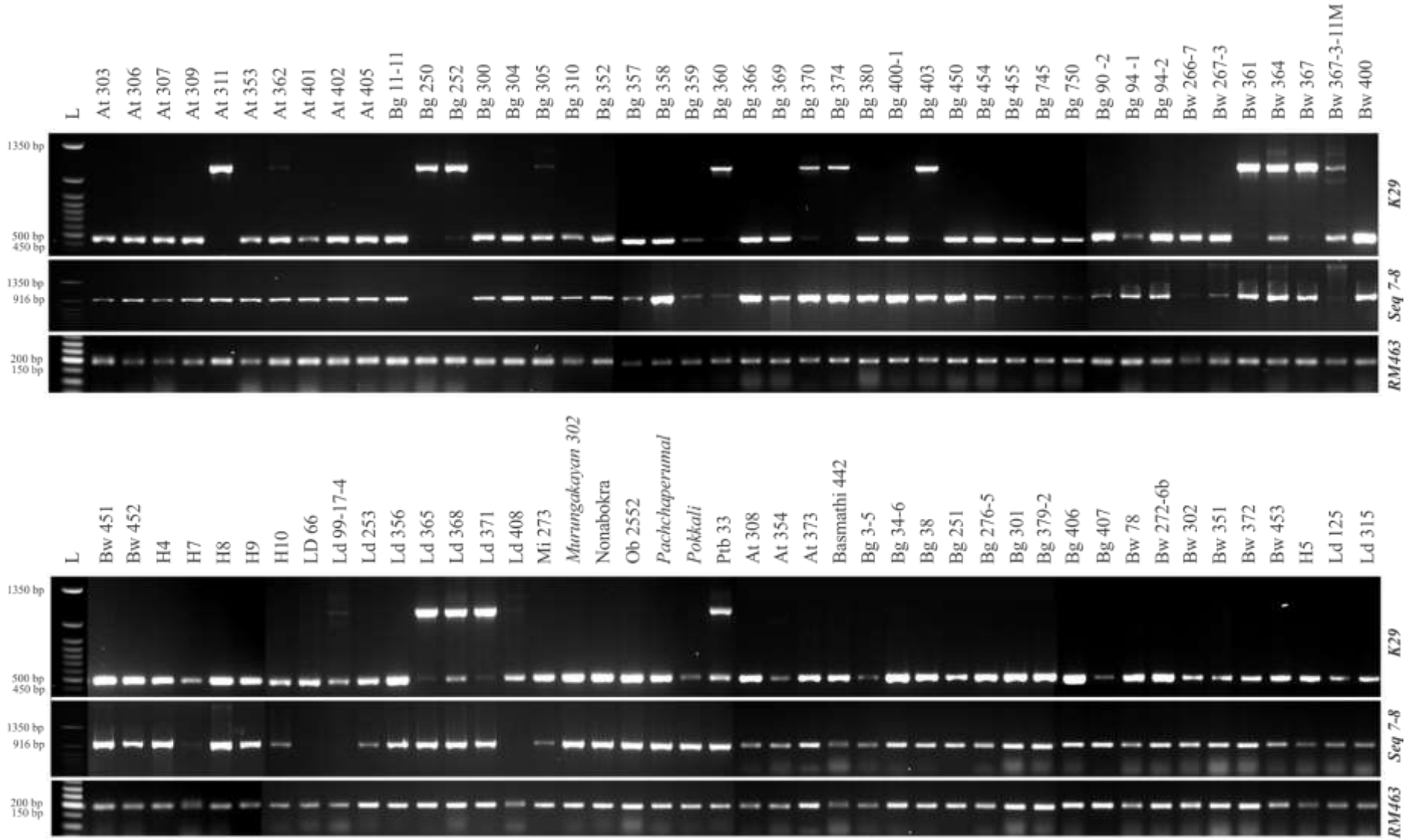


Figure 3.2 The polymorphism of three co-dominant DNA markers; *K29*, *Seq 7-8* and *RM463* in 90 rice cultivars. The band sizes are indicated at the left side of the Figure and the DNA marker names are indicated at the right side. The cultivar names are given at the top.

3.4.2 Organization of the breeding database using Pedimap

Worldwide plant genetics and breeding programs use Pedimap as the platform for maintaining breeding databases and pedigree visualization. In the RosBREED project (Weebadde *et al.*, 2010), the parental and progeny identification, tracing founders, and calculation of allelic representation are conducted with Pedimap. The pedigree displays using Pedimap is used to plan crosses in the Rosaceae research community (Peace *et al.*, 2014; Rosyara *et al.*, 2013), HIDRAS project (Evans *et al.*, 2011) and genetic visualization of *Arabidopsis thaliana* crosses (Paulo *et al.*, 2008).

The use of Pedimap as a pedigree visualization tool and a database for the decision-making process in rice breeding is described using three examples (Table 3.2). Selecting parentage, sketching out crossing schemes, estimating the probability of allelic segregation, and choosing compatible molecular markers for MAB can be achieved using Pedimap (Voorips *et al.*, 2012).

Example 1: Selecting parents for higher yield, BPH tolerance, short duration and white pericarp with diverse grain shapes

The Pedimap database created has a total of 224 input rice cultivars. There are 36 intermediate genotypes such as F1 and F2 that were not reported, but we included them to complete the pedigree in Pedimap. Thus, the database has a total of 188 rice cultivars with known identities with records (Table S3.1; Figure S3.1). In Example 1, we considered a scheme to select cultivars as parents with the parameters given in Table 3.1 for white pericarp, yield, BPH resistance, maturity period, and the grain shape. These thresholds defined a subpopulation of 26 cultivars (Figure 3.3). The variation of the yield is given in Figure 3.3A. According to the color shading given, the breeder can select the required parents for crossing to obtain higher yield levels. However, as shown in Figure 3.3B, only three cultivars show the complete resistance to BPH. If breeder plans to introgress the complete BPH resistance to the novel varieties, only Bg250, At307, and At306 are available as the sources of resistance. Figure 3.3C displays the variation for the maturity period. The breeder can choose the parents depending on his objective for the intended maturity period for the novel varieties. Example 1 was exclusively planned to breed for white pericarp. However, the grain shape is also important as a significant quality trait to become a successful variety in the market. Figure 3.3D shows the variation for grain shapes for the breeder to carry out the selection. If we consider all the traits and selected At307 as a parent

based on the pedigree visualization in Pedimap, At307 can provide the genetic basis for high yield, complete resistance to BPH, approximately three months for maturity and intermediate-bold shaped grains. If Bg450 was selected, the yield is still in the higher range with moderate resistance for BPH and short-round grains. However, Bg450 brings the alleles for an extended maturity period (Figure 3.3).

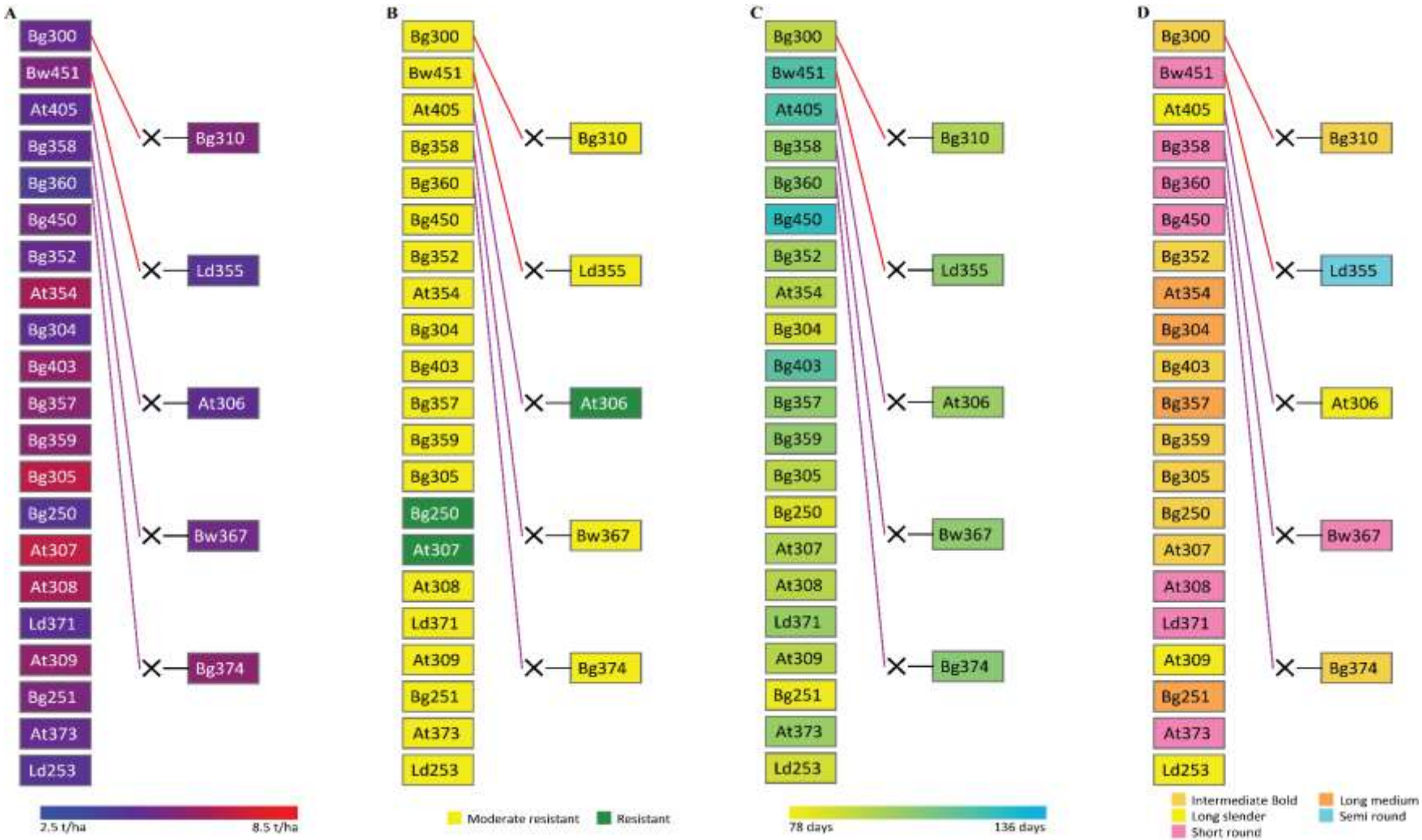


Figure 3.3 The pedigree visualization for Example 1 (Parents with white pericarp, yield ≥ 3.5 mt/ha, moderate or complete BPH resistance, maturity period ≤ 125 days, and diverse grain shapes). The selected pedigree is colored separately for four traits. A: Yield; B: Degree of resistance to brown planthopper (BPH); C: Maturity period; D: Grain shape. Female and male parentages are indicated by red and purple lines, respectively. The symbol '×' indicates the cross between two parents. The background colors of the cultivar-name boxes indicate the trait values, as shown in the colored legends below.

Example 2: Selecting parents for high/high-intermediate amylose content, higher yield, short duration, and resistance to blast disease

In Example 2, we considered a scheme to select cultivars as parents with the parameters given in Table 3.2 for high/high-intermediate amylose content, higher yield, short duration, and resistance to blast disease. These thresholds defined a subpopulation of 37 cultivars (Figure 3.4). The breeder can select the high yielding, short-duration, and blast-resistant cultivars as parents from pedigrees visualized in Figures 3.4A-C, respectively. The high, high-intermediate, and intermediate amylose contents are depicted in the pedigree given in Figure 3.4D. Only Bw351, At307, Bg407H, At308, and Bg252 show the complete resistance to blast (Figure 3.4C). However, At307 is the most promising parent with high yield (Figure 3.4A), short duration (Figure 3.4B), and high amylose content (Figure 3.4D) along with complete resistance to blast (Figure 3.4C). Also, Bg407H is the highest yielding (Figure 3.4A), blast-resistant (Figure 3.4C), and high in amylose content (Figure 3.4D). However, Bg407H is a long duration variety compared to At307. Therefore, the breeder may plan to cross At307 and Bg407H to accomplish the breeding objective of Example 2.

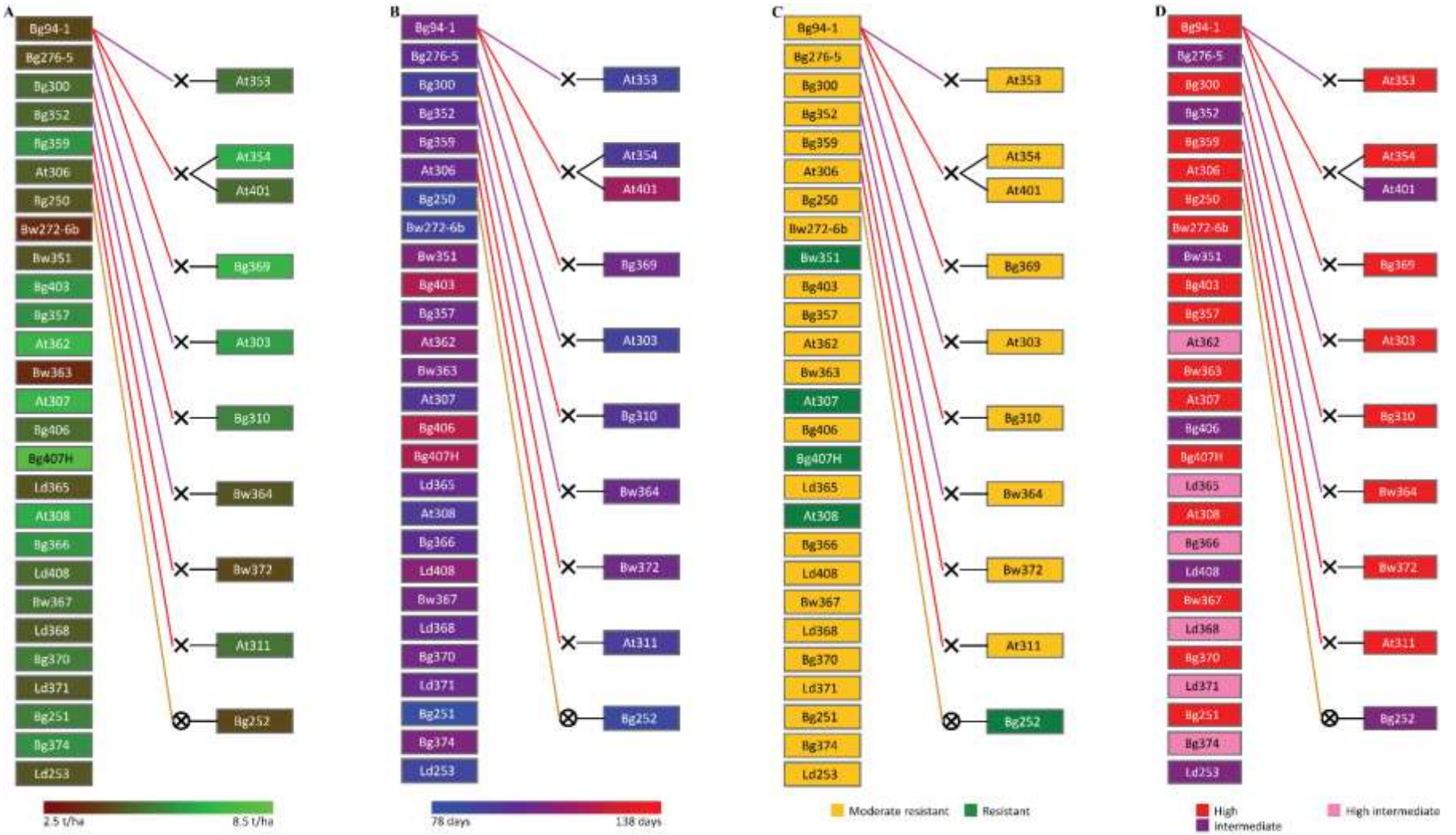


Figure 3.4 The pedigree visualization for Example 2 (Parents with high, high-intermediate, and intermediate amylose content, yield ≥ 3.5 mt/ha, moderate or complete resistance to rice blast disease and maturity period ≤ 125 days). The selected pedigree is colored separately for four traits. A: Yield; B: Maturity period; C: Degree of resistance to rice blast disease; D: Amylose content. Female and male parentages are indicated by red and purple lines, respectively. The symbol '×' indicates the cross between two parents, and '×' inside the circle represents selfing. The background colors of the cultivar-name boxes indicate the trait values, as shown in the colored legends below.

Example 3: Selecting parents for phosphorus deficiency tolerance, higher yield, short duration, resistance to both BPH and blast, and high/intermediate-high amylose content

We selected a set of rice cultivars from the Pedimap database based on the availability of ranked scores for phosphorus deficiency tolerance (PDT). Twenty-four cultivars contain the PDT ranks of high, moderate, and sensitive (Figure 3.5A). The same set was illustrated using Pedimap for yield (Figure 3.5B), maturity period (Figure 3.5C), degree of resistance to BPH (Figure 3.5D) and blast (Figure 3.5E), and amylose content (Figure 3.5F). If At362 is considered as a parent, it can bring resistance to PD, and BPH, moderate resistance to blast, high yield, average maturity period, and intermediate-high amylose content. Similarly, if Bg250 is selected, it can bring moderate resistance to PD and blast, resistance to BPH, moderate yield and shortest maturity period, and high amylose content (Figure 3.5).

A sample crossing scheme is shown in Figure 3.6 to produce a rice variety with high PDT, mean yield ≥5.0 mt/ha, maturity period ≤105 days, resistant to BPH and blast disease, and higher amylose content. Since there is no reported cultivar for high PDT with complete blast resistance (Figure 3.5), the illustrated crossing scheme in Figure 3.6 is proposed with two phases. In the first phase, the crossing of At362 and Bg250 followed by numerous rounds of selfing and selection at advanced generations would accomplish the breeding objective only without complete resistance to blast (i.e., a moderate level of blast resistance is possible). In the second phase, the selected RILs from phase 1 can be backcrossed to Bg252 as the donor parent to introgress the complete resistance to blast. The breeder can come up with diverse crossing schemes like the one given in Figure 3.6 to make effective decisions for breeding and maximize the resource utilization to release varieties in the shortest possible time. The breeder can select any number of parents that are needed to use as sources of resistance and other traits to start crossing. Also, the marker alleles and the IBD probabilities can be checked as illustrated in Figure 3.7A and Figure 3.7B, respectively.

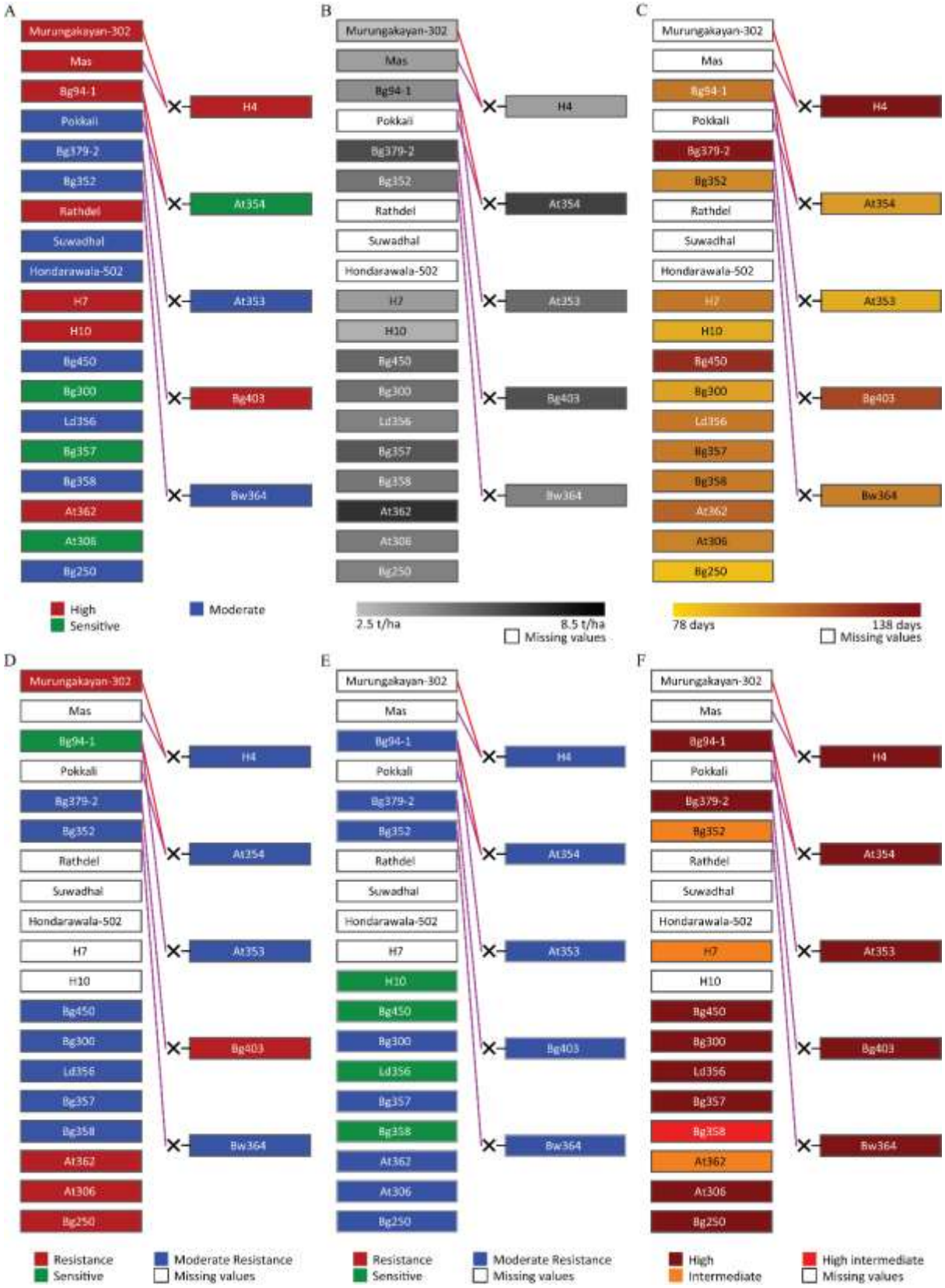


Figure 3.5 The pedigree visualization for Example 3 (Parents ranked for phosphorus deficiency tolerance). The selected pedigree is colored separately for six traits. A: PDT; B: Yield; C: Maturity period; D: Degree of resistance to BPH; E: Degree of resistance to BLAST; F: Amylose content. Female and male parentages are indicated by red and purple lines, respectively. The symbol '×' indicates the cross between two parents. The background colors of the cultivar-name boxes indicate the trait values, as shown in the colored legends below. The cultivars with missing-trait values are indicated by white boxes.

