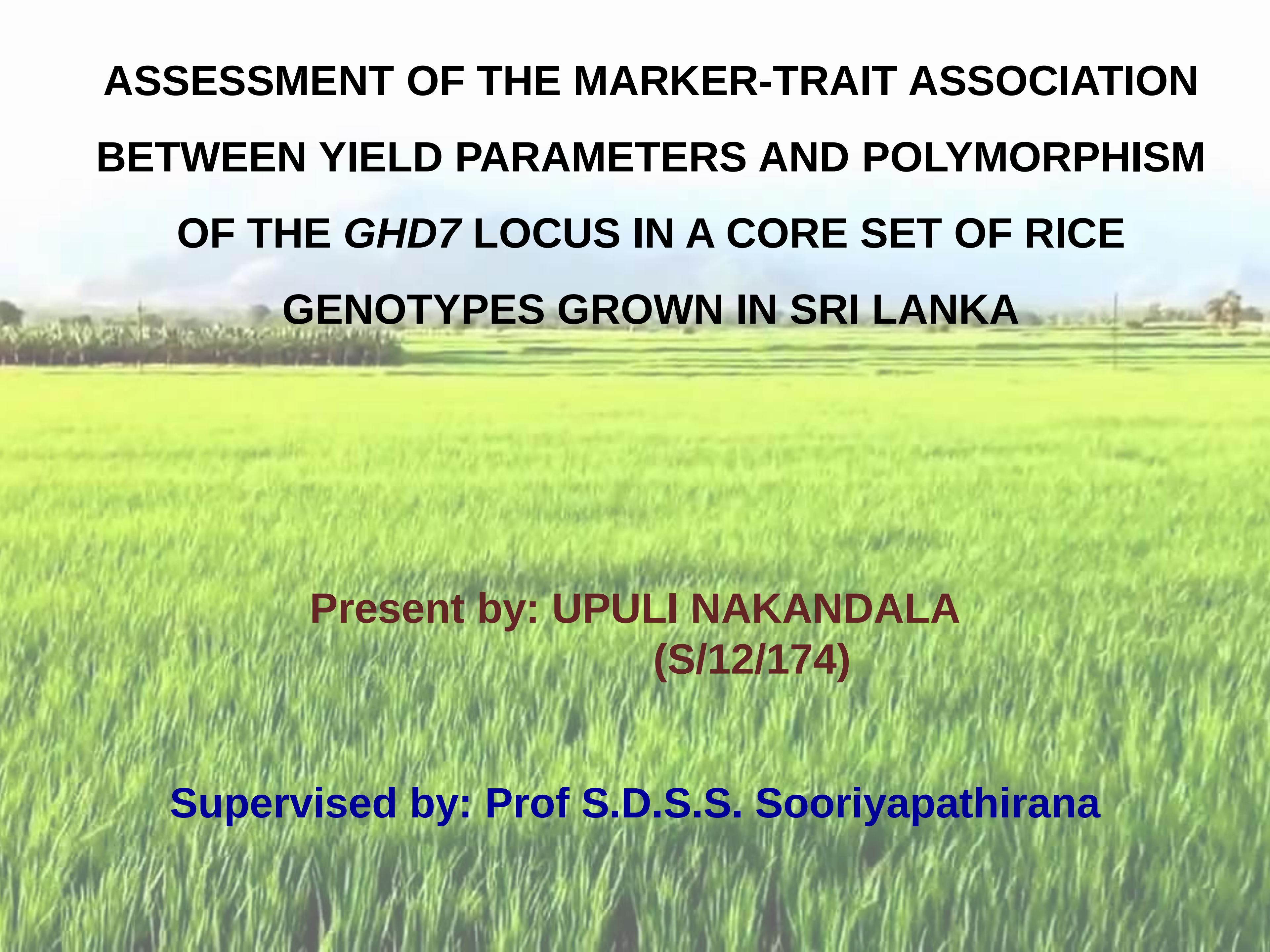
ASSESSMENT OF THE MARKER-TRAIT ASSOCIATION BETWEEN YIELD PARAMETERS AND POLYMORPHISM OF THE *GHD7* LOCUS IN A CORE SET OF RICE GENOTYPES GROWN IN SRI LANKA



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INTRODUCTION

- Rice is the staple food crop for nearly half of the world’s population

- 1.7 billion increase in the global population would be expected by 2025

- An increase in rice production would be required

- Genotype-based selection is much popular due to its high efficiency in plant breeding (Collard and Mackill, 2008)

- The concept of molecular markers and their mapping in relevant linkage groups is a requisite in molecular breeding (Jones *et al*., 1997)

Rice yield

- Rice yield is a complex agronomic trait (Yan *et al*., 2011)
    - Genetic constitution
    - Environment
- Grain yield, plant height and heading date are 3 important traits

Ghd7 QTL

- A pleiotropic QTL which regulates the above three traits
- It is positioned within the chromosome 