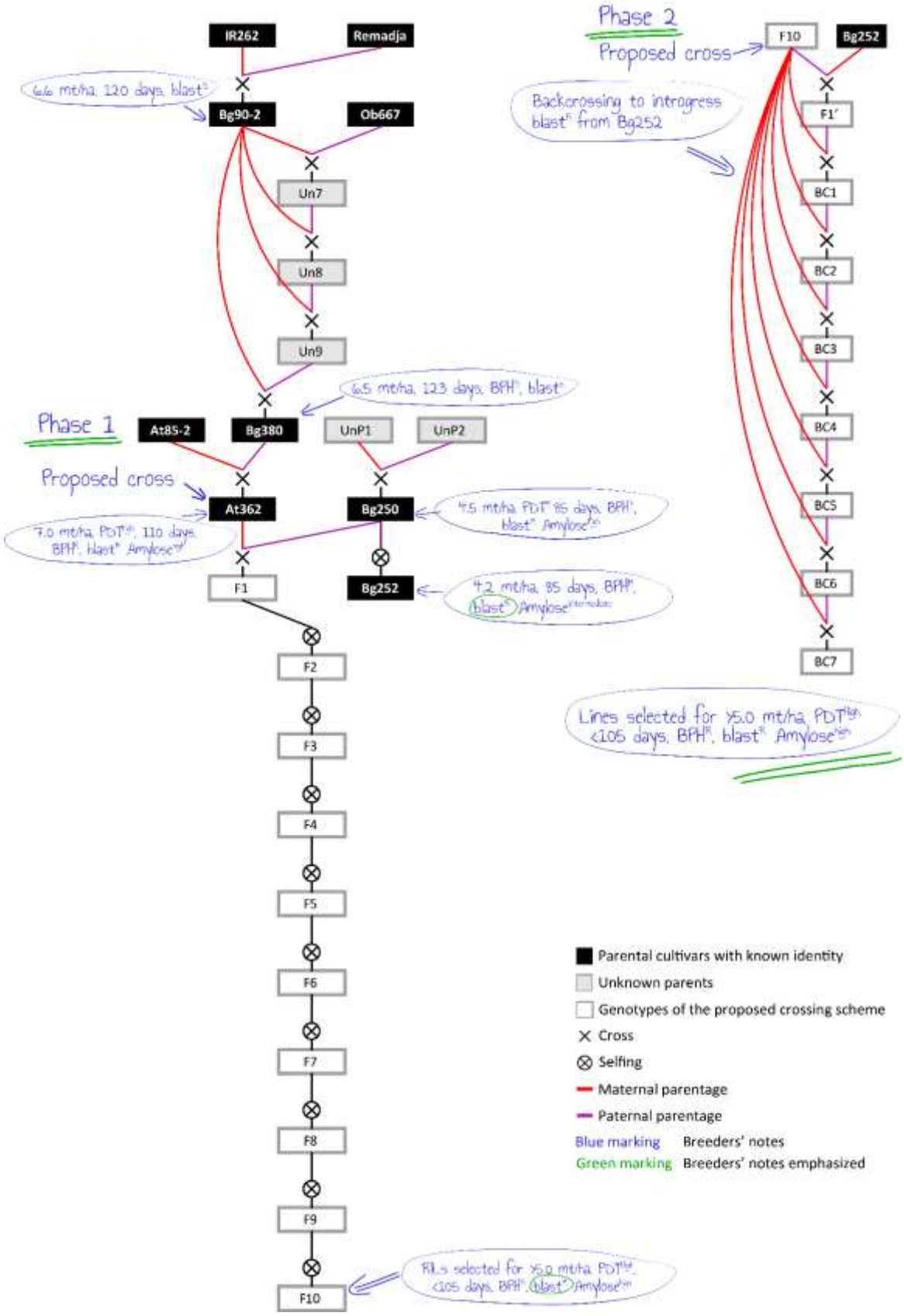
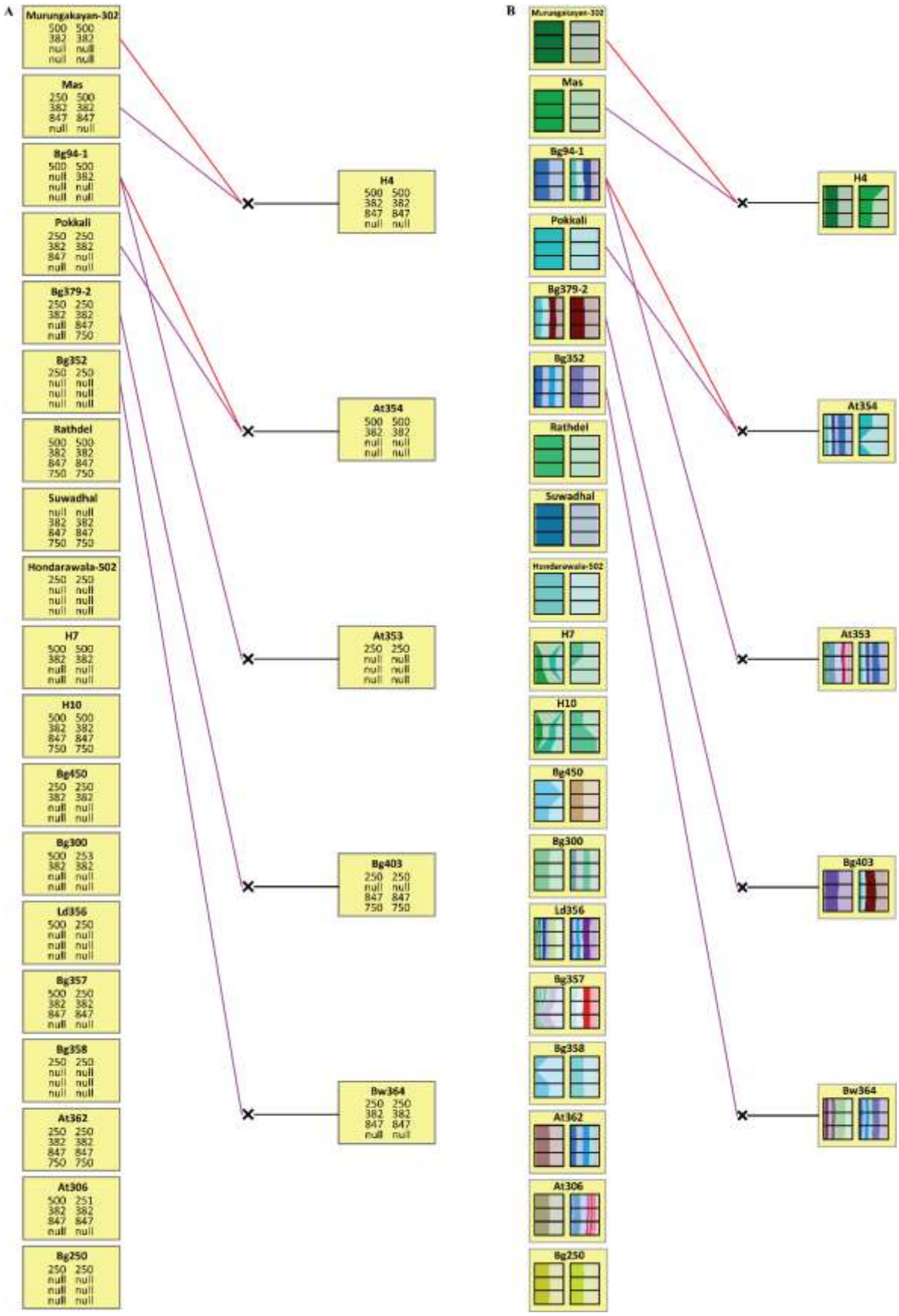


Figure 3.6 The pedigree visualization for planning a crossing scheme. Phase 1: Initial crossing of At362 and Bg250 and pedigree selection to obtain RILs with ≥5.0 mt/ha of mean yield, ≤105 day of maturity period, resistant to BPH, moderately resistant to blast and high level of amylose content. Phase 2: Then backcrossing with Bg252 as the donor parent to introgress the blast resistance.



3.7 Figure Visualization of selected marker genotypes and Identical by Descent (IBD). A: Marker alleles. The alleles of the DNA markers *K29-N*, *K41*, *K48*, and *K5-N* are given in vertical order.; B: IBD probabilities of four *Pup1* linked markers (on chromosome 12 at about 55 cM). Since the cultivar linkage maps are not available, we assumed 0.1 cM gap between adjacent markers for the representation of IBD values.

The decision-making process in breeding is a tedious task (Acquaah, 2012). The breeding germplasm is complicated with large numbers of improved varieties, traditional cultivars, landraces, and wild germplasm. Also, there can be large mapping populations and unreleased varieties due to various reasons. The numerous genotypes in breeding germplasm may have extensive records on agronomic data, pest and disease resistance, quality traits, availability of samples, geographic locations, and utilization in diverse breeding programs as parents (Davenport *et al.*, 2004; Peeters and Galwey, 1988). With the advent of DNA markers and sequencing technologies, a wealth of genomic information is also available (Nadeem *et al.*, 2018). However, one of the recurrent problems in any breeding germplasm in the world is most of the cultivars remain uncharacterized. Thus, they cannot be used directly in breeding activities. Traditionally, breeders keep records in field books. However, with the development of computer technology, data tabulation is becoming a common practice. However, given the highly complex nature of the datasets in breeding germplasm, data tables have a limited value to the breeders. The tables cannot graphically display complex pedigrees and variations of qualitative and quantitative traits along with DNA marker information. In this context, Pedimap provides a considerable advantage, as it can visualize pedigree relationships, trait variations, and any other useful information required for decision-making and planning crosses in breeding programs (Voorrips *et al.*, 2012). If all the available details on breeding germplasm are arranged as a database, the breeder can come up with subpopulations based on diverse traits and select the parents for improving multiple traits. However, simple spreadsheets or manually prepared note pages cannot be used to visualize the essential information and complex pedigrees. The breeding programs always suffered a lot when the breeder gets retired or moved to a different position (Sooriyapthirana *et al.*, 2019; USDA, 2015; Hmielowski, 2017). The newly hired breeder cannot practically go through the individual records of the existing breeding germplasm. Thus, there is a strong possibility that valuable breeding germplasm might get lost wasting time, resources, and courage of the retired breeder and his team. However, as a routine practice, if the breeder maintains and updates a Pedimap database for the developing germplasm of breeding materials, the newly hired breeders can go through and identify the value and gaps in the available material for him to plan further. The creation of a Pedimap database is simple, and a novice to informatics can curate and use Pedimap with a little training. Pedimap allows breeders to store data, fetch and visualize genomic information at any time with less effort and complete accuracy (Zhu *et al.*, 2017). The straightforward accessibility, direct data interpretation, ability to customize the views in multiple fashions, and editable output file

formats are the significant features of Pedimap. The graphic files created can be readily imported to image editing software for further visualizations and illustrations. Pedimap is also an open-source platform and can be easily obtained from the inventor. Even the breeders in developing countries can benefit from Pedimap (Voorrips *et al.*, 2012).

In the current study, we created a Pedimap database for the rice cultivars prominently used by breeding programs in Sri Lanka. With the available information, significant breeding decisions can be made as we explain in three examples (Figures 3.3-7). However, it is essential to characterize the cultivars for all the important traits, molecular markers and SNP haplotypes (Vanderzande *et al.*, 2019), so that breeding decisions can be effectively made (Jiang, 2013). The phenotyping methods must be standard and should follow common procedures across different locations so that the power of the Pedimap database would go up dramatically. Therefore, breeders should always follow the standard, globally acceptable phenomic platforms to characterize the material in breeding germplasm (Evans *et al.*, 2011; Fukai and Fischer, 2012).

3.5 CONCLUSION

The pedigree visualization with variations of phenotypic and molecular data using Pedimap is a user-friendly tool to plan rice breeding programs with higher accuracy and resource optimization. The present study explains the applicability of Pedimap as a decision-making tool to streamline the rice breeding programs in Sri Lanka. However, it is also important to note that accurate characterization of the breeding germplasm for phenotypic and molecular data is the critical prior step to harness the value of Pedimap for breeding.

CHAPTER 4

ASSESSMENT OF THE VARIATION OF PERFORMANCE INDICATORS UNDER P-STARVED CONDITIONS IN A CORE-SET OF RICE CULTIVARS AND THEIR DIVERSITY IN *PUP1*-LINKED DNA MARKER-HAPLOTYPES

4.1 ABSTRACT

The screening of PDT rice cultivars is important for sustainable rice production. If the performances (PDT indicators) of the rice cultivars can be correlated with *Pup*1 (major QTL linked to PDT in rice) haplotypes, MAB programs could be launched to produce PDT rice varieties. In the present study, we screened 27 rice cultivars (nine landraces and 18 improved varieties) under P-deficient soil conditions in two seasons (*Yala* and *Maha*, the major cropping seasons of Sri Lanka) based on shoot dry weight, and P utilization efficiency. To identify the basis for MAB of PDT, the *Pup1* haplotypes were assessed using 17 *Pup1* linked DNA markers and sequence polymorphism of the two marker loci (*K29* and *RM28102*). The PDT screening results revealed that the performance of the rice cultivars are highly seasonal dependent. The landrace *Madathawalu* and the old-improved variety H-4 showed highest trait values for yield, shoot dry weight, and P utilization efficiency in both *Yala* and *Maha* seasons. Remarkably *Madathawalu* and H-4 share a common *Pup1* haplotype demonstrating that MAB for PDT is possible using these two rice cultivars as parents. The present study also reports the *Pup1* linked marker haplotypes, sequence variants of *K29* and *RM8102*, and variation of the PDT indicators of the studied cultivars to be used in making breeding decisions and varietal selection for profitable and ecofriendly rice farming.

Keywords: Marker-assisted breeding for P efficient rice cultivars, phosphorus efficient rice varieties, phosphorus fertilizer crisis, *Pup1* QTL, rice landraces in Sri Lanka

4.2 INTRODUCTION

Phosphorus is one of the most important macronutrients for growth and development of rice plant (Halford, 1997). The problem associated with P as a macronutrient is the limited availability of P in the soil for the plants due to the fixation by Fe_2O_3, Al_2O_3, $CaCO_3$, $MgCO_3$, carboxyl ions, and humic substances (Wang *et al*., 2013). However, farmers do not recognize the fixation of P ions within the soil, and they add higher concentrations of P fertilizer for better growth and development of the plants. The applications of artificial P fertilizer causes two problems in rice farming (Bulluck and Ristaino, 2002; O'neil *et al*., 2012). Sri Lanka spends 0.3 billion US dollars annually (1.5 % of GDP) on importing P fertilizer to Sri Lanka, and farmers struggle to purchase P fertilizers due to their higher price (Aluwihare *et al*., 2016). Therefore, the government has to provide P fertilizer under a subsidized rate to the farmers creating an extra burden to the national economy. The additional P fertilizer incorporated to the soil would end up in runoff water; ultimately polluting the water bodies causing eutrophication and health hazards (Bulluck and Ristaino, 2002; O'neil *et al*., 2012). In addition to the higher price and environmental pollution caused by the heavy dependence on P fertilizer, the currently available P reserves in the world are depleting rapidly causing a massive fertilizer crisis in the near future (Cordell *et al*., 2009; FAO, 2015; Steen, 1998). Farmers need more and more P fertilizer in every season; however, P reserves are rapidly depleting which will place the future rice farming in a futile situation.

The only sustainable solution to face the indisputable P fertilizer crisis of the future rice farming is to produce PDT rice varieties through DNA MAB (i.e., molecular breeding). The genetics of the PDT has been studied in detail using world-wide rice germplasm (Ni *et al*., 1998; Wissuwa *et al*., 1998). Majumder *et al*., (1989) first reported that PDT is a quantitatively inherited trait. The major QTL underlying the PDT in rice has been identified and named *PUP1* by using a segregating progeny made by crossing PDT *Kasalath* and P deficiency sensitive *Nipponbare* as the parents (Ni *et al*., 1998; Wissuwa *et al*., 1998). Although some other minor QTLs were detected, *Pup1* was further validated as the major QTL with 80 % effect on the trait PDT. The effect of *Pup1* was further verified by Chin *et al*., (2010) and Wissuwa *et al*., (2002) by incorporating the *Pup1* allele of *Kasalath* to *Nipponbare* through backcrossing which demonstrated the increment of P uptake by 170 % and grain yield by 250 %.

Subsequently, the *Pup1* locus of *Kasalath* was molecularly characterized with respect to the counterpart allele in *Nipponbare*. The total length of the *Pup1* QTL in *Kasalath* is 423 kb, whereas *Nipponbare* has 293 kb allele difference due to the presence of a large deletion of about 130 kb (Heuer *et al*., 2009). The molecular markers within *Pup1*, the candidate genes and their relative physical and mapping distances were characterized and are publicly available (Chin *et al*., 2010,2011). One of the drawbacks in *Pup1* characterization studies is the underutilization of country-specific improved varieties and traditional landraces. It has been observed that such locally adapted rice varieties perform impressively in infertile soils. The local varieties may contain additional QTLs to *Pup1* or hitherto unknown haplotypes of the *Pup1*.

Aluwihare *et al*., (2016, 2018) have shown that some of the old improved rice cultivars and landraces in Sri Lanka possess novel *Pup1* haplotypes compared to the *Kasalath* type *Pup1* haplotype. This observation highlights the significance of studying the important local rice landraces and varieties (i.e., cultivars) for the PDT and then detect their *Pup1* haplotypes with respect to the association with PDT conferring traits. Thus, MAB can be swiftly carried out within the regional rice breeding programs because of the availability of locally present PDT promoting haplotypes. They would provide a strong basis for selecting more efficient rice varieties for local needs rather than just examining for the presence of the *Pup1* haplotype in the lines to be selected. Therefore, in the present study, we aimed to screen a set of rice landraces and improved rice varieties in Sri Lanka for PDT, identify their maker haplotypes of *Pup1* and characterize two key co-dominant sequence tagged sites (STS) sites within *Pup1* locus to facilitate the MAB of rice for PDT.

4.3 MATERIALS AND METHODS

4.3.1 Growing seasons

Based on the two seasons of monsoonal rains in Sri Lanka, rice farming is conducted under two major cropping seasons namely *Yala* and *Maha*. The growing season *Yala* runs from early April to late August with the South West monsoonal rains and the growing season *Maha* runs from late September to early March with the North-East monsoonal rains [DOA, Sri Lanka, 2006]. The growing and climatic conditions of the field where the trial was conducted are given in Table 4.1. The trials were conducted in *Maha* and *Yala* seasons of the year 2016/2017.

Table 4.1 Climatic parameters of the field location during the two seasons.

Location and Global Positioning System (GPS) Coordinates	Agro-Ecological Zone (AEZ)[a]	Season	Temperature (°C)	Total Rainfall (mm) of the Season	Sunshine hours (hrs/day)
Bathalagoda (N7°31′49.21″; E80°26′25.49″)	IL1a	*Maha* (Sept.-Mar, 2016/2017	21.3 -32.4	479.6	7.6-8.0
		Yala, (Apr. - Aug) 2017	24.3-32.2	408.4	6.2-8.0

[a]AEZ classification was obtained from Department of Agriculture, Sri Lanka (http://www.agridept.gov.lk/) (IL: Intermediate Low)

4.3.2 Rice cultivars, plant establishment and maintenance

The breeder seeds of 27 rice cultivars [landraces (L), old-improved (OI) and newly-improved (NI) varieties] were obtained from the Rice Research and Development Institute (RRDI) Bathalagoda, Sri Lanka (Table 4.2). The seeds were germinated and established in non-fertilized soil at RRDI (a separate block of the field is maintained as non-fertilized soil for last 45 years) (Kumaragamage and Indraratne, 2011; Sirisena and Wanninayake, 2014).

4.3.3 Data collection

4.3.3.1 Growth and yield parameters

Plant height (PLH), number of tillers (NT), flag leaf length (FLL), flag leaf width (FLW) and yield per plant were collected for both seasons. PLH was measured from the base of the plant to the tip of the top leaf in centimeters. These measurements were taken at flowering stage. The NT was also recorded at the flowering stage for all the cultivars. The FLL and FLW were measured after the flowering stage. The FLL was measured from the bottom of the leaf to the tip in centimeters. Maximum width of the leaf was taken as the FLW and measured in millimeters. Aerial parts of the plant were used to obtain the shoot dry weight (SDW). The plants were harvested at the flowering stage and the shoot parts were washed with distilled water in order to oven dry at 60 $^{\circ}$C to obtain a constant dry weight (grams).

4.3.3.2 PDT indicators

The plants from each cultivar were collected at the harvesting stage, roots were removed and oven dried until a constant weight was obtained. Weight of the oven dried samples were measured and taken as the SDW in grams. Oven dried shoots were ground in to fine powder and total of 0.5 grams (g) of powdered shoot material was taken for the digestion. The process of digestion was carried out using an acid mixture of conc. HNO_3 and conc. $HClO_4$ in a ratio of 4:1 respectively. The shoot P concentration (SPC) was obtained as the amount

of P (mg) in 1 g of shoot dry matter by following the phosphovanadate method described in Hanson, (1950). The shoot phosphorous uptake (SPU) was calculated using the formula described in Fageria *et al*., (1988) and Gunes *et al*., (2006). SPU was calculated for each variety in milligram (mg) per plant.

$$SPU = SDW \times SPC$$

PUE was calculated in g per milligrams in other words biomass produced per unit P accumulated in shoot (Rose and Wissuwa, 2012).

$$PUE = SDW/ SPU = 1/ SPC$$

Table 4.2 Rice genotypes screened for phosphorous deficiency tolerance.

Name of the genotype	Type of cultivar	Naturally preferred growing condition	Key characteristics
Herath banda	L	UL	Red pericarp, high amylose content
Kuruluthdu	L	UL	Red pericarp, good cooking quality, rich in protein and fiber, enhance male sexual potency, improved bladder function
Madathawalu	L	UL	Red pericarp, comparatively low glycemic index, recommended in Ayurvedic treatments
Masuran	L	UL	Red pericarp, bold grain type
Pachchaperumal	L	UL	Red pericarp, rich in protein, recommended for diabetes and cardiovascular diseases
Rath suwandel	L	UL	Red pericarp
Rathdel	L	UL	Recommended for cirrhosis, prevent formation of stones in the bladder and gall bladder
Sulai	L	UL	Red pericarp, good cooking quality, resistant to brown planthopper
Thatu vee	L	UL	Red pericarp
At308	NI	LL	Suitable grow under rain fed and irrigated farming conditions
At309	NI	LL	White pericarp, moderately resistant and resistant to blast and moderately resistant to brown planthopper
At311	NI	LL	Red elongated pericarp
At354	NI	LL	White pericarp
At373	NI	LL	White pericarp, short round grain, good cooking quality, resistance to gall midge
Bg310	NI	LL	White pericarp, high amylose content, resistant to blast, gall midge and brown planthopper
Bg359	NI	LL	Resistance to gall midge 1 and 2, rice blast disease and bacterial leaf blight, moderately tolerant to brown planthopper
Bg366	NI	LL	White pericarp, non-glutinous endosperm, resistant to bacterial leaf blight
Bg369	NI	LL	White pericarp, long grain shape, recommended for saline affected areas
Bw363	NI	LL	White pericarp, moderately tolerant to brown planthopper, blast and gall midge
Bw367	NI	LL	Short round grain type, moderately tolerant to ion toxicity, tolerant for lodging
Bw452	NI	LL	Red pericarp, little tall, tolerant to bronzing and submergence in low country wet zone
Ld356	NI	LL	Red pericarp, short round grains
Ld365	NI	LL	White pericarp
Ld371	NI	LL	Tolerant to seed discolorations and neck blast, resistant/moderately resistant to blast, gall midge and brown planthopper
H-10	OI	UL	Red pericarp, less photoperiod sensitivity
H-4	OI	UL	Resistant to rice blast disease
H-7	OI	UL	Better grain quality, higher response to nitrogen

Source: Rice Research and Development Institute, Bathalagoda, Sri Lanka; L: Landrace; NI: Newly Improved; OI: Old Improved (during 1950's); UL: upland; LL: lowland

4.3.4 Establishment of DNA marker haplotypes for the *Pup1* locus

4.3.4.1 DNA extraction and PCR

Immature leaf samples of rice cultivars (Table 4.2) were collected and subjected to DNA isolation using Dneasy® plant mini kit (Qiagen, Solna, Sweden). The isolated DNA of each cultivar was subjected to PCR amplification with the markers developed for the *Pup1* QTL (Table 4.3). The PCR conditions were provided in the Thermal Cycler (Takara, Japan) as follows; initial denaturation at 94 ℃ for 5 minutes (min), then 35 cycles of 94 °C for 30 seconds (sec) for denaturation, primer annealing temperature (Ta) (Table 4.3) for 90 sec, and 72 °C for 2 min, finally extension at 72 °C for 10 min. The PCR products were size separated using ethidium bromide stained 2.5 % agarose gel.

Table 4.3 *Pup1* linked markers assessed in the present study.

Marker	Forward and reverse primer sequences (5' → 3')	Band size (bp)	Ta (ºC)	Reference
K20	TCAGGTGATGGGAATCATTG TGTTCCAACCAAACAACCTG	240, 243	55	Chin *et al.*, (2010)
K29	CCATAGTAGCACAAGAAACCGACA GCTTCAATGAGCCCAGATTACGAA	850, 491	55	
K41	TGATGAATCCATAGGACAGCGT TCAGGTGGTGCTTCGTTGGTA	382	57	
K42	CCCGAGAGTTCATCAGAAGGA AGTGAGTGGCGTTTGCGAT	918	57	
K43	AGGAGGATGAGCCTGAAGAGA TCGCACTAACAGCAGCAGATT	912	57	
K46	TGAGATAGCCGTCAAGATGCT AAGGACCACCATTCCATAGC	523	57	
K48	CAGCATTCAGCAAGACAACAG ATCCGTGTGGAGCAACTCATC	847	57	
K52	ACCGTTCCCAACAGATTCCAT CCCGTAATAGCAACAACCCAA	505, 700	57	
K59	GGACACGGATTCAAGGAGGA TGCTTTCCATTTGCGGCTC	550	57	
RM28102	CACTAATTCTTCGGCTCCACTTTAGG GTGGAAGCTCCGAGAAAGTGC	168	55	
RM28073	GTGTTGGTGGTGATGAAGCAAGG GGACGAAGGATGTATGTGTCTGTACC	656	55	
K46-K1	TGAGATAGCCGTCAAGATGCT TGAGCCAGTAGAATGTTTTGAGG	342	57	Pariasca-Tanaka *et al.*, (2014)
K46-K2	CTGAAGTGAAAAGAATGACTAA TGAGCCAGTAGAATGTTTTGAGG	110, 433	57	
K46-3	TCCAAAGATCTCTGATTTTGGC GCTTTCCAACATCTCAAGGACT	400	57	
K46-CG1	CTAGAGTATCTCCACAGTCGTT AAGGACCACCATTCCATAGC	258	57	
K46-CG2	CCGAAGTAAGAAGAATGACGGA TGATCCAGGAGAATGTTTTGTGG	130, 433	57	
Ba76H14_7154	GAAACGGGGTCAAATAAGC GGGTTCGTCCAACAGGAGTA	292, 259	55	Heuer *et al.*, (2009)

4.3.4.2 Detection of the sequence polymorphism in *K29* and *RM28102*

The PCR products of *K29* and *RM28102* were purified using QIAquick® PCR Purification Kit (Catalog No: 28104, Qiagen, Hilden, Germany). The purified PCR products of the markers were subjected to 3× sanger sequencing in Macrogen Inc., South Korea.

4.3.5 Data analysis

4.3.5.1 Growth, Yield and PDT parameters

All the tested parameters were subjected to General Linear Model procedure (GLM) and Duncan's mean separation in SAS Version 9.4 (SAS Institute Cary, NC, USA).

4.3.5.2 *Pup1* DNA haplotypes

Based on the binary data for the DNA marker polymorphism, a dissimilarity matrix was constructed using the unweighted pair group method with arithmetic means (UPGMA) (Nei and Kumar, 2000) in Phylip package v3.697 (Felsentein, 2005) and the tree was visualized using FigTree v1.4.3. (Rambaut, 2018).

4.3.5.3 Detection of *K29* and *RM28102* haplotypes

Two separate alignments were constructed for the markers *RM28102* and *K29* using MEGA v7 software (Kumar *et al*., 2016) and the dendrograms were constructed using UPGMA algorithm. The obtained sequences were aligned using the MEGA software with a reference sequence obtained from the GenBank (https://www.ncbi.nlm.nih.gov/). The SNPs and the INDELs were identified from generated multiple sequence alignment.

4.4 RESULTS

4.4.1 Plant height and number of tillers

In the *Maha* season, mean PLH at flowering stage was significantly higher in *Kuruluthudu* and *Rathdel* compared to the rest of the cultivars. Whereas in *Yala* season, the significantly highest PLH was reported in LD356. NT was significantly highest in LD371 in both *Yala* and *Maha* seasons ($P< 0.05$) (Table 4.4). The landraces *Kuruluthudu* and *Rathdel* did not flower in *Yala* season and remained in the vegetative phase; thus their PLH and NT were not recorded and included in the analysis.

Table 4.4 Variation of the plant height and no. of tillers of the rice cultivars under P starved field condition.

Type	Cultivar	PLH (cm)		NT	
		Maha	***Yala***	***Maha***	***Yala***
L	*Herath banda*	79.0^{bcde}	93.7^{ef}	2.4^{h}	3.4^{bcde}
	Kuruluthudu	120.3^{a}	-	2.6^{gh}	-
	Madathawalu	79.6^{bcd}	67.3^{klm}	5.0^{abcd}	2.0^{ghij}
	Masuran	83.9^{bc}	94.5^{ef}	3.9^{cdefgh}	3.0^{cdefg}
	Pachchaperumal	73.3^{cdef}	115.2^{b}	3.6^{cdefgh}	3.6^{abcd}
	Rathdel	111.6^{a}	-	2.5^{h}	-
	Rath suwandel	56.9^{hij}	107.2^{cd}	4.5^{abcdef}	2.5^{efgh}
	Sulai	78.6^{bcde}	87.7^{ghi}	2.6^{gh}	1.5^{hij}
	Thatuvee	91.8^{b}	84.6^{hi}	2.3^{h}	1.5^{hij}
NI	At308	54.4^{ij}	65.1^{lmn}	3.5^{cdefgh}	2.1^{fghij}
	At309	59.2^{ghij}	61.8^{no}	5.9^{ab}	2.8^{defg}
	At311	61.1^{fghi}	57.8^{o}	5.3^{abc}	3.5^{bcde}
	At354	69.3^{defgh}	71.1^{jk}	4.9^{abcde}	3.8^{abcd}
	At373	51.9^{ij}	64.3^{mn}	5.8^{ab}	2.3^{fghi}
	Bg310	46.0^{j}	69.4^{jkl}	2.8^{fgh}	2.8^{defg}
	Bg359	86.3^{bc}	62.0^{no}	3.5^{cdefgh}	3.0^{cdefg}
	Bg366	65.3^{efghi}	73.7^{j}	3.0^{fgh}	3.1^{bcdef}
	Bg369	72.0^{cdefg}	70.9^{j}	3.1^{efgh}	2.0^{ghij}
	Bw363	81.8^{bcd}	83.4^{i}	3.9^{cdefgh}	3.0^{cdefg}
	Bw367	68.4^{defgh}	97.4^{e}	3.4^{defgh}	1.1^{j}
	Bw452	53.5^{ij}	72.7^{j}	2.9^{fgh}	2.3^{fghi}
	Ld356	74.0^{cdef}	147.6^{a}	4.0^{cdefgh}	1.4^{ij}
	Ld365	63.3^{fghi}	108.7^{c}	4.4^{bcdefg}	3.9^{abc}
	Ld371	81.4^{bcd}	88.9^{gh}	6.1^{a}	4.6^{a}
OI	H-10	86.4^{bc}	71.3^{jk}	4.9^{abcde}	4.2^{ab}
	H-4	82.3^{bcd}	91.2^{fg}	4.0^{cdefgh}	2.8^{defg}
	H-7	82.5^{bcd}	103.1^{d}	5.1^{abcd}	4.0^{abc}

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

4.4.2 Flag leaf size and yield

The landrace *Herath banda* and the newly improved variety BW452 took at least 8 – 10 weeks to flower and produce panicles in *Yala* season. By that time all the other plots were harvested, and therefore the plots of *Herath banda* and BW452 were heavily exposed to birds, pests, and diseases and thereby we were not able to collect the yield data for these two cultivars in *Yala* season (Table 4.5).

The longest flag leaf size was reported for *Kuruluthudu* in *Maha* season and *Herath banda* in *Yala* season; however, the widest flag leaf was observed in BW367 in both *Yala* and *Maha* seasons. The significantly highest yields were reported by newly improved cultivars under *Yala* and *Maha* seasons where AT354 reported 11.91 g per plant in *Yala* season and AT306 reported 19.53 in *Maha* season (Table 4.5).

The yield per plant is an ideal trait to identify PDT cultivars under P starved conditions. The grand mean of the yield reported for a cultivar in both seasons was six grams per plant. When this yield level was considered as the expected threshold to declare the PDT cultivars; *Pachchaperumal*, *Madathavalu*, Bg369, AT354, H-10 and H-4 provided higher yield than six grams per plant, thereby can be considered as promising cultivars providing above average yield under P starved conditions (Figure 4.1). A total of one OI, one land race and seven NI provided a mean yields of less than six grams per plant in both seasons, showing the negative effect of P limitation on the yield (Table 4.5; Figure 4.1).

Table 4.5: Variation of the size of flag leaf and yield under P starved field condition

Origin	Cultivar	FLL (cm)		FLW (cm)		Yield (g/plant)	
		Maha	***Yala***	***Maha***	***Yala***	***Maha***	***Yala***
L	*Herath banda*	24.06^{de}	40.62^{a}	7.94^{cdef}	12.63^{bc}	3.40^{op}	-**
	Kuruluthudu	37.37^{a}	-*	11.44^{bc}	-*	8.98^{bc}	-*
	Maathawalu	26.08^{cd}	38.02^{ab}	8.81^{cdef}	12.50^{bc}	7.97^{cd}	11.11^{b}
	Masuran	23.92^{de}	33.69^{c}	8.13^{cdef}	10.63^{cde}	2.08^{rs}	6.84^{d}
	Pahhaperumal	19.55^{efg}	30.34^{de}	6.38^{defgh}	8.20^{hi}	9.43^{bc}	11.76^{b}
	Rath suwandel	20.13^{efg}	30.46^{de}	6.13^{defgh}	8.50^{g}	1.38^{tu}	5.56^{ef}
	Rathel	30.81^{b}	-*	9.63^{cde}	-*	8.08^{cd}	-*
	Sulai	22.78^{def}	23.04^{gh}	8.88^{cdef}	10.63^{cde}	4.36^{kl}	7.55^{c}
	Thatu vee	28.92^{bc}	32.27^{cd}	7.88^{cdef}	10.63^{cde}	3.54^{no}	6.25^{de}
NI	At308	16.88^{fgh}	19.67^{hi}	8.38^{cdef}	10.00^{cde}	3.16^{opq}	19.53^{a}
	At309	16.26^{fgh}	19.22^{hi}	9.19^{cdef}	7.88^{hij}	7.22^{e}	4.17^{j}
	At311	17.89^{fgh}	21.69^{ghi}	7.31^{cdefg}	7.88^{hij}	5.05^{j}	3.55^{k}
	At354	22.58^{def}	26.23^{f}	10.5^{bcd}	11.13^{bcd}	11.91^{a}	7.81^{c}
	At373	15.22^{fgh}	18.35^{hi}	9.25^{cde}	11.00^{bcd}	4.46^{kl}	5.56^{ef}
	Bg310	16.69^{fgh}	20.48^{hi}	8.75^{cdef}	10.38^{cde}	0.84^{uv}	11.22^{b}
	Bg359	23.60^{def}	19.84^{hi}	8.93^{cdef}	10.13^{cde}	5.35^{ij}	5.21^{gh}
	Bg366	22.71^{def}	26.23^{f}	11.31^{bc}	13.25^{ab}	4.22^{lm}	4.81^{ghi}
	Bg369	22.26^{def}	26.68^{f}	8.50^{cdef}	10.13^{cde}	9.26^{bc}	7.81^{c}
	Bw363	17.58^{fgh}	33.11^{cd}	5.86^{defgh}	7.50^{hij}	0.92^{uv}	4.17^{j}
	Bw367	17.99^{fgh}	21.94^{ghi}	13.00^{a}	13.63^{ab}	3.54^{no}	5.85^{ef}
	Bw452	17.58^{fgh}	20.59^{hi}	8.63^{cdef}	12.00^{bc}	1.79st	-**
	Ld356	21.94^{def}	31.62^{cd}	8.06^{cdef}	10.00^{cde}	1.05^{uv}	5.21gh
	Ld365	19.16^{efg}	19.95^{hi}	9.44^{cde}	11.63^{bcd}	9.70^{bc}	4.17^{j}
	Ld371	22.32^{def}	33.69^{cd}	7.25^{defgh}	7.63^{hij}	1.70st	4.17^{j}
OI	H-10	22.78^{def}	31.53^{cd}	7.56^{cdef}	9.75^{def}	6.06^{fgh}	6.51^{de}
	H-4	26.68^{cd}	30.90^{de}	10.44^{bcd}	10.50^{cde}	6.26^{fg}	6.25^{de}
	H-7	22.39^{def}	32.92^{cd}	5.50^{efgh}	7.50^{hij}	3.34^{op}	5.21^{gh}

* These landraces did not flower and therefore did not collect the data in *Yala* season.

** The data was unable to collected since the seeds got exposed to birds, pests, and diseases.

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

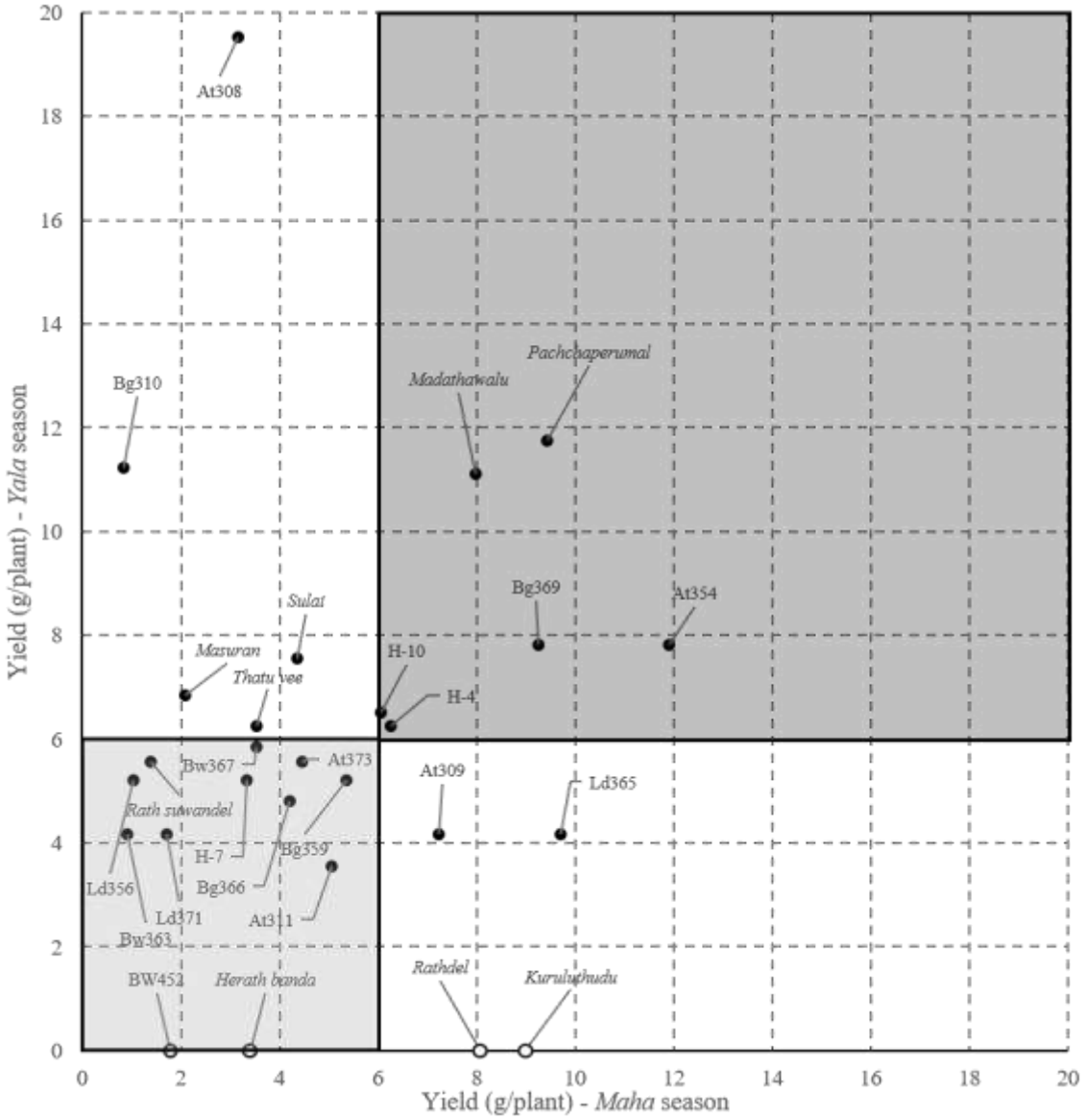


Figure 4.1 The scatter plot drawn between the mean yield of the cultivars assessed in *Maha* and *Yala* seasons. Six grams per plant is considered as the threshold for identifying the high yielding cultivars under P starved conditions. Dark gray area represents the high yielding cultivars in both seasons and light gray area represent the low yielding cultivars in both seasons. (For BW452, *Herath banda*, *Rathdel* and *Kuruluthudu*, Data were only available in *Maha* season)

4.4.3 Variation on the PDT indicators

The significantly highest SDW was identified in *Rathdel* in *Maha* season and *Herath banda* in *Yala* season. However, under the Duncan's grouping of means, many other cultivars also showed the significantly higher mean SWD values with overlapping groups (Table 4.6). The variation of the SPC, SPU and PUE indicated that the studied rice cultivars are variable and mostly seasonal specific in responding to P sparse conditions in the soil. The inverse of SPC is defined as PUE. Higher SPC or SPU means plants accumulate higher amount of P without partitioning into required tissues. Therefore, the relationship between SDW (amount of

biomass generated under P limited conditions in the context of present study) and PUE would provide the basis to identify PDT of the rice cultivars. As given in previous studies, we used 7 g of SDW per plant and 0.5 g/mg of PUE as the two thresholds (i.e., independent culling levels) (Chankaew *et al*., 2019; Vandamme *et al*., 2016) to identify the PDT and Phosphorus deficiency (PD) sensitive cultivars (Figure 4.2).

In the *Maha* season, *Kuruluthudu*, *Madathawalu*, *Masuran*, and seven improved cultivars showed SDW and PUE above the thresholds indicating that they were PD tolerant. Similarly, in *Yala* season, *Madathawalu*, *Thatuvee*, *Sulai*, and ten improved cultivars were PD tolerant. The landrace *Madathawalu*, OI cultivar H-4 and NI cultivars Bg366, Ld359, At373, Bw367 and Bg366 were PDT in both seasons (Figures 4.2A and 2B). Bg359 in *Maha* season, and Bw452 in *Yala* season were found to be PD sensitive. Other cultivars got switched back and forth in two moderately PDT classes (PUE > 0.5 g/mg, SDW < 7 g or PUE < 0.5 g/mg and SWD > 7 g) in *Maha* and *Yala* seasons (Figures 4.2A and B).

Table 4.6 Variation of PDT indicators under P starved field condition.

Type	Cultivar	SDW (g/plant)		SPC (mg/g)		SPU (mg/plant)		PUE (g/mg)	
		Maha	***Yala***	***Maha***	***Yala***	***Maha***	***Yala***	***Maha***	***Yala***
L	*Herath banda*	3.34^{efg}	29.17^{a}	1.61^{ab}	1.49^{bcde}	5.37^{ab}	41.69^{a}	0.62^{ab}	0.67^{bcdef}
	Kuruluthudu	16.78^{a}	-*	1.12^{b}	-*	18.62^{ab}	-*	0.89^{ab}	-*
	Madathawalu	15.66^{a}	14.13^{bcd}	2.02^{ab}	1.48^{bcde}	30.90^{ab}	20.65^{abcd}	0.50^{b}	0.68^{bcdef}
	Masuran	7.16^{abcdef}	6.76^{hijk}	1.42^{ab}	1.38^{bcde}	10.47^{ab}	22.91^{abc}	0.71^{ab}	0.72^{bcdef}
	Pachchaperumal	3.84^{cdefg}	5.89^{ijkl}	0.59^{b}	1.73^{abcd}	2.29^{b}	$10.00^{cdefghi}$	1.69^{a}	0.58^{cdef}
	Rathdel	15.66^{a}	-*	2.10^{ab}	-*	35.48^{a}	-*	0.48^{b}	-*
	Rath suwandel	2.88^{fg}	3.85^{l}	0.93^{b}	1.12^{cde}	2.66^{ab}	$9.77^{cdefghi}$	1.07^{ab}	0.89^{bcde}
	Sulai	$6.76^{abcdefg}$	7.08^{ghijk}	1.64^{ab}	0.88^{de}	11.22^{ab}	$10.35^{cdefghi}$	0.61^{ab}	1.14^{bcd}
	Thatuvee	$6.45^{abcdefg}$	9.12^{defghi}	0.86^{b}	0.89^{de}	5.31^{ab}	14.29^{bcdef}	1.16^{ab}	1.13^{bc}
NI	At308	3.54^{defg}	4.17^{l}	1.10^{a}	1.83^{abcd}	12.59^{ab}	7.59^{efghi}	0.91^{b}	0.55^{def}
	At309	4.73^{bcdefg}	5.62^{jkl}	1.40^{ab}	1.32^{bcde}	6.68^{ab}	7.50^{efghi}	0.71^{ab}	0.76^{bcdef}
	At311	11.61^{ab}	12.88^{cde}	2.34^{ab}	1.41^{bcde}	26.61^{ab}	5.43^{hi}	0.43^{b}	0.71^{bcdef}
	At354	7.49^{abcdef}	10.47^{defgh}	2.19^{ab}	1.81^{abcd}	16.22^{ab}	15.85^{bcdef}	0.46^{b}	0.55^{def}
	At373	8.81^{abcde}	8.71^{efghij}	1.69^{ab}	1.74^{abcd}	14.45^{ab}	18.20^{abcde}	0.59^{ab}	0.57^{cdef}
	Bg310	2.37^{g}	4.17^{l}	1.04^{b}	1.73^{abcd}	2.34^{b}	7.16^{fghi}	0.96^{ab}	0.58^{cdef}
	Bg359	4.73^{bcdefg}	8.32^{efghij}	2.72^{ab}	0.84^{de}	12.88^{ab}	6.84^{fghi}	0.37^{b}	1.20^{b}
	Bg366	8.03^{abcdef}	7.85^{fghijk}	1.70^{ab}	1.74^{abcd}	13.49^{ab}	13.65^{bcdefg}	0.59^{ab}	0.57^{cdef}
	Bg369	5.12^{bcdefg}	6.10^{ijkl}	1.10^{b}	0.92^{de}	5.43^{ab}	5.62^{ghi}	0.91^{ab}	1.09^{bcde}
	Bw363	4.78^{bcdefg}	7.00^{ghijk}	1.31^{ab}	1.81^{abcd}	5.96^{ab}	$12.59^{bcdefgh}$	0.76^{ab}	0.55^{def}
	Bw367	11.22^{abc}	11.48^{cdef}	1.99^{ab}	1.44^{bcde}	22.13^{ab}	8.32^{defghi}	0.50^{b}	0.70^{bcdef}
	Bw452	$5.68^{abcdefg}$	5.19^{kl}	1.78^{ab}	2.12^{abc}	10.00^{ab}	15.85^{bcdef}	0.56^{ab}	0.47^{ef}
	Ld356	7.07^{abcdef}	10.47^{defgh}	1.22^{b}	0.92^{de}	8.04^{ab}	4.95^{i}	0.82^{ab}	1.09^{bcde}
	Ld365	8.31^{abcdef}	10.72^{defgh}	1.60^{ab}	2.60^{a}	16.41^{ab}	27.23^{ab}	0.62^{ab}	0.39^{f}
	Ld371	$6.60^{abcdefg}$	10.00^{defgh}	1.31^{ab}	0.99^{de}	8.71^{ab}	$10.23^{cdefghi}$	0.76^{ab}	1.02^{bcde}
OI	H-10	10.83^{abc}	17.18^{bc}	1.84^{ab}	2.17^{ab}	19.28^{ab}	22.91^{abc}	0.54^{ab}	0.46^{f}
	H-4	10.47^{abcd}	20.18^{ab}	1.55^{ab}	1.09^{de}	16.22^{ab}	21.88^{abc}	0.65^{ab}	0.92^{bcdef}
	H-7	$6.16^{abcdefg}$	8.41^{efghij}	1.61^{ab}	0.52^{e}	9.89^{ab}	4.37^{i}	0.62^{ab}	1.92^{a}

* These landraces did not flower and therefore did not collect the data in *Yala* season
Means denoted by same letters within each column are not significantly different at P<0.05.

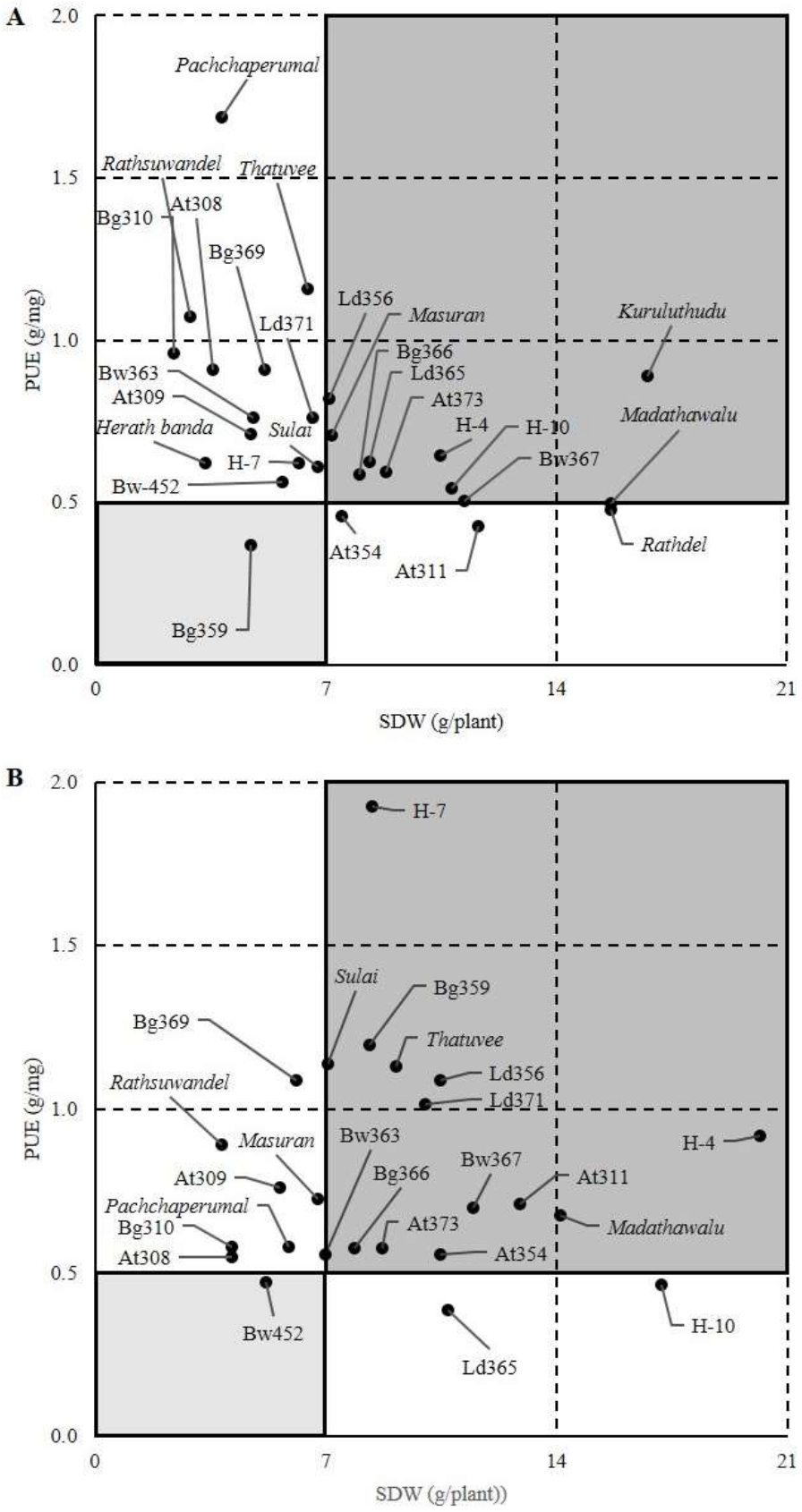


Figure 4. 2 The scatter plot drawn between SDW and PUE of the rice cultivars A: *Maha* season B: *Yala* season. Two threshold lines are drawn at 7 g/plant of SDW and 0.5 g/mg of PUE. The PDT cultivars are indicated in dark gray box and the PD sensitive cultivars are indicated in light gray box.

4.4.4 Diversity of *Pup 1* linked DNA marker haplotypes

The UPGMA cluster (C) analysis resulted 12 clusters (*Pup1*-C1 to *Pup1*-C12 in Figure 4.3). Cluster *Pup1*-C1 only contains two Sri Lankan landraces which are moderately tolerant or tolerant to P deficiency. *Pup1*-C2 contains two PDT rice cultivars, *Madathawalu* and H-4 and rest of the two are moderately tolerant cultivars (At 373 and *Rath Suwandel*). Cluster *Pup1*-C3 contains only moderately tolerant rice genotypes. Within *Pup1*-C4 moderately tolerant Bg359 and At354 grouped distantly from the sensitive cultivar At309. *Pup1*-C11 contains the most genetically distinct PDT genotypes (At311 and Bw367). Within the *Pup1*-C8, Bw363 which is a PD sensitive cultivar showed clear genetic distance from the rest of the cultivars within the cluster.

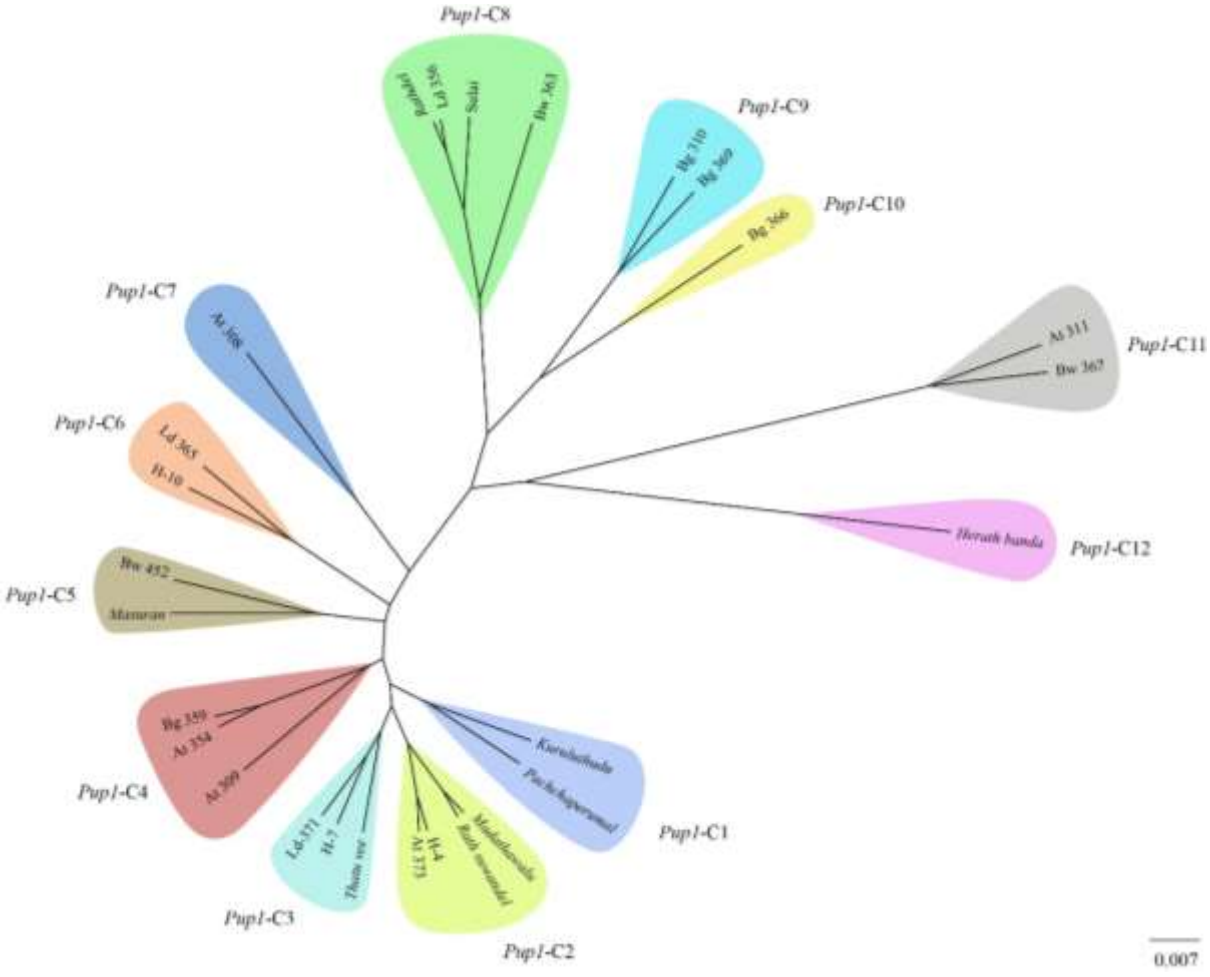


Figure 4.3 The clustergram constructed for the 27 rice cultivars based on the *Pup1* linked marker haplotypes. The dissimilarity matrix was constructed using the unweighted pair group method with arithmetic means (UPGMA) (Nei and Kumar, 2000).

4.4.5 *K29* and *RM 28102* based sequence diversity

The sequence polymorphism at *K29* locus generated eight clusters; *K29*-C1 contains the PDT cultivar H-4. However, the overall clustering structure does not coincide with the PDT variation shown in Figure 4.2 for *Maha* and *Yala* seasons (Figure 4.3). The sequence polymorphism at *RM 28102* generated eight clusters and a big paraphyletic group in *RM28102*-C1 (Figure 4.5). There, *Madathawalu* and *Rath suwadal* are apparent as unique haplotypes. However, the overall clustering structure is not correlated with PDT diversity structure shown in Figure 4.2.

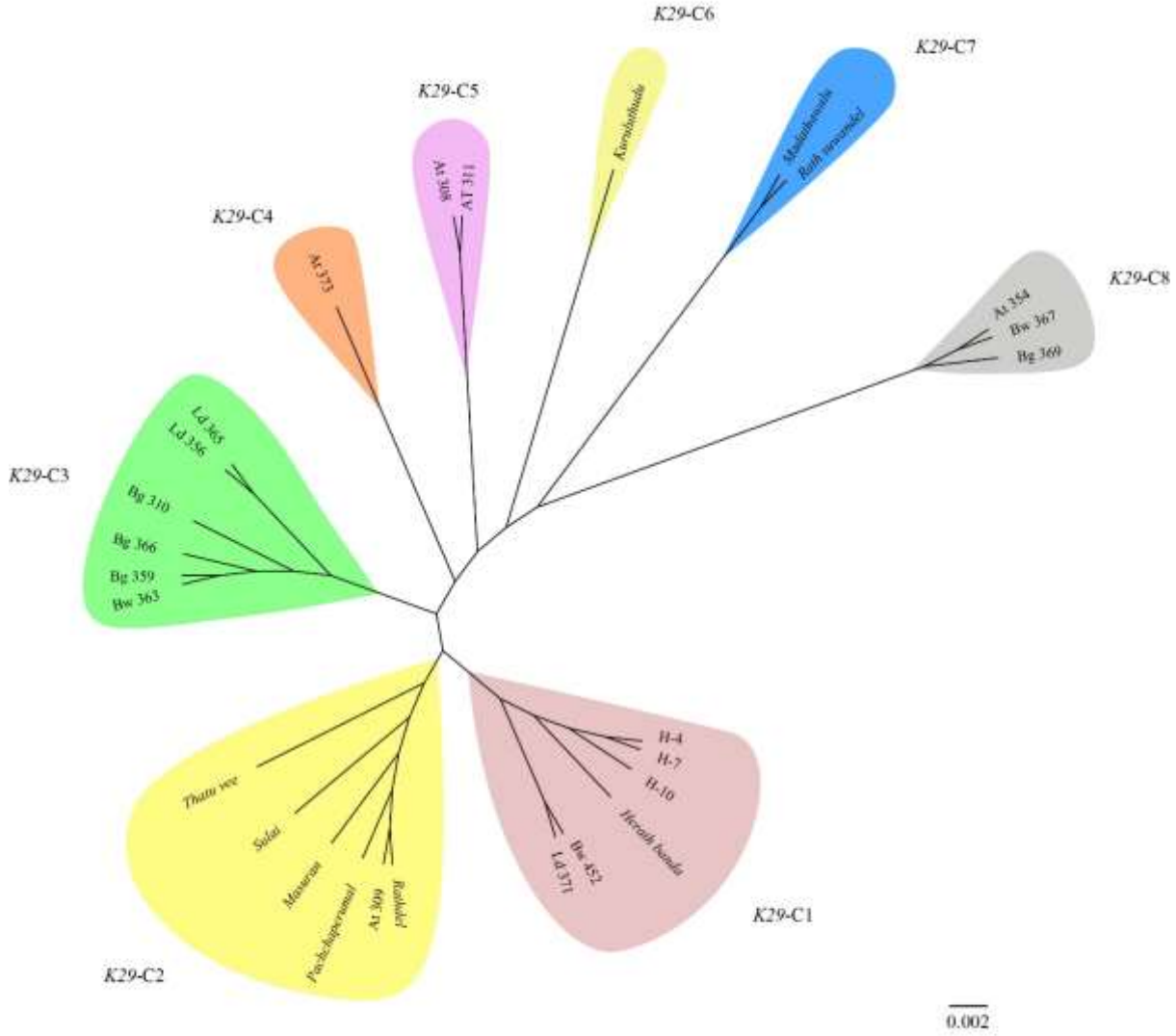


Figure 4.4 The clustergram constructed for rice cultivars based on the sequence polymorphism at *K29* locus. The cluster labels are indicated at each cluster.

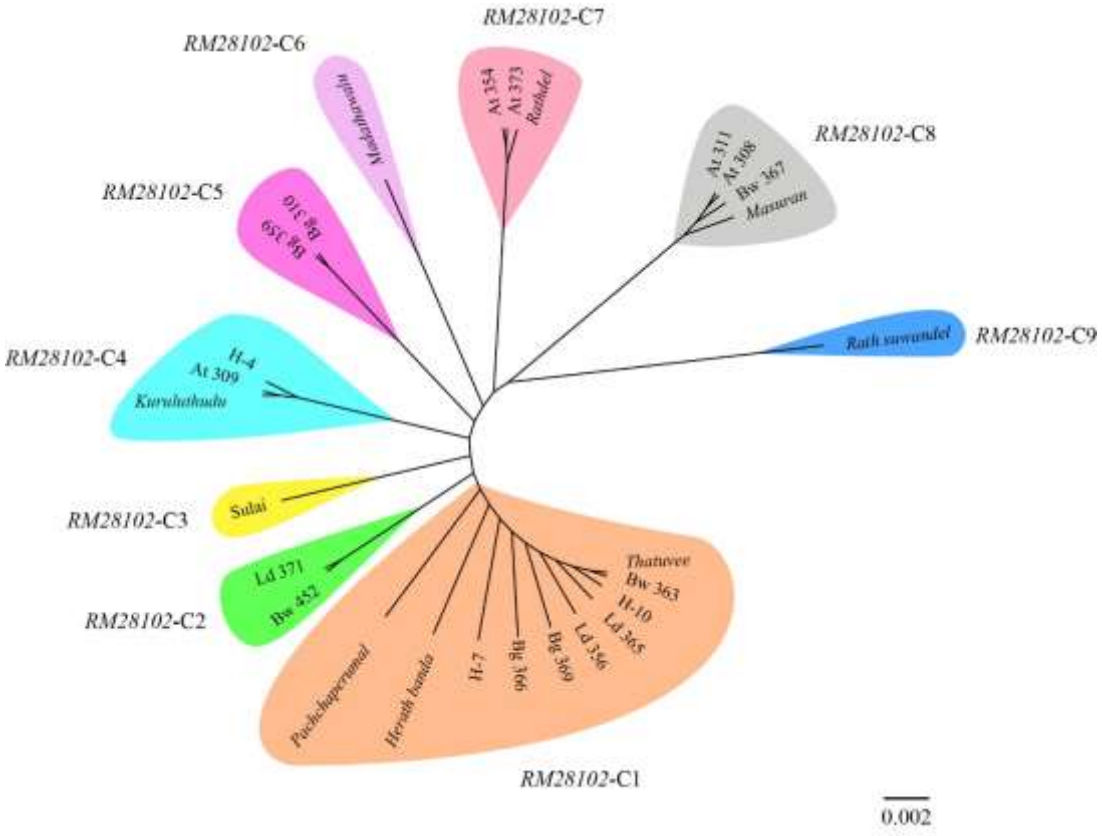


Figure 4.5 The cluster diagram constructed for rice cultivars based on the sequence polymorphism at *RM 28102* locus. The cluster labels are indicated at each cluster.

4.5 Discussion

The identification of PDT rice cultivars are important to use as parents in breeding programs and also to recommend as varieties for organic rice farming. Even in conventional rice farming, P efficient rice cultivars are important to reduce the cost of production and environmental pollution, and as answers to the P fertilizer crisis in the future (Cordell *et al*., 2009; O'neil *et al*., 2012; Wissuwa and Ae, 2001). Worldwide rice genetics and breeding programs have screened diverse germplasm for PDT. In Sri Lanka, Aluwihare *el al*., (2016) have screened 30 rice cultivars for PDT. In the present study, we assessed 27 cultivars including few varieties **(**Rathdel, H-4, H-7, H-10, At354, Ld356**)** from Aluwihare *el al*., (2016) to further identify useful PDT parents /cultivars for rice breeding and production in Sri Lanka. Nine landraces were incorporated into the present study because they have untapped genetic resources for various resistant or tolerant traits (Chin *et al*., 2010; Fageria and Santos, 2002; Herath *et al*., 1982). Fifteen newly improved varieties were also incorporated to check the PDT. As indicated in Table 4.2 and the rice varietal descriptions in RRDI Sri Lanka, the selected rice cultivars in the present study are crucial for rice breeding and production in Sri Lanka.

As explained by Majumder *et al*., (1989) and subsequent studies, PDT is a quantitative trait (Ni *et al*. 1998; Wissuwa and Ae, 2001; Wissuwa *et al*. 1998, 2002) and heavily dependent on the *Pup1* haplotype present in the cultivar, environment and the environment × QTL interaction. The PLH and the NT have shown very high variability and cultivar specificity indicating that they are less sensitive to PD imposed (Table 4.4). Similarly, FLL and the FLW are also following the varietal specific pattern and seem to be less affected by the PD conditions (Table 4.5). The yield under P limited conditions is very important as a selection parameter in breeding and screening. However, only six cultivars were identified as providing above average yield under P sparse conditions. As reported in many studies, the frequent practice is to identify PDT cultivars based on SDW and PUE of the varieties (Aluwihare *et al*., 2016; Wissuwa *et al*., 1998)**.** In the present study, we identified PDT cultivars for *Maha,* and *Yala* seasons (Figure 4.2A and 2B). Our results are in line with the results presented in Aluwihare *el al*., (2016) as both studies identified H-4 as PDT.

SDW is the ideal parameter for selection because it expresses the amount of biomass produced under P sparse conditions, and PUE is also ideal as it reflects the partitioning of P into sink tissues under low P conditions. However, it is important to select simultaneously based on yield and the PDT indicators for more robust screening outcomes (Fageria and Knupp, 2013; Wissua and Ae, 2001). In both *Maha* and *Yala* seasons, if yield and PDT indicators are considered simultaneously, H-4 and *Madathawalu* can be considered as the stable PDT rice cultivars (Figures 4.1,2).

The haplotype analysis at *Pup1* QTL for rice cultivars and the sequence polymorphism-based diversity analysis for two linked markers revealed variable diversity structures. This kind of higher diversity within *Pup1* QTL is reported in many studies (Chin *et al*., 2010, 2011; Heuer *et al*., 2009) and has also been linked to the very high transposon activity going on near and within *Pup1* QTL **(**Heuer *et al*., 2009).rrag5 The haplotype and sequence diversity observed in the present study also proved that rice germplasm in Sri Lanka is very highly diverse at this locus (Aluwihare *el al*., 2016) and imply the possibility of mining novel haplotypes for PDT. For MAB of rice for PDT, it is interesting to note that the stable PDT H-4 and *Madathawalu* (as indicated by yield and PDT indicators) are positioned within the *Pup1*-C2 cluster. Aluwihare *el al*., (2016) reported that H-4 bears different *Pup1* QTL haplotype to *Kasalath*. The tolerant haplotype in H-4 was inherited form *Murungakayan*, another PDT landrace in Sri Lanka (Aluwihare *el al*., 2016; RRDI, Sri Lanka). Therefore, it is logical to

assume that *Madathaawalu* also shares the same haplotype as in H-4 and *Murungakayan*. The present study thereby proves the utmost importance of screening locally available landraces and the cultivars and subsequent correlation with *Pup1* haplotype to lay a platform for MAB of rice (Aluwihare *et al*., 2017, 2018; Chin *et al*., 2010).

4.6 CONCLUSIONS

The screening of a core-set of rice cultivars under PD soil conditions revealed that the rice cultivars *Kuruluthudu*, *Madathawalu*, *Masuran*, At373, Bg366, Bw367, H-4, H-10, Ld356, and Ld365 are PDT in *Maha* season and *Madathawalu*, *Sulai*, *Thatuvee*, At-311, At354, At373, Bg359, Bg366, Bw363, Bw367, H-4, H-7, Ld356, and Ld371 are PDT in *Yala* season based on the PDT indicators, SDW and PUE. When the yield parameters are considered along with the PDT indicators, the landrace *Madathawalu* and old improved variety H-4 were found to be PDT and providing above average yield in both *Maha* and *Yala* seasons. When the haplotype diversity of 17 DNA markers linked to *Pup1* QTL were considered, H-4 and *Madathawalu* got clustered together indicating that *Pup1* linked DNA markers could be used for MAB, if H-4 or/and *Madathawalu* are used as breeding parents. In the health-conscious organic rice industry, *Madathawalu* is a frequently grown famous landrace. According to the PDT screening results of the present study, *Madathawalu* can be recommended to grow under zero or minimum P fertilizer-application regimes in rice farming.

CHAPTER 5

ASSESSMENT OF THE SEQUENCE-BASED HAPLOTYPE-VARIANTS IN SELECTED DNA MARKER LOCI FOR THE MOLECULAR BREEDING OF RESISTANT RICE VARIETIES TO BROWN PLANTHOPPER

5.1 ABSTRACT

Brown planthopper is the most devastating insect pest of rice. The BPH damage is trivial that farmers may end up with zero or below average yields. The application of insecticide would only lead to the emergence of virulent biotypes, thus MAB of BPH resistant rice varieties is the only feasible solution. Although, the molecular genetics of BPH resistance and the linked DNA makers are known; the breeders struggle to use them in MAB due to the limited ability to identify length-based allele polymorphisms in gel electrophoresis. Therefore, the present study was conducted to assess the employability of haplotype variants of the DNA markers linked to BPH resistant genes using a set of resistant and moderately resistant rice cultivars. The BPH resistant landraces *Murungakayan302*, *PtB33*, *Sulai* and moderately resistant Bg300, and Bw367 cultivars in Sri Lanka were assessed using six DNA makers linked to BPH resistant genes. The band length polymorphism using 2.5 % agarose gel electrophoresis and haplotype variants using 3× sequence-reads of the PCR products of DNA markers were assessed. The DNA markers *RM246* and *C3-14* provide *Murungakayan302* and *Sulai* specific haplotypes respectively. The assessed DNA markers yielded polymorphic bands that are closely positioned in agarose gels making the band scoring cumbersome and ambiguous. The sequences of PCR products provided distinct haplotypes for the rice cultivars. Thus, the introgression of BPH resistance or moderate resistance into rice cultivars can be undertaken using the sequence-based haplotypes in place of the band polymorphisms.

Keywords: BPH, *Bph2*, *Bph3*, *Bph4*, *Murungakayan302*, *PtB33*, *Sulai*

5.2 INTRODUCTION

Rice productivity is affected by many biotic and abiotic stresses. Attack by BPH, Nilaparvata lugens Stål can be considered as one of the most devastating biotic stresses in rice farming (Dyck and Thomas, 1979). BPH sucks an enormous amount of photosynthetic assimilates of the rice plant by piercing into the parenchymal cells of the phloem tissue (Sōgawa, 1982). Mature BPH infested rice plants show chlorosis of stems, wilting of leaves, lowered productivity and ultimate death of the entire plant (Du *et al*., 2009). Extensive removal of the phloem sap and the deposition of ammonia from the salivary secretions of BPH result in the circular patches leading to severe 'hopperburns' (Krishnaiah, 2014; Sōgawa and Cheng, 1979). Functional damage done by BPH with respect to the rice plant physiology has given rise to a greater reduction in grain yield (Nagadhara *et al*., 2003). Furthermore, BPH acts as a vector of two viruses; Ragged Stunt Virus and Grassy Stunt Virus (Du *et al*., 2009; Ling, 1972). These viral diseases also account for a considerable loss of rice yields (Cabauatan *et al*., 2009).

BPH can rapidly adapt to the host plant resistance, an enhancement of the pest virulence against host defence mechanism (Horgan, 2009). Four discrete BPH strains (Biotypes) that are virulent to the host resistant genes have been identified (Jena and Kim, 2010). Local BPH populations of Sri Lanka have recorded with different virulence patterns and closer adaptations to the rice varieties from which they were collected (Claridge and Hollander, 1980). Moreover, outbreaks of BPH due to rigorous destruction of rice plants via large-scale pest occurrences have been recorded especially in the South Western and Eastern Sri Lanka resulting in huge disparities in rice production (Fernando *et al*., 1979; Horgan, 2009). The outbreaks have been more persistent along with the introduction of high yielding rice cultivars and improved agronomic practices (Sōgawa and Cheng, 1979).

Extensive damage caused by BPH outbreaks can be eliminated by applying insecticides and planting resistant varieties. However, insecticides have also been identified to induce the outbreaks, which result in detrimental effects on rice farming (Gallagher *et al*., 1994). Outbreaks have a strong correlation with the development of resistance in BPH towards particular insecticides. Insecticide such as Imidacloprid was earlier used as an effective solution against the outbreaks; however, later it became the major contributory factor promoting the BPH outbreaks due to the development of resistance (Matsumura *et al*., 2008).

Hence, the strong correlation of outbreaks with the resistance of BPH towards the insecticides has left the rice growers with the solitary option of cultivating the resistant rice varieties.

BPH genes/loci in rice genome for the resistance to diverse BPH biotypes have been characterized (Cheng *et al*., 2013; Hou *et al*., 2011; Hu *et al*., 2016; Jing *et al*., 2017; Yang *et al*., 2012;). The linked DNA markers have also been reported for each gene (Chang-Chao *et al*., 2006; Jairin *et al*., 2007). However, utilization of these markers in country specific or regional breeding programs is tricky as the expected length polymorphisms are depending on the platform of gel electrophoresis, running conditions and concentration of the gel material (Lee *et al*., 2012; Szoke *et al*., 1999). For the exact band size determination for DNA markers such as microsatellites, 6 % or higher denaturing polyacrylamide gel electrophoresis with silver staining is required. Manufacturing and utilization of large vertical gel units are currently not practiced and the only option is to use higher % (e.g.: 2.5 % or more) agarose gel electrophoresis or sequencing of the PCR products of the DNA markers to select the specific haplotypes for MAB (Salmaso *et al*., 2005; Wang *et al*., 2003;). Therefore, the present study was conducted as a ground-breaking attempt to see the applicability of BPH resistant specific haplotype variants in marker loci in comparison to the band lengths in agarose gel electrophoresis using a panel of BPH resistant and moderately resistant rice cultivars.

5.3 MATERIALS AND METHODS

5.3.1 Plant material and DNA extraction

Three rice landraces that were confirmed to contain BPH resistant gene/s were selected. Two newly improved rice varieties; Bg300 and Bw367 (reported as moderately resistant to BPH) were also selected (Table 5.1) ([RRDI, Bathalagoda, Sri Lanka]). Henceforth, the landraces and varieties are referred to as cultivars. The breeder seeds of the cultivars were collected from RRDI. The seeds were germinated, and the seedlings were maintained for two weeks to obtain immature leaf material for DNA extraction. The collected leaves were ground into fine powder samples in liquid nitrogen using mortar and pestle. The genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Solna, Sweden).

Table 5.1 BPH tolerant and sensitive rice cultivars assessed for microsatellite polymorphism.

Variety	Type	Age (Months)	BPH resistant gene/s present	Remarks
Murungakayan302	Landrace (Sri Lanka)	4	*Bph2* (Kaneda *et al*.1981; Lakshminarayana and Kush, 1977)	Susceptible to BPH biotype 3. (Kaneda *et al*.1981) Resistant to BPH biotype 2. (Kaneda *et al*.1981)
PtB33	Landrace. (*Pathambi*, India)	4	*Bph2* (Angeles *et al*.1986) *Bph3* (Horgan *et al*. 2015; Jairin *et al*.2007; Sidhu and Khush,1978)	Highly resistant to BPH populations in many Asian countries.(Seshu and Kauffmann1980) Resistant to BPH and Green Leaf Hopper.(Nugaliyadde *et al*. 2000)
Sulai	Landrace (Sri Lanka)	4-4.5	*Bph4* (Hu *et al*. 2016)	Red rice. Moderate Resistant to BPH. Prone to lodging.
Bg300	Newly improved variety (Bg 367-7//IR 841/Bg 276-5)	3	PtB33 has been used to give BPH resistance to Bg300 (Nugaliyadde *et al*. 2000, 2004)	Resistant to gold midge, blast and bacterial blight. Moderately resistant to BPH. Intermediate bold grain shape with white color pericarp
Bw367	Newly improved variety (Bw 361/ Bg 358)	3.5	Bg379-2 has been used to provide resistance to Bw 367	Moderately susceptible or moderately resistant to gold midge and bacterial blight. resistant or moderately resistant to blast and BPH, Short round grain shape with white color pericarp

5.3.2 PCR, DNA sequencing and data analysis

PCR was conducted with a panel of DNA markers that are linked to BPH resistant genes (Table 5.2). PCR conditions were provided by using a Thermal Cycler (Takara, Japan) with following criteria; initial denaturation at 94 °C for 5 mins followed by 35 cycles of denaturation at 94 °C for 30 sec, primer annealing at temperature (Ta) (Table 5.2) for 1 min, extension at 72 °C for 2 mins and final extension at 72 °C for 10 mins. PCR products were size separated in 2.5 % agarose gel electrophoresis.

PCR products were purified using a QIAquick® PCR purification kit (Catalog No: 28104, Qiagen, Hilden, Germany) and subjected to 3× DNA sequencing in Macrogen Inc., South Korea. The sequences obtained were used to carry out a reference sequence search in NCBI Nucleotide Blast Tool. Then, the sequences obtained for each marker was aligned with the reference sequences retrieved via Clustal W algorithm in Mega 7 (Kumar *et al*., 2016). Sequences were manually inspected for any inaccuracies in automated sequencing. Next, forward and reverse end noise was eliminated, and consensus sequences were set. Ambiguous regions were eradicated, and our sequences alone were aligned using the Clustal W algorithm to detect the unique haplotypes of the five varieties affecting the BPH resistance and susceptibility.

Table 5.2 DNA markers assessed for identification of the haplotypes linked to BPH tolerance

Resistant Gene	Marker	Primer Sequences (5'→3')	T_a (C°)	Reference
Bph2	*RM463*	TTCCCCTCCTTTTATGGTGC TGTTCTCCTCAGTCACTGCG	55°	Li-Hong *et al*. (2006)
Bph2	*RM7102*	TTGAGAGCGTTTTTAGGATG TCGGTTTACTTGGTTACTCG	55°	Li-Hong *et al*. (2006)
Bph2	*RM1246*	CTCGATCCCCTAGCTCTC TCACCTCGTTCTCGATCC	55°	Li-Hong *et al*. (2006)
Bph3	*RM589*	ATCATGGTCGGTGGCTTAAC CAGGTTCCAACCAGACACTG	55°	Liu *et al*. (2016)
Bph4	*RM217*	ATCGCAGCAATGCCTCGT GGGTGTGAACAAAGACAC	55°	Kawaguchi *et al*. (2001)
Bph4	*C3-14*	GGCAAAATTAGACGGCACG GAATATGCATTTTGTTTGGAG	55°	Hu *et al*. (2015)

5.4 RESULTS AND DISCUSSION

5.4.1 Band-length polymorphisms of the DNA markers for selecting BPH tolerance in breeding

The resistant cultivar *Murungakayan302*, Ptb33 and *Sulai*, and the moderately resistant cultivars Bg300 and Bw367 contain two different bands (i.e. alleles) for the *Bph2* linked markers *RM1246*, *RM463* and *RM7102*. For the *Bph3* linked marker *RM589* and *Bph4* linked markers *RM217* and *C3-14* contains three alleles (Figure 5.1). The specific band found in *Sulai* for the marker *C3-14* can be used to introgress the *Sulai* specific allele to the rice cultivars. Also, the other bands detected for the six markers can be used to check the introgression of BPH resistant alleles to the rice varieties in the future breeding programs. However, even at 2.5 % agarose gel electrophoresis, the bands were less resolved in to close positions making the scoring difficult.

5.4.2 Haplotype sequence-variants of marker loci for selecting BPH tolerance in breeding

For *RM1246* marker, *Murungakayan302* and Ptb33 shared unique haplotypes whereas *Sulai*, Bg300 and Bw367 shared a common haplotype. For *RM463*, three haplotypes were identified in which *Murungakayan302* contains a unique haplotype. For *RM7102*, *Murungakayan302*, *Sulai* and Bg300 contains unique haplotypes whereas Ptb33, Bw367 share a common haplotype. For *Bph3* linked marker *RM589*, *Murungakayan302*, Ptb33, and *Sulai* possess unique haplotypes whereas Bg300 and Bw367 share a common haplotype. *RM217*, *Bph4* linked marker, provides unique haplotypes for *Murungakayan302* and Ptb33. However, *Sulai* haplotype is also seen in Bg300 and Bw367. For *C3-14* marker, which is linked to *Bph4*, unique haplotypes identified for the *Murungakayan302*, Ptb33, and *Sulai*. However, shared haplotype was observed in Bg300 and Bw367 (Figure 5.2; Table 5.3).

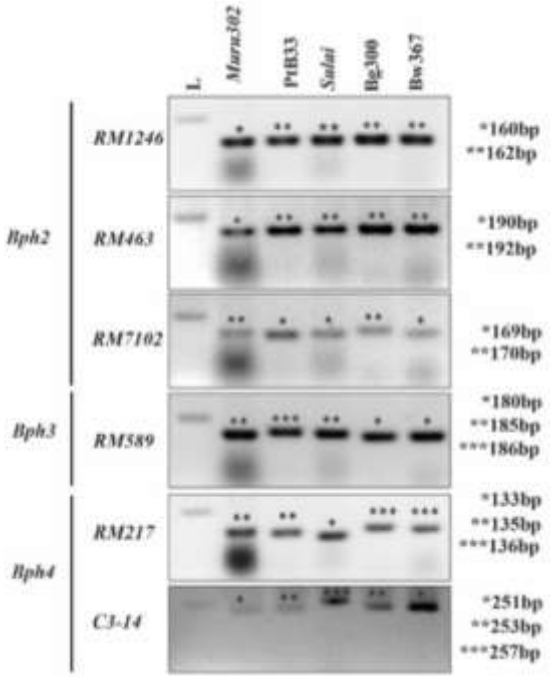


Figure 5.1 The polymorphism of BPH resistance linked DNA markers. The corresponding BPH resistant gene (*Bph2*, *Bph3*, and *Bph4*) and marker names are given on the left side and variety names are given on the top. The approximate band size is denoted on the right side. The number of star marks (*) represent the polymorphic bands separately in the increasing order of their sizes given in base pairs (bps).

The introgression of the alleles of BPH resistance genes from the rice landraces to improved cultivars is the only viable solution to combat against BPH (Jena and Kim 2010). The MABC is the most pragmatic approach to introgress the favourable alleles from landraces to the cultivated germplasm (Bouchez *et al*., 2002; Frisch and Melchinger, 2001). However, the DNA markers available in the public domain cannot be directly used in breeding programs. The length polymorphisms are ideal for the selection (Yang *et al*., 1994) however, the length difference among marker bands are too small to detect with limited facilities available in developing countries. In the present study, with 2.5 % agarose gel electrophoresis, the polymorphic bands were observed, however, their close positions on the gels made it difficult to call the exact band sizes. This demands difficult and time-consuming polyacrylamide gel electrophoresis for the resolution of alleles in breeding programs that often have many individuals to be genotyped for marker assisted selection. In addition to the tediousness of the process of electrophoresis; the laboratory chemicals, equipment and conditions greatly affect the detection of 1-20 bp band length differences accurately. Therefore, the most feasible solution is, if 2.5 % or higher percentage agarose gel electrophoresis is not capable of detecting the maker allelic status, the sequencing of PCR products of the marker loci and select based on the sequence haplotypes (Varshney *et al*., 2009). As the sequencing cost is getting low, DNA sequencing facilities both locally and

internationally are now greatly facilitating the large-scale sequencing of populations as a routine tool in breeding (He *et al*., 2014).

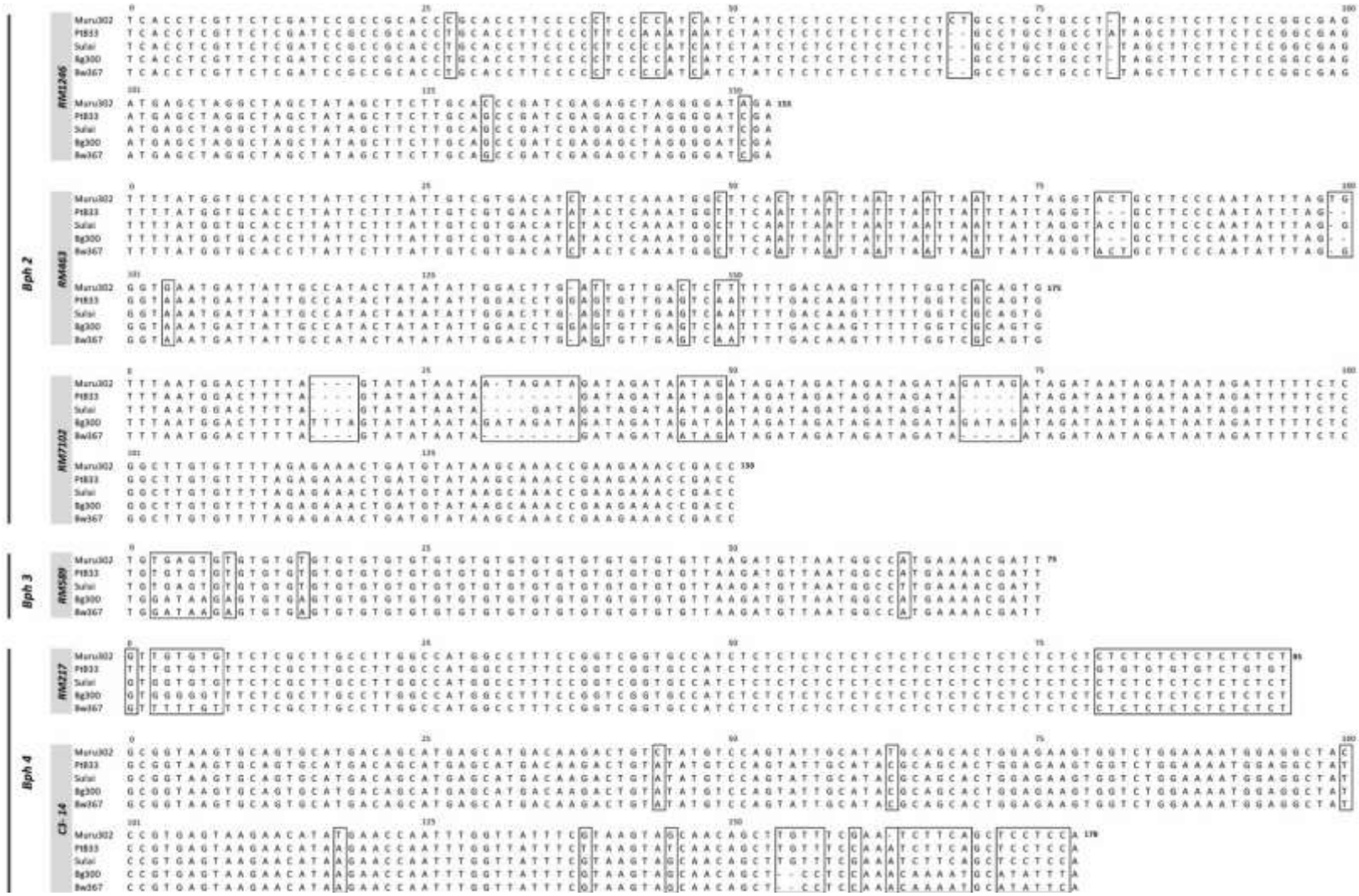


Figure 5.2 The sequence alignments of six maker loci for five rice cultivars. The marker names and linked BPH resistant genus are shown in the left. The primer regions were trimmed off from the alignment. The relative base positions (in 25-base intervals) are given above the sequence and total length of each alignment is given at the end just after the last base of each sequence. The useful polymorphic sites for marker assisted breeding of BPH resistance are shown within boxes drawn over the alignments.

Table 5.3 SNPs and INDEL based haplotypes of the marker loci for the selection for BPH tolerance in breeding

BPH resistant gene	Marker	Position (bp)*	*Muru302*	PtB33	*Sulai*	Bg300	Bw367
Bph2	***RM1246***	27	C	T	T	T	T
(PtB33, *Muru02*)		39	C	T	C	C	C
		43-44	CC	AA	CC	CC	CC
		47	C	A	C	C	C
		68-69	CT	--	--	--	--
		81	-	A	-	-	-
		130	C	G	G	G	G
		151	A	C	C	C	C
		37	C	A	C	A	C
		49	C	T	C	T	C
	RM463	54	C	A	A	A	A
		58	A	T	A	T	A
		62	A	T	A	T	A
		66	A	T	A	T	A
		70	A	T	A	T	A
		80-82	ACT	---	ACT	---	ACT
		99-100	TG	--	-G	--	-G
		104	G	A	A	A	A
		138	-	G	-	G	-
		140	T	G	G	G	G
		146	C	G	G	G	G
		149-150	TT	AA	AA	AA	AA
		170	A	G	G	G	G
	RM7102	16-19	----	----	----	TTTA	----
		30-37	A-TAGATA	--------	----GATA	GATAGATA	--------
		46-49	ATAG	ATAG	ATAG	GATA	ATAG
		69-73	GATAG	-----	-----	GATAG	-----
Bph3	***RM589***	3-7	TGAGT	TGTGT	TGAGT	GATAA	GATAA
		9	T	T	T	A	A
		15	T	T	T	A	A
		64	A	A	T	A	A
Bph4	***RM217***	1	G	T	G	G	G
(*Sulai*)		3-8	TGTGTG	TGTGTT	GGTGTG	GGGGGT	TTTTGT
		80-95	(CT)8	(GT)5(CT)1(GT)2	(CT)8	(CT)8	(CT)8
	C3-14	44	C	A	A	A	A
		63	T	C	C	C	C
		100	C	T	T	T	T
		118	T	A	A	A	A
		138	G	T	G	G	G
		145	G	T	G	G	G
		154-157	TGTT	TGTT	TGTT	--CC	--CC
		160	G	C	G	C	C
		163	-	A	A	A	A
		164-169	TCTTCA	TCTTCA	TCTTCA	CAAAAT	CAAAAT
		172-177	TCCTCC	TCCTCC	TCCTCC	ATATTT	ATATTC

*Position (bp) as in the alignment shown in Figure 5.2

5.6 CONCLUSIONS

The present study proved that the DNA markers linked to BPH resistant genes provide polymorphic bands that are closely positioned in high concentration agarose gel electrophoresis. This makes the band scoring difficult which hinders the transferability of polymorphic band data across different laboratories. However, the sequencing of PCR products of the assessed markers provided clearly distinguishable haplotypes for the studied resistant and moderately resistant cultivars. Therefore, in MAB for BPH resistance of rice, the introgression of BPH resistance or moderate resistance into novel cultivars can be swiftly practiced using the sequence-based haplotypes rather than using the band polymorphisms detected in gel electrophoresis.

CHAPTER 6
CONCLUSIONS AND FUTURE DIRECTIONS

The research was aimed to construct a breeding database as a tool to facilitate decision-making in breeding. The output of the Pedimap; the constructed database, can be efficiently used to plan new rice breeding programs and select suitable markers in MAS. The pedigree visualizations of Pedimap boost up the efficiency of breeding decision-making in parentage selection, marker identification, and constructing breeding plans by avoiding complicated, time-consuming, tedious methods od decision making in conventional breeding. Simple data handling and higher storing capability enable it to be used as a massive-scale data handling platform to record all the progeny data in breeding programs.

The next section of the study focused on revealing a core-set of rice cultivars which can survive under PD soil conditions using SDW and PUE as the PDT indicators. The rice cultivars *Kuruluthudu, Madathawalu, Masuran,* At373, Bg366, Bw367, H-4, H-10, Ld356, and Ld365 are PDT in *Maha* season and *Madathawalu, Sulai, Thatuvee,* At-311, At354, At373, Bg359, Bg366, Bw363, Bw367, H-4, H-7, Ld356, and Ld371 are PDT in *Yala* season. The old improved variety H-4 and landrace *Madathawalu* were recognized as PDT varieties together with a yield of above-average in both *Maha* and *Yala* seasons. From the diverse the haplotypes of 17 *Pup1*-linked DNA markers, H-4 and *Madathawalu* got clustered together. It indicates that H-4 or/and *Madathawalu* can be used as breeding parents in MAB with the use of *Pup1* linked DNA markers. Besides, the landrace *Madathawalu* can be used to grow under zero or minimum P fertilizer-application regimes as a frequently grown rice variety in the organic rice industry. The identification of the marker haplotypes of BPH resistant genes reveals that DNA sequencing and haplotype analysis is an effective strategy for screening the target progeny in MAB, which is conducted by directing the introgression of BPH resistant genes from BPH resistant landraces to the improved sensitive varieties.

Furthermore, the study has identified that *RM246* provides a *Murungakayan302* specific allele, and *C3-14* implements a specific haplotype to *Sulai*, which contains the resistant gene *bph4*. The length polymorphism of the gel bands is also assessed. *C3-14* is the only suitable

DNA marker to use in MAB of rice for *BPH* tolerance because all of the other five DNA markers did not show length polymorphism in agarose gel electrophoresis.

Construction of the consummate, organized, fully informative rice-breeding database ultimately contributes to the accomplishment of the fundamental goal of this study. The assembled database can be further enhanced on a massive scale by focusing on rice cultivation at the industrial level. The current database contains only RRDI improved rice varieties and their pedigrees along with, phenotypic and molecular marker data. However, hundreds of landraces and improved rice varieties are spread throughout the country, and all are not adequately evaluated to date. Therefore, all the accessible landraces and improved varieties have to be consolidated and evaluated to expand the capacity of the database according to pedigree history, phenotypic parameters, and molecular marker data along with SNP profiles. The developed database can also be expanded to evaluate the worldwide rice germplasm.

The accuracy of the gene introgression in MAB directly depends upon the number of DNA markers using the screening. For accurate monitoring of the gene introgression at an appropriate QTL, the establishment of the DNA marker alleles for the whole genome of each variety for 1 to 5 centimorgan (cM) intervals are recommended (Van de Weg *et al*., 2004; Bink *et al*., 2008). It is suggested that the DNA marker coverage of the target genome should be increased according to a high-resolution linkage marker map in order to obtain accurate progeny screening. Since very little genome coverage was done in this study, it is crucial to enhance the coverage, and evaluate more QTL-linked DNA markers to make the screening more useful. With the changing consumer preferences and breeding priorities, introgression of multiple genes in breeding planning becomes more realistic in up-to-date breeding programs. Besides that, due to the promotion of inducing resistance towards a selected single defensive gene in improved rice varieties, multiple gene introgression by gene pyramiding could be considered today (Singh *et al*., 2001).

Identification of the parental lineages for multiple trait introgression becomes somewhat difficult, due to the unavailability of a reliable phenotyping protocol (Evans *et al*., 2011) for rice breeding. So that, the introducing of the rice phenotyping protocol based on the characterization catalog of rice, which is introduced by the Plant Genetic Resources Center in Sri Lanka is vital for the evaluation of multiple traits. In addition, several improvements

in the software are suggested for future endeavors. As it is a specified software, understanding and applying of it is bit complicated to the beginners. If Pedimap can be developed as user-friendly, easy handling, handheld application, then the ease-of-use of the database can be further increased. Rather than handling only with computers, it can be developed as a mobile application, which supports android and IOS operating systems. Since it is an applicable worldwide database, an online version of the database can be launched with features including data storage, modify data, download data and maintain personal datasets as an ordered, well-developed online database (Figure 6.1).

The utilization of marker haplotypes using SNP data is highly accurate (Varshney *et al*., 2009) instead of using DNA marker scores. All the useful QTL-linked DNA marker haplotypes can be assessed through the entire local and worldwide rice germplasm, and recorded in Pedimap. This haplotype analysis will be extremely beneficial to the MAB. Pedimap analysis involves only the data visualization, so the statistical analysis to unveil the relationship between marker data with phenotypic values (Marker- trait association) needed to be estimated. This analysis can be done by using a QTL analyzer like FlexQTL^{TM} (Bink *et al.*, 2008). At the same time, the corresponding dataset can be shared with both applications, and the results can be used together in the plant breeding decision-making process (Figure 6.1).

This study solely focused on two QTLs, including PDT, and BPH resistance. However, in a meticulous study, at least all major QTLs and their behavior must be investigated. Then QTL analysis has to be conducted to reveal the relationship between DNA marker data along with the phenotypic traits. This study is conducted as a pilot study by using sample datasets, including 27 local rice varieties for the *Pup1*-linked haplotype identification and five varieties for BPH resistance gene analysis. Development of tolerance to PD and resistance against BPH in agronomically worthy rice varieties sounds more excellent prospects in the industrial rice farming. The whole local varietal diversity should be covered to enrich the applicability of the database. All the available /or representative rice germplasm can be used to validate DNA markers further accurately. Phylogenetic analysis such as SNP-INDEL profiling, phylogenetic trees, and dendrogram constructions can be performed, as well as principal component analysis and a scatter plot analysis with the high-density DNA marker maps and more significant sample numbers for conclusive results.

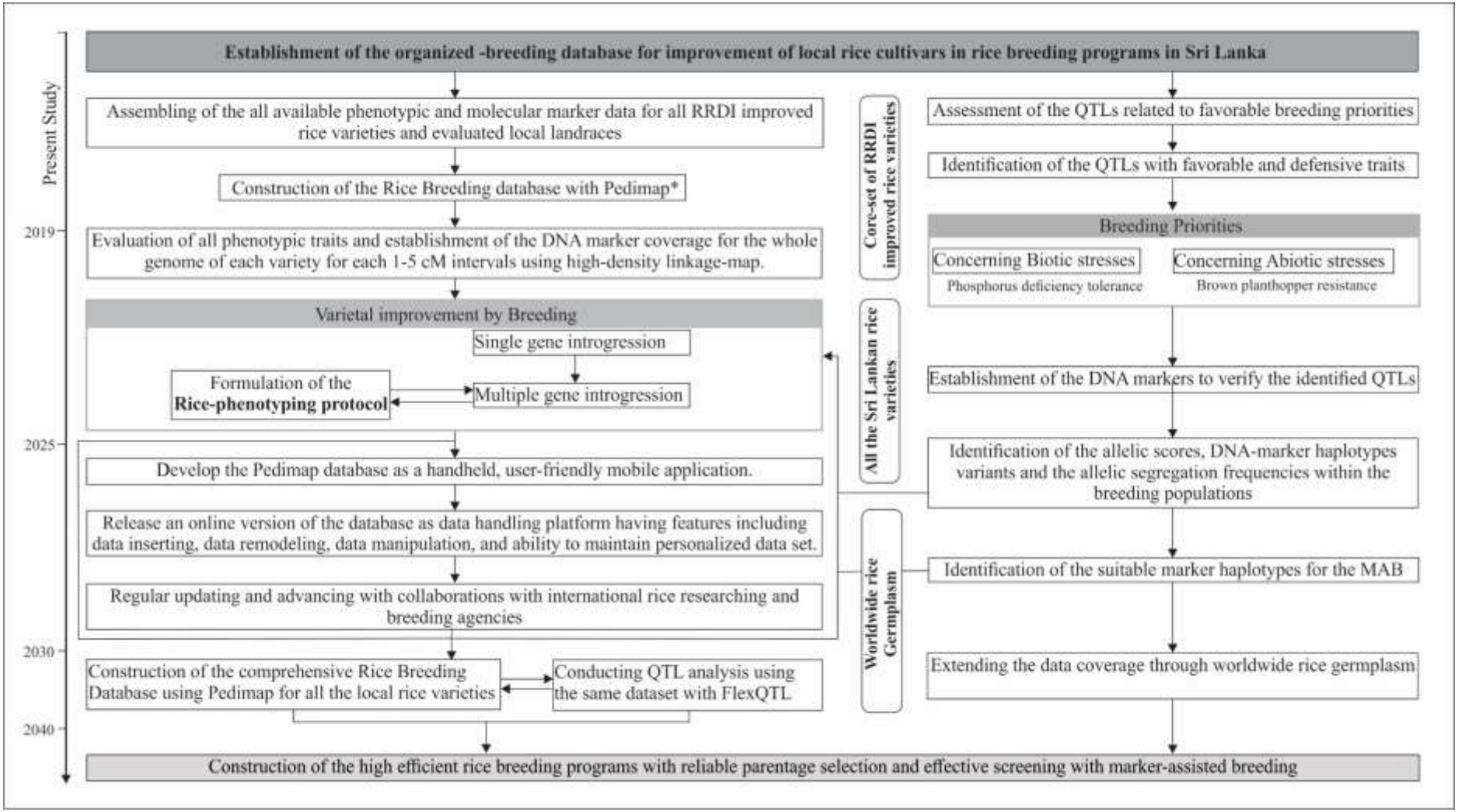


Figure 6.1 Deployment plan of the Pedimap as an organized rice breeding database with expanded coverage of local and worldwide rice germplasm. The proposed timeline is shown on the left side. The * mark indicates the current stage of the project. Suggested plant to molecular genetical analysis for the QTL and related marker identification is indicated in the right column of the illustration, while the steps in the development of the Pedimap database are indicated in the left column.

REFERENCES

Acquaah, G. (2012) *Principles of plant genetics and breeding*, 2nd edn, pp 281-334. Oxford, UK: Wiley-Blackwell.

Acquaah, G. (2015) Conventional plant breeding principles and techniques. In: *Advances in plant breeding strategies: Breeding, biotechnology and molecular Tools*, pp 115-158. Cham, Switzerland: Springer.

Agritech (2019, December 18) Expert system for paddy: weed management. Retrieved from: http://www.agritech.tnau.ac.in/expert_system.

Akkaya, M.S., Bhagwat, A.A. and Cregan, P.B. (1992) Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, **132**, 1131-1139.

Ali, A.M.S. (2006) Rice to shrimp: land use/land cover changes and soil degradation in Southwestern Bangladesh. *Land Use Policy*, **23,** 421-435.

Allard, R.W. (1960) *Principles of plant breeding*. New York: John Wiley and Sons.

Aluwihare, Y.C., Chamikara, M.D.M., Dissanayake, D.R.R.P., Dissanayake, M.D.M.I.M., Karannagoda, N.N.H., Dayananda, A.G.M.L.K., Sirisena, D.N. *et al*. (2017) DNA sequence polymorphism of *Pup1* linked *K20-1* STS region can be effectively used in molecular breeding of rice for phosphorus deficiency tolerance. *J. Natl. Sci. Found.* **45**, 413-425.

Aluwihare, Y.C., Chamikara, M.D.M., Dissanayake, D.R.R.P., Karannagoda, N.N.H., Sirisena, D.N., Samarasinghe, W.L.G., Rajapakse, S. *et al*. (2015) Validation of *K46*, a *Pup1*-linked marker, using a selection of Sri Lankan rice (*Oryza sativa* L.) germplasm for marker assisted selection towards phosphorous deficiency tolerance. *Ceylon J. Sci. Biol. Sci.* **44**, 45-54.

Aluwihare, Y.C., Chamikara, M.D.M., Dissanayake, D.R.R.P., Karunarathne, S.I., Ananda, G.K.S., Kannangara, S.K., Madhukalpani, O.V.S. *et al*. (2018) Identification of *Pup1* QTL linked DNA marker haplotypes for molecular breeding of phosphorous deficiency tolerant rice varieties. *J. Natl. Sci. Found.* **46**, 495-504.

Aluwihare, Y.C., Ishan, M., Chamikara, M.D.M., Weebadde, C.K., Sirisena, D.N., Samarasinghe, W.L.G. and Sooriyapathirana, S.D.S.S. (2016) Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. *Rice Science,* **23,** 184-195.

Angeles, E.R., Khush, G.S. and Heinrichs, E.A. (1986) Inheritance of resistance to planthoppers and leafhopper in rice. In: *Rice Genetics I* (IRRI, ed), pp. 537–549. Los Baños, Philippines: IRRI.

Athwal, D.S., Pathak, M.D., Bacalangco, E.H. and Pura, C.D. (1971) Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. 1. *Crop Sci.* **11,** 747-750.

Babu, R., Nair, S.K., Prasanna, B.M. and Gupta, H.S. (2004) Integrating marker-assisted selection in crop breeding–prospects and challenges. *Current Science,* **87,** 607-619.

Bader, J.S. (2001) The relative power of SNPs and haplotype as genetic markers for association tests. *Pharmacogenomics.* **2**, 11-24.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U. *et al*. (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS One,* **3,** e3376. doi: 10.1371/journal.pone.0003376.

Bennett, R.L., Steinhaus, K.A., Uhrich, S.B., O'Sullivan, C.K., Resta, R.G., Lochner-Doyle, D., Markel, D.S. *et al*. (1995) Recommendations for standardized human pedigree nomenclature. *J. Genet. Couns.* **4,** 267-279.

Bink, M.C.A.M., Boer, M.P., Ter Braak, C.J.F., Jansen, J., Voorrips, R.E. and Van de Weg, W.E. (2008) Bayesian analysis of complex traits in pedigreed plant populations. *Euphytica,* **161**, 85-96.

Borojevic, S. (1990) *Principles and methods of plant breeding*. Amsterdam, Netherlands: Elsevier.

Bouchez, A., Causse, M., Gallais, A. and Charcosset, A. (2002) Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genetics*, **162**, 1945-1959.

Breseghello, F. and Coelho, A.S.G. (2013) Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *J. Agric. Food Chem.* **61**, 8277-8286.

Briggs, F.N. and Knowles, P.F. (1967) *Introduction to plant breeding*. New York: Reinhold Publishing Corporation.

Brown, J. and Caligari, P. (2008) *An introduction to plant breeding*. Oxford, UK: Blackwell Publishing, Ltd.

Brun-Samarcq, L., Gallina, S., Philippi, A., Demenais, F., Vaysseix, G. and Barillot, E. (1999) CoPE: a collaborative pedigree drawing environment. *Bioinformatics (Oxford, England)*, **15,** 345-346.

Bulluck III, L.R. and Ristaino, J.B. (2002) Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. *Phytopathology*, **92,** 181-189.

Cabauatan, P.Q., Cabunagan, R.C. and Choi, I.R. (2009) Rice viruses transmitted by the brown planthopper *Nilaparvata lugens Stål*. In: *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (Heong, K.L. and Hardy, B. eds), pp. 357-368. Los Banos, Philippines: International Rice Research Institute.

Calingacion, M., Laborte, A., Nelson, A., Resurreccion, A., Concepcion, J.C., Daygon, V.D., Mumm, R., *et al.* (2014) Diversity of global rice markets and the science required for consumer-targeted rice breeding. *PloS One*, **9**, e85106.

Capell, T., Bassie, L. and Christou, P. (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. In *Proceedings of the National Academy of Sciences*, **101**, 9909-9914.

Central Bank of Sri Lanka (2017) Annual report of central bank of Sri Lanka in 2017. Available on-line at: https://www.cbsl.gov.lk/en/publications/economic-and-financial-reports/annual-reports/annual-report-2017.

Central Bank of Sri Lanka (2018) Annual report of central bank of Sri Lanka in 2018. Available on-line at: https://www.cbsl.gov.lk/en/publications/economic-and-financial-reports/annual-reports/annual-report-2018.

Chahal, G.S. and Gosal, S.S. (2002) *Principles and procedures of plant breeding*. Oxford, UK: Alpha Science Ltd.

Chang-Chao Su, Hu-Qu Zhai, Chun-Ming Wang, Li-Hong Sun and Jian-Min Wan (2006) SSR mapping of brown planthopper resistance gene *Bph9* in *Kaharamana*, an *indica* rice (*Oryza sativa* L.). *Acta Genetica Sinica*, **33,** 262-268.

Chankaew, S., Monkham, T., Pinta, W., Sanitchon, J., Kaewpradit, W. and Srinives, P. (2019) Screening tolerance to phosphorus deficiency and validation of *Phosphorus uptake 1* (*Pup1*) gene-linked markers in Thai indigenous upland rice germplasm. *Agronomy*, **9**, 81. doi: 10.3390/agronomy9020081.

Cheng, E., Elliott, B. and Meral Ozsoyoglu, Z. (2008) Scalable computation of kinship and identity coefficients on large pedigrees. *Computational Systems Bioinformatics*, **7,** 27-36.

Cheng, X., Zhu, L. and He, G. (2013) Towards understanding of molecular interactions between rice and the brown planthopper. *Molecular Plant*, **6**, 621-634.

Chin, J.H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetiyono, J., Moeljopawiro, S., Wissuwa, M. *et al*. (2011) Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application. *Plant physiol.* **156**, 1202-1216.

Chin, J.H., Lu, X., Haefele, S.M., Gamuyao, R., Ismail, A., Wissuwa, M. and Heuer, S. (2010) Development and application of gene-based markers for the major rice QTL *Phosphorus uptake 1*. *Theor. Appl. Genet.* **120**, 1073-1086.

Claridge, M.F. and Hollander, J.D. (1980) The biotypes of the rice brown planthopper, *Nilaparvata lugens*. *Ent. exp. & appl. 2*, **27,** 23-30.

Clark, B. H. (1999) Marketing Performance Measures: history and interrelationships. *Journal of Marketing Management*, **15,** 711–732.

Coburn, J.R., Temnykh, S.V., Paul, E.M. and McCouch, S.R. (2002) Design and application of microsatellite marker panels for semiautomated genotyping of rice (*Oryza sativa* L.). *Crop Sci.* **42,** 2092-2099.

Collard, B.C. and Mackill, D.J. (2007) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B.* **363**, 557-572.

Collard, B.C., Jahufer, M.Z.Z., Brouwer, J.B. and Pang, E.C.K. (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, **142**, 169-196.

Complex Trait Consortium (2003) The nature and identification of quantitative trait loci: a community's view. *Nat. Rev. Genet.* **4,** 911.

Cordell, D., Drangert, J.O. and White, S. (2009) The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, **19**, 292-305.

Cramer, H. H. (1967) Plant protection and world crop production. *Bayer Pflanzenschutz Leverkusen,* **20**, 1-524.

Crosbie, T.M., Eathington, S.R., Johnson, G.R., Edwards, M., Reiter, R., Stark, S., Mohanty, R.G. *et al*. (2008) Plant breeding: past, present, and future. In *Plant Breeding: The Arnel R. Hallauer International Symposium* (Lamkey, K.R. and Lee, M., eds). doi: 10.1002/9780470752708.ch1.

Crossa, J., de Los Campos, G., Pérez, P., Gianola, D., Burgueño, J., Araus, J.L., Makumbi, D. *et al*. (2010) Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers. *Genetics*, **186,** 713-724.

Crow, J.F. (1998) 90 years ago: the beginning of hybrid maize. *Genetics*, **148**, 923-928.

Curtis, D. (1990) A program to draw pedigrees using LINKAGE or LINKSYS data files. *Ann. Hum. Genet.* **54,** 365-367.

D. Vinebrooke, R. L., Cottingham, K., Norberg, Marten Scheffer, J., I. Dodson, S., C. Maberly, S. and Sommer, U. (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos*, **104**, 451-457.

Davenport, G., Ellis, N., Ambrose, M. and Dicks, J. (2004) Using bioinformatics to analyse germplasm collections. *Euphytica*, **137**, 39-54.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. and Blaxter, M.L. (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet. **12***, 499-510.

Davis, K.F., Gephart, J.A. and Gunda, T. (2016) Sustaining food self-sufficiency of a nation: the case of Sri Lankan rice production and related water and fertilizer demands. *Ambio, **45,*** 302-312.

Dawe, D. (2002) The changing structure of the world rice market, 1950–2000. *Food Policy,* **27**, 355-370.

De Datta, S.K., Gomez, K.A. and Descalsota, J.P., 1988. Changes in yield response to major nutrients and in soil fertility under intensive rice cropping. *Soil Sci.* **146**, 350-358.

De Janvry, A. and Sadoulet, E. (2002) World poverty and the role of agricultural technology: direct and indirect effects. *Journal of development studies*, **38**, 1-26.

Department of Agriculture (DOA), Sri Lanka (2006) Climate of rice growing regions in Sri Lanka. Available on-line at: http://www.agridept.gov.lk/.

Desta, Z.A. and Ortiz, R. (2014) Genomic selection: genome-wide prediction in plant improvement. *Trends in plant science,* **19,** 592-601.

Dhanapala, M.P. (2007) Bridging the rice yield gap in Sri Lanka. In *Proceedings of the symposium on Expert Consultation on Bridging the Rice Yield Gap in the Asia-Pacific Region*, 5–8 October 1999, Bangkok, Thailand. pp. 135–145.

Dharmarathna, W.R.S.S., Herath, S. and Weerakoon, S.B. (2014) Changing the planting date as a climate change adaptation strategy for rice production in Kurunegala district, Sri Lanka. *Sustain. Sci.* **9,** 103-111.

Doni, F., Al-Shorgani, N.K.N., Abuelhassan, N.N., Isahak, A., Zain, C.R.C.M. and Yusoff, W.M.W. (2013) Microbial involvement in growth of paddy. *Curr. Res. J. Biol. Sci.* **5**, 285-290.

Du, B., Zhang, W., Liu, B., Hu, J., Wei, Z., Shi, Z., He, R. *et al*. (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 22163-22168.

Dudbridge, F., Carver, T. and Williams, G.W. (2004) Pelican: pedigree editor for linkage computer analysis. *Bioinformatics,* **20,** 2327-2328.

Duvick, D.N. (1984) Progress in conventional plant breeding. In: *Gene manipulation in plant improvement*, pp. 17-31. Boston, MA: Springer.

Dyck, V.A. and Thomas, B. (1979) The brown planthopper problem. In: *Brown planthopper: threat to rice production in Asia*, pp. 3-17. Los Banos, Philippines : International Rice Research Institute.

Eathington, S.R., Crosbie, T.M., Edwards, M.D., Reiter, R.S. and Bull, J.K. (2007) Molecular markers in a commercial breeding program. *Crop Sci.* **47**, S-154.

Eberhart, S.A. and Gardner, C.O. (1966) A general model for genetic effects. *Biometrics,* **22**, 864–881.

Edwards, D., Forster, J.W., Chagne, D. and Batley, J. (2007) What is SNPs? In: *Association Mapping in Plants* (Or- aguzie, N.C., Rikkerink, E.H.A., Gardiner, S.E. and de Silva, H.N., eds), pp. 41-52. Berlin: Springer.

Edwards, M.D., Stuber, C.W. and Wendel, J.F. (1987) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. numbers, genomic distribution and types of gene action. *Genetics,* **116,** 113-125.

Elazegui, F. and Islam, Z. (2003) *Diagnosis of common diseases of rice.* Philippines: International Rice Research Institute.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. and Mitchell, S.E. (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS One,* **6**, e19379. doi: 10.1371/journal.pone.0019379.

Evans, K., Guan, Y., Luby, J., Clark, M., Schmitz, C., Brown, S., Orcheski, B. *et al*.(2011) Large-scale standardized phenotyping of apple in RosBREED. In *Proceedings of the IV International Conference Postharvest Unlimited*, pp. 233-238.

Fageria, N.K. and Knupp, A.M. (2013) Upland rice phenology and nutrient uptake in tropical climate. *J. Plant Nutr.* **36**, 1-14.

Fageria, N.K. and Moreira, A. (2011) The role of mineral nutrition on root growth of crop plants. In: *Advances in Agronomy. Vol. 110,* pp. 251-331. Burlington: Academic Press.

Fageria, N.K. and Santos, B. (2002) Lowland rice genotypes evaluation for phosphorus use efficiency. *J. Plant Nutr.* **25,** 2793-2802.

Fageria, N.K., Baligar, V.C. and Li, Y.C. (2008) The role of nutrient efficient plants in improving crop yields in the twenty first century. *J. Plant Nutr.* **31,** 1121-1157.

Fageria, N.K., Wright, R.J. and Baligar, V.C. (1988) Rice cultivar evaluation for phosphorus use efficiency. *Plant and Soil Science,* **111,** 105-109.

Falconer, D.S. (1981) *Introduction to quantitative genetics*. Longman Group Ltd, New York.

FAO (2008) Land and plant nutrition management service. Available on-line at: http://www.fao.org/ag b/agl /agll/spush.

Farmer, B.H. (1979) The 'green revolution 'in South Asian ricefields: environment and production. *The Journal of Development Studies,* **15,** 304-319.

Farrell, T. C., Fox, K. M., Williams, R. L. and Fukai, S. (2006) Genotypic variation for cold tolerance during reproductive development in rice: screening with cold air and cold water. Field *Crop Res.* **98**, 178–194.

Fehr, W.R. (1987) *Principles of cultivar development, vol 1, Theory and technique*, 5p. New York: Macmillan Publishing Company.

Felsenstein, J. (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Seattle: Department of Genome Sciences, University of Washington.

Fernando, H., Senodhera, D., Elikawela, Y., de Alwis, H. M. and Kudagamage, C. (1979) Varietal resistance to the brown planthopper in Sri Lanka. In: *Brown Planthopper: Threat to Rice Production in Asia*, pp. 241-249. Los Banos, Philippines : International Rice Research Institute.

Flowers, T.J. and Flowers, S.A. (2005) Why does salinity pose such a difficult problem for plant breeders? *Agric. Water Manag*. **78**, 15–24.

Food and Agriculture Organization of the United Nations (FAO) (2015) *Rice market monitor,* **18,** 1-10.

Food and Agriculture Organization of the United Nations (FAO) (2018) *Rice market monitor,* **21,** 1-3.

Fowler, S. and Thomashow, M.F. (2002) Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *The Plant Cell,* **14**, 1675-1690.

Frisch, M. and Melchinger, A.E. (2001) Marker-assisted backcrossing for introgression of a recessive gene. *Crop sci.* **41,** 1485-1494.

Fukai, S. and Fischer, K.S. (2012) Field phenotyping strategies and breeding for adaptation of rice to drought. *Front. Physiol.* **3**, 282.

Fukuda, M., Nagumo, F., Nakamura, S. and Tobita, S. (2012) Alternative fertilizer utilizing methods for sustaining low input agriculture. In *Soil Fertility*. IntechOpen.

Gallagher, K.D., Kenmore, P.E. and Sogawa, K. (1994) Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice. In *Planthoppers*, pp. 599-614. Boston, MA; Springer.

Garbe, J.R. and Da, Y. (2008) PediGrach User Manual Version 2.1. Department of Animal Science, University of Minnesota.

Gazal, A., Dar, Z.A., Lone, A.A., Abidi, I. and Ali, G. (2015) Molecular breeding for resilience in maize - a review. *J. Appl. & Nat. Sci.* **7**, 1057-1063.

Ghosh, B., Md, N.A. and Gantait, S. (2016) Response of rice under salinity stress: a review update. *Rice research: open access*, 1-8.

Gnanamanickam, S.S. and Mew, T.W. (1992) Biological control of blast disease of rice (*Oryza sativa* L.) with antagonistic bacteria and its mediation by a Pseudomonas antibiotic. *Japanese Journal of Phytopathology*, **58**, 380-385.

Gokidi, Y., Bhanu, A.N. and Singh, M. (2016) Marker assisted recurrent selection: an Overview. *Advances in Life Sciences*, **5**, 6493-6499.

Gore, M., Bradbury, P., Hogers, R., Kirst, M., Verstege, E., Van Oeveren, J., Peleman, J. *et al*. (2007) Evaluation of target preparation methods for single-feature polymorphism detection in large complex plant genomes. *Crop Sci.* **47**, S-135.

Gore, M.A., Chia, J.M., Elshire, R.J., Sun, Q., Ersoz, E.S., Hurwitz, B.L., Peiffer, J.A. *et al*. (2009) A first-generation haplotype map of maize. *Science*, **326**, 1115-1117.

Gunes, A., Inal, A., Alpaslan, M. and Cakmak, I. (2006) Genotypic variation in phosphorus efficiency between wheat cultivars grown under greenhouse and field conditions. *Soil Science and Plant Nutrition*, **52,** 470-478.

Gupta, M., Chyi, Y.S., Romero-Severson, J. and Owen, J.L. (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theor. Appl. Genet.* **89,** 998-1006.

Gupta, P.K., Roy, J.K. and Prasad, M. (2001) Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current Science*, **80**, 524-535.

Hakim, M.A., Juraimi, A.S., Begum, M., Hanafi, M.M., Ismail, M.R. and Selamat, A. (2010) Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *Afr. J. Biotechnol.* **9**, 1911–1918.

Halford, I.C.R. (1997) Soil Phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research*, **35**, 39-227.

Hallauer, A.R. (1967) Development of Single-Cross Hybrids from Two-Eared Maize Populations 1. *Crop Sci.* **7,** 192-195.

Hanson, W.C. (1950) The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. *J. Sci. Food Agric.* **1,** 172-173.

Harlan, J. (1976) Genetic resources in wild relatives of crops 1. *Crop sci.* **16**, 329-333.

Harlan, J.R. and de Wet, J.M. (1971) Toward a rational classification of cultivated plants. *Taxon*, **20**, 509-517.

Harris, D., Tripathi, R.S. and Joshi, A. (2002) On-farm seed priming to improve crop establishment and yield in dry direct seeded rice. In: *Direct seeding: Research Strategies and Opportunities* (Pandey, S., Mortimer, M., Wade, L., Tuong, T.P., Lopes, K. and Hardy, B., eds), pp. 231–240. Manila, Philippines: International Research Institute.

He, D.H., Lin, Z.X., Zhang, X.L., Nie, Y.C., Guo, X.P., Zhang, Y.X. and Li, W. (2007) QTL mapping for economic traits based on a dense genetic map of cotton with PCR-based markers using the interspecific cross of *Gossypium hirsutum*× *Gossypium barbadense*. *Euphytica*, **153**, 181-197.

He, J., Zhao, X., Laroche, A., Lu, Z.X., Liu, H. and Li, Z. (2014) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. *Front. Plant Sci.* **5,** 484. doi: 10.3389/fpls.2014.00484.

Heffner, E.L., Lorenz, A.J., Jannink, J.L. and Sorrells, M.E. (2010) Plant breeding with genomic selection: gain per unit time and cost. *Crop sci.* **50,** 1681-1690.

Heffner, E.L., Sorrells, M.E. and Jannink, J.L. (2009) Genomic selection for crop improvement. *Crop Sci.* **49,** 1-12.

Heinrichs, E.A. (1994) *Biology and management of rice insects*. Int. Rice Res. Inst.

Herath Banda, R.M., Dhanapala, M.P., de Silva G.C.A. and Hossain, M. (1998). Constraints increasing rice production in Sri Lanka. In *Proceedings of the workshop on Prioritization of Rice Research*. IRRI, Los Banõs, Laguna, Philippines, pp. 20-22.

Herath, H., Hardaker, J.B. and Anderson, J.R. (1982) Choice of varieties by Sri Lanka rice farmers: comparing alternative decision models. *Am. J. Agric. Econ.* **64**, 87-93.

Heuer, S., Lu, X., Chin, J.H., Tanaka, J.P., Kanamori, H., Matsumoto, T., Leon, T.D. *et al*. (2009) Comparative sequence analyses of the major quantitative trait locus *phosphorus up take 1* (*Pup1*) reveal a complex genetic structure. *Plant Biotechnology Journal*, **7**, 456-471.

Hittalmani, S., Parco, A., Mew, T.V., Zeigler, R.S. and Huang, N. (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.* **100,** 1121-1128.

Hmielowski, T. (2017) Challenges to developing cultivars in the public sector. *Crops, Soils, Agronomy News*, **62**, 4-5.

Ho, N.K. (1996) Introducing integrated weed management in Malaysia. In: *Herbicides in Asian Rice: Transitions in Weed Management* (Naylor, R., ed), pp. 167-182. Stamford University, California and IRRI, Philippines.

Hogan, R. (2018) *A practical guide to database design*, 1p. USA: Chapman and Hall/ CRC.

Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P., 1977. *The world's worst weeds: distribution and biology.* University Press of Hawaii.

Horgan, F. (2009) Mechanisms of resistance: a major gap in understanding planthopper-rice interactions. In *Planthoppers: New threats to the sustainability of intensive rice*, 62p.

Horgan, F.G., Ramal, A.F., Bentur, J.S., Kumar, R., Bhanu, K.V., Sarao, P.S., Iswanto, E.H. *et al*. (2015) Virulence of brown planthopper (*Nilaparvata lugens*) populations from South and South East Asia against resistant rice varieties. *Crop Protection*, **78**, 222-231.

Hospital, F. and Charcosset, A. (1997) Marker-assisted introgression of quantitative trait loci. *Genetics*, **147,** 1469–1485.

Hou Ly, Yu, P., Xu, Q., Yuan Xp, Yu Hy, Wang Yp, Wang Ch *et al*. (2011) Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, *Nilaparvata lugens* Stål in rice. *Rice Science*, **18**, 238-242.

Hsiao, T.C. (1982) The soil plant atmosphere continuum in relation to drought and crop production. In: *Drought resistance in crops with emphasis on rice*, pp. 39–52. Los Banõs, Philippines: IRRI.

Hu, J., Xiao, C. and He, Y. (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. *Rice*, **9**, 1-12.

Hu, J., Xiao, C., Cheng, M., Gao, G., Zhang, Q. and He, Y. (2015) Fine mapping and pyramiding of brown planthopper resistance genes *QBph3* and *QBph4* in an introgression line from wild rice *O. officinalis*. *Mol. Breeding*, **35**, 3. doi: 10.1007/s11032-015-0228-2.

Huang, J., Levine, A. and Wang, Z. (2013) Plant abiotic stress. *The Scientific World Journal*, **2013,** 1–2.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N. *et al*. (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* **95,** 313-320.

Huke, R.E. and E.H. Huke (1997) *Rice area by type of culture: South, Southeast, and East Asia*. Los Ban˜ os, Philippines: IRRI.

Iezzoni, A., Weebadde, C., Luby, J., Chengyan Yue, van de Weg, E., Fazio, G., Main, D. *et al*. (2009) RosBREED: Enabling Marker-Assisted Breeding in Rosaceae. In *Proceedings of the International Symposium on Molecular Markers in Horticultural Species*, Corvallis, Oregon (USA), pp. 389-394.

IPCC (2007) *Climate change 2007: Impacts, adaptation and vulnerability.* Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. (Parry, M.L., Canziani, O.F., Palutikof, J.P., Van der Linden, P.J. and Hanson, C.E., eds), 976p. Cambridge, UK: Cambridge University Press.

Irz, X., Lin, L., Thirtle, C. and Wiggins, S. (2001) Agricultural productivity growth and poverty alleviation. *Development policy review*, **19,** 449-466.

Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S., Bennett, J. and Craufurd, P.Q. (2009) Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J. Exp. Bot.* **61**, 143-156.

Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A. and Toojinda, T. (2007) Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. *Mol. Breeding*, **19**, 35-44.

Jannink, J.L., Lorenz, A.J. and Iwata, H. (2010) Genomic selection in plant breeding: from theory to practice. *Brief. Funct. Genomics*, **9,** 166-177.

Jayarathne, H.S.M., Rathnayake, P.G.R.G., Ranaweera, L.T., Nizam, R., Rathnayake, R.M.S.K., Kannangara, S.K., Udawela, U.A.K.S., et al. (2019) Assessment of the variation of performance indicators under p-starved conditions in a core-set of rice cultivars and their diversity in *Pup1*-linked DNA marker-haplotypes. *J. Natl. Sci. Found. Sri.* In review.

Jayne, T. (1993) *Sources and effects of instability in the world rice market*, MSU International Development Paper No. 13, 104p. Michigan State University, USA: Department of Agricultural Economics and Department of Economics.

Jena, K.K. and Kim, S.M. (2010) Current status of brown planthopper (BPH) resistance and genetics. *Rice*, **3**, 161-171.

Jiang, G.L. (2013a) Plant marker-assisted breeding and conventional breeding: challenges and perspectives. *Adv. Crop Sci. Tec.* **1**, e106. doi :10.4172/2329-88631000e106.

Jiang, G.L. (2013b) Molecular markers and marker-assisted breeding in plants. In: *Plant breeding from laboratories to fields*. IntechOpen. doi: 10.5772/52583.

Jing, S., Zhao, Y., Du, B., Chen, R., Zhu, L. and He, G. (2017) Genomics of interaction between the brown planthopper and rice. *Curr. Opin. Insect Sci.* **19,** 82-87.

Johnson, G.R. and Mumm, R.H. (1996) Marker assisted maize breeding. In *Proceedings of the 51st Corn and Sorghum Conference,* pp. 75-84. Washington, DC: American Seed Trade Association.

Jones, P., Garcia, B., Furches, A., Tuskan, G. and Jacobson, D. (2019) Plant host-associated mechanisms for microbial selection. *Front. plant sci.* **10**, 862.

Jongdee, B., Pantuwan, G., Fukai, S. and Fischer, K. (2006) Improving drought tolerance in rainfed lowland rice: an example from Thailand. *Agric. Water Manag.* **80**, 225-240.

Judson, R., Stephens, J.C. and Windemuth, A. (2000) The predictive power of haplotypes in clinical response. *Pharmacogenomics*, **1**, 15-26.

Just, R.E. and Zilberman, D. (1988) The effects of agricultural development policies on income distribution and technological change in agriculture. *Journal of Development Economics*, **28**, 193-216.

Kabis, A. and Khush, G.S. (1988) Genetic analysis of resistance to brown planthopper in rice (*Oryza sativa* L.). *Plant Breed.* **100**, 54-58.

Kaneda, C., Ito, K. and Ikeda, R. (1981) Screening of rice cultivars for resistance to the brown planthopper, *Nilaparvata lugens* Stål., by three biotypes. *Japan. J. Breed.* **31**, 141-151.

Karim, R.S., Man, A.B. and Sahid, I.B. (2004) Weed problems and their management in rice fields of Malaysia: an overview. *Weed Biol. Manag.* **4**, 177-186.

Kariuki, C.M., van Arendonk, J.A.M., Kahi, A.K. and Komen, H. (2017) Multiple criteria decision-making process to derive consensus desired genetic gains for a dairy cattle breeding objective for diverse production systems. *J. Dairy Sci.* **100,** 4671-4682.

Kato, H. (1976) Some topics in a disease cycle of rice blast and climatic factors. In: *Climate and Rice*, pp. 417-425. International Rice Research Institute.

Kato, H. (2001) Rice blast disease. *Pesticide outlook*, **12,** 23-25.

Kawaguchi, M., Murata, K., Ishii, T., Takumi, S., Mori. N. and Nakamura, C. (2001) Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph4* to the rice chromosome 6. *Breeding Science*, **51**, 13-18.

Kaya, M.D., Okçub, G., Ataka, M. and Kolsar, Ö. (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.* **24**, 291–295.

Kearsey, M.J. and Pooni, H.S. (1998) The genetical analysis of quantitative traits. Surrey, SR, UK: Stanley Thornes (Publishers) Ltd.

Keith, J.M., McRae, A., Duffy, D., Mengersen, K. and Visscher, P.M. (2008) Calculation of IBD probabilities with dense SNP or sequence data. *Genet. Epidemiol.* **32**, 513-519.

Kempe, K. and Gils, M. (2011) Pollination control technologies for hybrid breeding. *Mol. Breeding*, **27**, 417-437.

Khalil, M.S., Moubasher, H. and Hasan, F.F. (2014) Biological control of rice blast disease by *Streptomyces flavotricini*. *Res. J. Pharm. Biol. Chem. Sci*. **5**, 1453-1461.

Khush, G.S. (1977) Disease and insect resistance in rice. *Advances in Agronomy*, **29**, 265-341.

Khush, G.S. (1995) Breaking the yield frontier of rice. *Geo Journal*, **35**, 329-332.

Khush, G.S., Karim, A.R. and Angeles, E.R. (1985) Genetics of resistance of rice cultivar *ARC10550* to Bangladesh brown planthopper teletype. *J. Genet.* **64**, 121-125.

Koreth, J., O'Leary, J.J. and O'D. McGee, J.A.M.E.S. (1996) Microsatellites and PCR genomic analysis. *The Journal of pathology*, **178**, 239-248.

Krishnaiah, N.V. (2014) A global perspective of rice brown planthopper management I-crop-climatic requirement. *Intl. J. of Molecular Zoology*. **4**, 45-55.

Kuan C.Y., Ann L.S., Ismail A.A., Leng T., Fee C.G. and Hashim K. (1990) Crop loss by weeds in Malaysia. In *Proceedings of the Third Tropical Weed Science Conference*, December 4-6, Malaysian Plant Protection Society, Kuala Lumpur, Malaysia. pp 1–21.

Kumar, A., Dixit, S., Ram, T., Yadaw, R.B., Mishra, K.K. and Mandal, N.P. (2014) Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *J. Exp. Bot.* **65**, 6265-6278.

Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870-1874.

Kumaragamage, D. and Indraratne, S. (2011) Systemic approach to diagnosing fertility problems in soils of Sri Lanka. *Communication in soil science and plant analysis*, **42**, 2699-2715.

Labrada, R. (2003) The need for improved weed management in rice, *FOA*. Available on-line at: http://www.fao.org/3/y4751e0l.html.

Laksminarayana, A. and Khush, G.S. (1977) New genes for resistance to the brown planthopper in rice 1. *Crop Sci.* **17**, 96-100.

Lamkey, K.R. and Edwards, J.W. (1999) Quantitative genetics of heterosis. In *Proceeding on Agronomy Conference and Presentations, Iowa State University, USA.* pp.31–48. Available on-line at: http://lib.dr.iastate.edu/agron_conf/65.

Lee, P.Y., Costumbrado, J., Hsu, C.Y. and Kim, Y.H. (2012) Agarose gel electrophoresis for the separation of DNA fragments. *J. Vis. Exp.* **62**, e3923. doi: 10.3791/3923.

Li-Hong, S.U.N., Chun-Ming, W.A.N.G., Chang-Chao, S.U., Yu-Qiang, L.I.U., Hu-Qu, Z.H.A.I. and Jian-Min, W.A.N. (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). *Acta Genetica Sinica*, **33**, 717-723.

Ling, K.C. (1972) *Rice virus diseases*, pp. 78-102, Los Banos, Philippines: International Rice Research. Institute.

Liu, Y., Chen, L., Liu, Y., Dai, H., He, J., Kang, H., Pan, G. *et al*. (2016) Marker assisted pyramiding of two brown planthopper resistance genes, *Bph3* and *Bph27(t)*, into elite rice cultivars. *Rice*, **9**, 27. doi: 10.1186/s12284-016-0096-3.

Lu, L., Yan, W., Xue, W., Shao, D. and Xing, Y. (2012) Evolution and association analysis of *Ghd7* in rice. *PLoS One*, **7,** e34021.

Lutz, W. and Qiang, R. (2002) Determinants of human population growth. *Phil. Trans. R. Soc. Lond. B.* **357**, 1197-1210.

Mackill, D.J. (1996) Rainfed lowland rice improvement, 242p. Manila, Philippines: International Rice Research Institute.

Majumder, N., Borthakur, D. and Rakshit, S. (1989) Heterosis in rice under phosphorus stress. *Indian J. Genet. Plant Breed.* **49**, 231-235.

Matsumura, M., Takeuchi, H., Satoh, M., Sanada-Morimura, S., Otuka, A., Watanabe, T. and Van Thanh, D. (2008) Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera* in East and South-east Asia. *Pest Manag. Sci.* **64**, 1115-1121.

McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B. *et al.* (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA research*, **9**, 199-207.

McNally, K.L., Childs, K.L., Bohnert, R., Davidson, R.M., Zhao, K., Ulat, V.J., Zeller, G. *et al.* (2009) Genomewide SNP variation reveals relationships among landraces and modern varieties of rice. In *Proceedings of the National Academy of Sciences*, **106**, 12273-12278. doi: 10.1073pnas.0900992106.

Mengel, K., Kirkby, E.A., Kosegarten, H. and Appel, T. (2001) *Principles of plant nutrition.* Kluwer, Dordrecht: Springer.

Meuwissen, T.H. and Goddard, M.E. (2001) Prediction of identity by descent probabilities from marker-haplotypes. *Genet. Sel. Evol.* **33**, 605-634.

Miles, C.M. and Wayne, M. (2008) Quantitative trait locus (QTL) analysis. *Nature Education*, **1**, 208.

Moody, K. and De Datta, S.K. (1982) Integration of weed control practices of rice in tropical Asia. In: *Weed Control in Small Farms* (Soerjani, M. eds), pp 34-47. BIOTROP Special Publication No. 15. SEAMEO–BIOTROP, Bogor, Indonesia.

Morgante, M., Hanafey, M. and Powell, W. (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature genetics*, **30**, 194-200.

Morita, S., Shiratsuchi, H., Takahashi, J. and Fujita, K. (2004) Effect of high temperature on ripening in rice plants: analysis of the effects of high night and high day temperatures applied to the panicle and other parts of the plant. *Jpn. J. Crop Sci.* **73**, 77–83 [in Japanese with English summary].

Mullis, K.B. (1990) The unusual origin of the polymerase chain reaction. *Scientific American*, **262**, 56-65.

Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**, 651–681.

Muthayya, S., Sugimoto, J.D., Montgomery, S. and Maberly, G.F. (2014) An overview of global rice production, supply, trade, and consumption. *Ann. Ny. Acad. Sci.* **1324**, 7-14.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., *et al.* (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnol. Equip.* **32**, 261-285.

Nagadhara, D., Ramesh, S., Pasalu, I.C., Rao, Y.K., Krishnaiah, N.V., Sarma, N.P., Bown, D.P. *et al.*(2003) Transgenic *indica* rice resistant to sap-sucking insects. *Plant Biotechnology Journal*, **1**, 231-240.

Nakandala, N.D.U.S., Karunarathne, S.I., Ranaweera, L.T., Wijesundara, W.W.M.U.K., Senevirathna, R.W.K.M., Rathnayake, R.M.S.K., Udawela, U.A.K.S., *et al.* (2019) Assessment of the association between yield parameters and polymorphic sites of the *Ghd7* locus in a core-set of rice cultivars grown in Sri Lanka. *Ceylon J. Sci.* **48**, 121-132.

Nasu, S., Suzuki, J., Ohta, R., Hasegawa, K., Yui, R., Kitazawa, N., Monna, L. *et al*. (2002) Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (*Oryza sativa, Oryza rufipogon*) and establishment of SNP markers. *DNA research*, **9**, 163-171.

Nei, M. and Kumar, S. (2000) *Phylogenetic inference: distance methods. Molecular evolution and phylogenetics*, 89p. Oxford, UK: Oxford University Press.

Newell, M. A. and Jannink, J.L. (2014) Genomic selection in plant breeding. In: *Crop Breeding*, pp. 117-130. New York, NY: Humana Press.

Ni, J.J., Wu, P., Senadhira, D. and Huang, N. (1998) Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **97,** 1361-1369.

Nugaliyadde, L., Des Abeysiriwardena, D.S., Samanmalee, L.G.A., Pathirana, R. and Wilkins, R.M. (2004) Inheritance of resistance in rice to brown planthopper: its implications on rice varietal improvement in Sri Lanka. Available on-line at: http://ag.udel.edu/delpha/7674.pdf

Nugaliyadde, L., Dissanayake, N., Mitrasena, J. and Wijesundera, D.S.A. (2000) Advances of pest and disease management of rice in Sri Lanka: A review. In *Proceedings of the Annual Symposium*, Department of Agriculture, Peradeniya, Sri Lanka, pp. 402-422.

O'neil, J., Davis, T.W., Burford, M.A. and Gobler, C. (2012) The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. *Harmful Algae*, **14**, 313-334.

O'Toole, J.C. (1982) Adaptation of rice to drought prone environments. In: *Drought resistance in crops with emphasis on rice*, 195–213. Los Banõs, Philippines: IRRI.

Ou, S.H. (1985) *Rice diseases* (2nd Edn), pp.370. New England, Commonwealth Mycological Institute.

Ou, S.H. and Rivera, C.T. (1969) Virus diseases of rice in Southeast Asia. In *Symposium of Virus Diseases in Rice Plant Proc.*

Pariasca-Tanaka, J., Chin, J.H., Dramé, K.N., Dalid, C., Heuer, S. and Wissuwa, M. (2014) A novel allele of the P-starvation tolerance gene *OsPSTOL1* from African rice (*Oryza glaberrima* Steud) and its distribution in the genus *Oryza*. *Theor. Appl. Genet.* **127**, 1387-1398.

Paterniani, E. and Vencovsky, R. (1977) Reciprocal recurrent selection in maize (*Zea mays L.*) based on testcrosses of half-sib families. *Maydica*, **22**, 141-152.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E. and Tanksley, S.D. (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature*, **335**, 721-726.

Pathak, M.D. and Khan, Z.R. (1994) *Insect pests of rice.* Int. Rice Res. Inst.

Pathak, M.D. and Khush, G.S. (1979) Studies of varietal resistance in rice to the brown planthopper at the International Rice Research Institute. In: *Brown Planthopper: Threat to Rice Production in Asia*, pp 285-301. Los Banos, Philippines: International Rice Research Institute.

Pathak, M.D., Cheng, C.H. and Fortuno, M.E. (1969) Resistance to *Nephotettix impicticeps* and *Nilaparvata lugens* in varieties of rice. *Nature*, **223**, 502-504.

Paulo, M.J., Boer, M., Huang, X., Koornneef, M. and Van Eeuwijk, F. (2008) A mixed model QTL analysis for a complex cross population consisting of a half diallel of two-way hybrids in *Arabidopsis thaliana*: analysis of simulated data. *Euphytica.* **161**, 107-114.

Peace, C.P., Luby, J.J., Van de Wag, W.E., Bink, M.C.A.M. and Iezzoni A.F. (2014) A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach, and sweet cherry. *Tree Genet. Genomes*, **10**, 1679-1694.

Peeters, J.P. and Galwey, N.W. (1988) Germplasm collections and breeding needs in Europe. *Econ. Bot.* **42**, 503-521.

Peng, S., Cassman, K.G., Virmani, S.S., Sheehy, J. and Khush, G.S. (1999) Yield potential trends of tropical rice since the release of *IR8* and the challenge of increasing rice yield potential. *Crop Sci.* **39**, *1552-1559.*

Peng, S., Tang, Q. and Zou, Y. (2009) Current status and challenges of rice production in China. *Plant Prod. Sci.* **12**, 3-8.

Peng, T., Sun, X. and Mumm, R.H. (2014) Optimized breeding strategies for multiple trait integration: II. Process efficiency in event pyramiding and trait fixation. *Mol. Breeding,* **33**, 105-115.

Poehlman, J.M. and Sleper, D.A. (1995) *Breeding field crops.* Iowa, USA: Iowa State University Press.

Poland, J.A. and Rife, T.W. (2012) Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome,* **5**, 92-102.

Powell, W., Machray, G.C. and Provan, J. (1996) Polymorphism revealed by simple sequence repeats. *Trends in plant science*, **1**, 215-222.

Prasad, P.V.V., Staggenborg, S.A. and Ristic, Z. (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. *Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes*, (responseofcrops), pp.301-355.

Price, A.H. (2006) Believe it or not, QTLs are accurate! *Trends in plant science*, **11**, 213-216.

Rabbani, M.A., Maruyama, K., Abe, H., Khan, M.A., Katsura, K., Ito, Y., Yoshiwara, K. *et al*. (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant physiol.* **133**, 1755-1767.

Ragot, M., Bonierbale, M. and Weltzien, E. (2018) *From Market Demand to Breeding Decisions: a framework. Lima (Peru).* CGIAR gender and breeding initiative. GBI Working Paper. No. 2. Available on-line at: https://www.rtb.cgiar.org/gender-breeding-initiative.

Rajendran, K., Tester, M. and Roy, S. J. (2009) Quantifying the three main components of salinity tolerance in cereals. *Plant Cell Environ.* **32**, 237–249.

Rambaut, A. (2018) FigTree v1. 4.2, a graphical viewer of phylogenetic trees. Available on-line at : http://tree.bio.ed.ac.uk/software/figtree/.

Rathnayake, R.M.S.K., Rathnayake, P.G.R.G., Jayarathne, H.S.M., Nizam, R., Ranaweera, L.T., Kannangara, S.K., Udawela, U.A.K.S., *et al.* (2019) Assessment of the sequence-based haplotype-variants in selected DNA marker loci for the molecular breeding of resistant rice varieties to brown plant hopper. *Sri Lankan Journal of Agriculture and Ecosystems.* **1**, 2-15.

Reddy, I.N.B.L., Kim, B.K., Yoon, I.S., Kim, K.H. and Kwon, T.R. (2017) Salt tolerance in rice: focus on mechanisms and approaches. *Rice Science*, **24**, 123-144.

Ribaut, J.M., De Vicente, M.C. and Delannay, X. (2010) Molecular breeding in developing countries: challenges and perspectives. *Curr. Opin. Plant Biol.* **13**, 213-218.

Rice Knowledge Bank (2019, December 18) Rice doctor: Fact sheets: Bacteria blight. Retrieved from: http://www.knowledgebank.irri.org/decision-tools/.

Rice Research and development Institute (RRDI) (2019, June 5) Rice Cultivation. In *Department of Agriculture, Sri Lanka,* Retrieved from http:// www.doa.gov.lk/rrdi/.

Risch, N. and Merikangas, K. (1996) The future of genetic studies of complex human diseases. *Science,* **273**, 1516-1517.

Röder, M.S., Plaschke, J., König, S.U., Börner, A., Sorrells, M.E., Tanksley, S.D. and Ganal, M.W. (1995) Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet*. **246**, 327-333.

Rombach, M.C., Aguda, R.M., Shepard, B.M. and Roberts, D.W. (1986) Infection of rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopathogenic Hyphomycetes (Deuteromycotina). *Environmental Entomology*, **15**, 1070-1073.

Rose, T.J., Rose, M.T., Pariasca Tanaka, J., Heuer, S. and Wissuwa, M. (2011) The frustration with utilization: why have improvements in internal phosphorus utilization efficiency in crops remained so elusive? *Frontiers in Plant Science*, **2,** 73. doi: 10.3389/ fpls.2011.00073.

Rose, T.J., Wissuwa, M. (2012) Rethinking internal phosphorus utilization efficiency: A new approach is needed to improve PUE in grain crops. *Advances in Agronomy*, **116**, 186-211.

Rosyara, U.R., Bink, M.C.A.M., Van de Weg, E., Zhang, G., Wang, D., Sebolt, A., Dirlewanger, E., *et al.* (2013) Fruit size QTL identification and the prediction of parental QTL genotypes and breeding values in multiple pedigreed populations of sweet cherry. *Mol. Breeding*, **32**, 875-887.

Roy, S. J., Negrao, S. and Tester, M. (2014) Salt resistant crop plants. *Curr. Opin. Biotechnol.* **26,** 115–124.

RRDI (2018) *Recommended rice varieties in Sri Lanka (1958-2016).* Bathalagoda, Ibbagamuwa, Sri Lanka: Rice Research and Development Institute, Department of Agriculture.

Saha, S., Garg, R., Biswas, A. and Rai, A.B. (2015) Bacterial diseases of rice: an overview. *J. Pure Appl. Microbiol.* **9**, 725-736.

Salmaso, M., Faes, G., Segala, C., Stefanini, M., Salakhutdinov, I., Zyprian, E., Toepfer, R. *et al*. (2005) Genome diversity and gene haplotypes in the grapevine (*Vitis vinifera* L.), as revealed by single nucleotide polymorphisms. *Mol. Breeding.* **14**, 385-395.

Samanta, S., Pal, B. and Pal, D.K. (2011) Land suitability analysis for rice cultivation based on multi-criteria decision approach through GIS. *Int. J Sci. Emerging Tech.* **2**, 2-20.

Sanchez, P.A. (2002) Soil fertility and hunger in Africa. *Science,* **295**, 2019-2020.

Sasaki, T. (2011) Power of conventional rice breeding. *Breeding Science*, **61**, 317.

Satake, T. and Yoshida, S. (1978) High temperature-induced sterility in indica rices at flowering. *Jpn. J. Crop Sci.* **47**, 6–17.

Seck, P.A., Diagne, A., Mohanty, S. and Wopereis, M.C. (2012) Crops that feed the world 7: Rice. *Food Sec.* **4**, 7-24.

Segal, R. and Le Nguyet, M. (2019) *Unfair Harvest: The state of rice in Asia.* Oxford, UK: Oxfam International.

Semagn, K., Bjornstad, A. and Ndjiondjop, M.N. (2006) An overview of molecular marker methods for plants. *Afr. J. Biotechnol.* **5**, 2540-2568.

Servin, B., Martin, O.C. and Mezard, M. (2004) Toward a theory of marker-assisted gene pyramiding. *Genetics*, **168**, 513-523.

Seshu, D.V. and Kauffmann, H.E. (1980) *Differential response of rice varieties to the brown planthopper in international screening tests.* Los Banos, Laguna, Philippines: International Rice Research Institute.

Sharma, P.N., Torii, A., Takumi, S., Mori, N. and Nakamura, C. (2004) Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens Stål*) resistance genes *Bph1* and *Bph2* on rice chromosome 12. *Hereditas*, **140**, 61-69.

Sharopova, N., McMullen, M.D., Schultz, L., Schroeder, S., Sanchez-Villeda, H., Gardiner, J., Bergstrom, D. *et al*. (2002) Development and mapping of SSR markers for maize. *Plant molecular biology*, **48**, 463-481.

Shen, J., Li, R., Zhang, F., Fan, J., Tang, C. and Rengel, Z. (2004) Crop yields, soil fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture system on a calcareous soil. *Field Crops Res.* **86**, 225-238.

Shetty, P.K. (2004) Socio-ecological implications of pesticide use in India. *Econ. Polit. Wkly*. 5261-5267.

Sidhu, G.S. and Khush, G.S. (1978) Genetic analysis of brown planthopper resistance in twenty varieties of rice, *Oryza sativa* L. *Theor. Appl. Genet. Theor. Appl. Genet.* **53**, 199-203.

Singh, A., Parmar, N., Kuhad, R.C. and Ward, O.P. (2011) Bioaugmentation, biostimulation, and biocontrol in soil biology. In: *Bioaugmentation, Biostimulation and Biocontrol* (Singh, A., Parmar, N. and Kuhad, R.C., (Eds.), pp. 1-23. Germany: Springer: Berlin/Heidelberg.

Singh, G.P., Srivastava, M.K., Singh, R.V. and Singh, R.M. (1977) Variation in quantitative and qualitative losses caused by bacterial blight in different rice varieties. *Ind. Phytopath.* **30**, 180-185.

Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S. *et al*. (2001) Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar *PR106*. *Theor. Appl. Genet.* **102**, 1011-1015.

Sirisena, D.N. and Wanninayake, W.M.N. (2014) Identification of promising rice varieties for low fertile soils in the low country intermediate zone in Sri Lanka. *Annals of Sri Lanka Department of Agriculture*, **14**, 95-105.

Skamnioti, P. and Gurr, S.J. (2009) Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol.* **27**, 141-150.

Smith Jr, R.J. (1983) Weeds of major economic importance in rice and yield losses due to weed competition. In *Proceeding of the conference Weed Control in Rice*. IRRN, pp. 19-36.

Sobrino, B., Bríon, M. and Carracedo, A. (2005) SNPs in forensic genetics: a review on SNP typing methodologies. *Forensic Science International*. **154**, 181-194.

Sōgawa, K. (1982) The rice brown planthopper: feeding physiology and host plant interactions. *Ann. Rev. EntomoL*. **27**, 49-73.

Sogawa, K. (2015) Planthopper outbreaks in different paddy ecosystems in Asia: man-made hopper plagues that threatened the green revolution in rice. In *Rice Planthoppers*, pp. 33-63. Dordrecht, Netherlands: Springer.

Sogawa, K. and Cheng, C.H. (1979) Economic thresholds, nature of damage, and losses caused by the brown planthopper. In *Brown planthopper: threat to rice production in Asia*, pp. 125-142. Los Banos, Philippines: The International Rice Research Institute.

Song, Q., Jia, G., Zhu, Y., Grant, D., Nelson, R.T., Hwang, E.Y., Hyten, D.L. (2010) Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1. 0) in soybean. *Crop Science*. **50**, 1950-1960.

Song, Q.J., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis, J., Gill, B.S. *et al*. (2005) Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.* **110**, 550-560.

Sooriyapathirana, S.D.S.S., Ranaweera, L.T., Perera, H.A.C.R., Weebadde, C.K., Finn, C.E., Bassil, N.V. and Hancock, J.F. (2019) Using SNP/INDEL diversity patterns to identify a core group of genotypes from FVC11, a superior hybrid family of *Fragaria virginiana Miller* and F. *chiloensis* (L.) *Miller*. *Genetic Resources and Crop Evolution*, **66,** 1691-1698.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Of Mol. Biol.* **98**, 503-517.

Steen, I. (1998) Phosphorus availability in the 21st century: management of a non-renewable resource. *Phosphorus Potassium*, **217,** 25-31.

Swain, D.K., Herath, S., Pathirane, A. and Mittra, B.N. (2005) Rainfed lowland and flood-prone rice: a critical review on ecology and management technology for improving the productivity in Asia. In *Proceeding of the international Symposium on Role of Water Sciences in Transboundary River Basin Management*, Thailand.

Szoke, M., Sasvari-Szekely, M. and Guttman, A. (1999) Ultra-thin-layer agarose gel electrophoresis: I. Effect of the gel concentration and temperature on the separation of DNA fragments. *J. Chromatogr.* **A830**, 465-471.

Tahir, R.M.A., Afzal, M., Sarwar, G. and Noorka, I.R. (2019) Smart nutrition management of rice crop under climate change environment. In *Protecting Rice Grains in the Post-Genomic Era*. IntechOpen.

Tanksley, S.D. and Nelson, J.C. (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* **92**, 191-203.

Tao, F., Yokozawa, M., Hayashi, Y. and Lin, E. (2003) Future climate change, the agricultural water cycle, and agricultural production in China. *Agric. Ecosyst. Environ.* **95**, 203-215.

Tautz, D. (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic acids research*, **17**, 6463-6471.

Thiele, H. and Nürnberg, P. (2004) HaploPainter: a tool for drawing pedigrees with complex haplotypes. *Bioinformatics*, **21**, 1730-1732.

Thompson, E.A. (2013) Identity by descent: variation in meiosis, across genomes, and in populations. *Genetics*, **194**, 301-326.

Thomson, M.J., Ismail, A.M., McCouch, S.R. and Mackill, D.J. (2009) Marker assisted breeding. In *Abiotic Stress Adaptation in Plants*, pp 451-469. Dordrecht: Springer.

Tuteja, N. and Gill, S.S. (2016) *Abiotic stress response in plants*. USA: John Wiley and Sons.

USDA (2015) USDA roadmap for plant breeding March 11, 2015: final report. Office of the chief scientist research, education, and economics mission area. United States department of agriculture. Available on-line at : http://www.usda.gov/wps/portal/usda/ usdahome?navid=OCS.

USDA-ARS (2008) Research databases bibliography on salt tolerance. *George E. Brown, Jr. Salinity Lab.* US Department of Agriculture, Agric. Res. Serv. Riverside, CA.

Van Berloo, R. and Hutten, R.C.B. (2005) Peditree: pedigree database analysis and visualization for breeding and science. *Journal of Heredity*, **96**, 465-468.

Van de Weg, W.E., Voorrips, R.E., Finkers, R., Kodde, L.P., Jansen, J. and Bink, M.C.A.M. (2004) Pedigree genotyping: a new pedigree-based approach of QTL identification and allele mining. *Acta Hort.* **663**, 45-50.

Van Dijk, T., Noordijk, Y., Dubos, T., Bink, M.C., Meulenbroek, B.J., Visser, R.G. and van de Weg, E. (2012) Microsatellite allele dose and configuration establishment (MADCE): an integrated approach for genetic studies in allopolyploids. *BMC Plant Biology*, **12,** 25.

Van Nguyen, N. and Ferrero, A. (2006) Meeting the challenges of global rice production. *Paddy Water Environ.* **4,**1–9.

Vandamme, E., Rose, T., Saito, K., Jeong, K. and Wissuwa, M. (2016) Integration of P acquisition efficiency, P utilization efficiency and low grain P concentrations into P-efficient rice genotypes for specific target environments. *Nutr. Cycl. Agroecosyst.* **104,** 413-427.

Vanderzande, S., Howard, N.P., Cai, L., Da Silva Linge, C., Antanaviciute, L., Bink, M.C.A.M., Johannes, W., *et al.* (2019) High-quality, genome-wide SNP genotypic data for pedigreed germplasm of the diploid outbreeding species apple, peach, and sweet cherry through a common workflow. *PLoS One*, **14**, e0210928.

Varshney, R.K., Nayak, S.N., May, G.D. and Jackson, S.A. (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology*, **27**, 522-530.

Velasquez, M. and Hester, P.T. (2013) An analysis of multi-criteria decision-making methods. *Int. J. Oper. Res.* **10**, 56-66.

Visscher, P.M., Haley, C.S. and Thompson, R. (1996) Marker-assisted introgression in backcross breeding programs. *Genetics*, **144**, 1923-1932.

Voorrips, R.E. (2007) Pedimap: instruction manual. Netherlands: Plant Research International. Available on-line at: https://www.wur.nl/en/show/Pedimap.htm.

Voorrips, R.E., Bink, M.C. and Van de Weg, W.E. (2012) Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. *Journal of Heredity*, **103**, 903-907.

Walisinghe B.R., Gunaratne, L.H.P., Prasada, D.V.P. and Sarthaj, I.Z. (2010) Analysis of the consumer preferences for rice-based noodles and biscuits. *Annals of Sri Lanka Department of Agriculture*, **12**, 201-211.

Wang, D., Shi, J., Carlson, S.R., Cregan, P.B., Ward, R.W. and Diers, B.W. (2003) A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. *Crop sci.* **43,** 828-1832.

Wang, X., Liu, F., Tan, W., Li, W., Feng, X. and Sparks, D.L. (2013) Characteristics of phosphate adsorption-desorption onto ferrihydrite: comparison with well-crystalline Fe (hydr) oxides. *Soil Science*, **178**, 1-11.

Wang, Y., Xue, Y. and Li, J. (2005) Towards molecular breeding and improvement of rice in China. *Trends in Plant Science*, **10,** 610-614.

Watanabe, S., Hideshima, R., Xia, Z., Tsubokura, Y., Sato, S., Nakamoto, Y., Yamanaka, N. *et al*. (2009) Map-based cloning of the gene associated with the soybean maturity locus *E3*. *Genetics*, **182,** 1251-1262.

Weebadde, C., Crisosto, C., Fazio G., Gallardo, K., Main, D., Peace, C., Reighard, G., *et al.* (2010) Use of PediMap in RosBREED for visualizing genetic and phenotypic data across pedigrees in Rosaceous crops. *HortScience.* **45**, S55-S55.

Whitehead, S.R., Turcotte, M.M. and Poveda, K. (2017) Domestication impacts on plant–herbivore interactions: a meta-analysis. *Phil. Trans. R. Soc. B.* **372**, 20160034. doi: 10.1098/rstb.2016.0034.

Wijewardena, J.D.H. (2005) Improvement of plant nutrient management for better farmer livelihood, food security and environment in Sri Lanka. *Improving plant nutrient management for better farmer livelihoods. Food Security and Environmental Sustainability,* pp. 73-93. Beijing.

Wissuwa, M. and Ae, N. (2001) Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*, **120**, 43-48.

Wissuwa, M., Wegner, J., Ae, N. and Yano, M. (2002) Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. *Theor. Appl. Genet.* **105**, 890-897.

Wissuwa, M., Yano, M. and Ae, N. (1998) Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **97**, 777-783.

Xu, Y. (2010) *Molecular plant breeding*. Wallingford, United Kingdom: Cab International press.

Xu, Y. and Crouch, J.H. (2008) Marker-assisted selection in plant breeding: from publications to practice. *Crop sci.* **48**, 391-407.

Xu, Y., Lu, Y., Xie, C., Gao, S., Wan, J. and Prasanna, B.M. (2012) Whole-genome strategies for marker-assisted plant breeding. *Mol. Breeding*, **29**, 833-854.

Yan, C., Ding, Y., Wang, Q., Liu, Z., Li, G., Muhammad, I. and Wang, S. (2010) The impact of relative humidity, genotypes and fertilizer application rates on panicle, leaf temperature, fertility and seed setting of rice. *J. Agr. Sci.* **148**, 329–339.

Yang, G.P., Maroof, M.S., Xu, C.G., Zhang, Q. and Biyashev, R.M. (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Mol. Gen. Genet.* **245**, 187-194.

Yang, L., Li, R.B., Li, Y.R., Huang, F.K., Chen, Y.Z., Huang, S.S., Huang, L.F. *et al*. (2012) Genetic mapping of *bph20* (t) and *bph21* (t) loci conferring brown planthopper resistance to *Nilaparvata lugens* Stål in rice (*Oryza sativa* L.). *Euphytica*, **183**, 161-171.

Yin, X., Kroff, M. J. and Goudriann, J. (1996) Differential effects of day and night temperature on development to flowering in rice. *Ann. Bot.* **77,** 203–213.

Yoshida, S. (1978) Tropical Climate and its Influence on Rice. *IRRI Research Paper Series 20.* Los Baños, The Philippines: IRRI.

Yu, J., Jung, S., Cheng, C.H., Ficklin, S.P., Lee, T., Zheng, P., Jones, D. *et al*. (2013) CottonGen: a genomics, genetics and breeding database for cotton research. *Nucleic acids research*, **42**, D1229-D1236.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D. *et al*. (2006) Genetic basis of drought resistance at reproductive stage in rice : separation of drought tolerance from drought avoidance. *Genetics*, **172**, 1213-1228.

Zamir, D. (2001) Improving plant breeding with exotic genetic libraries. *Nat. Rev. Genet.* **2**, 983-989.

Zeng, L. H. and Shannon, M. C. (2000) Salinity effects on seedling growth and yield components of rice. *Crop Sci*. **40,** 996–1003.

Zhu, T., Liang, C., Meng, Z., Sun, G., Meng, Z., Guo, S., and Zhang, R. (2017) CottonFGD: an integrated functional genomics database for cotton. *BMC Plant Biol.* **17**, 101.

APPENDIX I

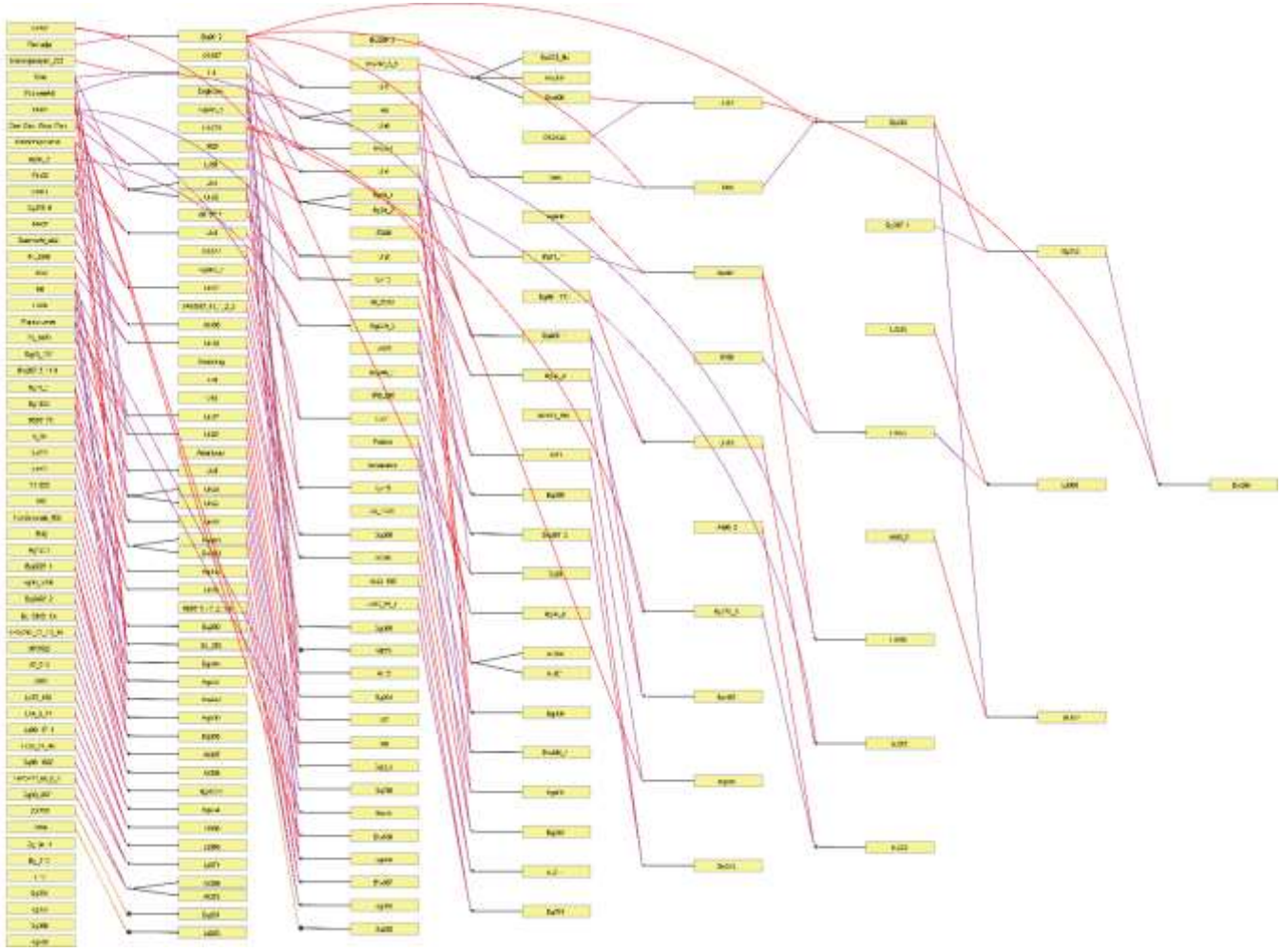


Figure S3.1 Visualization of the entire pedigree of the rice cultivars in the rice breeding germplasm of Sri Lanka. Female and male parentages are indicated by red and purple lines, respectively. The symbol '×' indicates the cross between two parents, and '×' inside the circle represents selfing (http://bit.do/PedimapFig1).

APPENDIX II

Table S3.1 Varietal data (http://bit.do/PedimapS1)

Table S3.2 Marker data (http://bit.do/PedimapS2)

Table S3.3 Pedimap input data file (http://bit.do/PedimapS3)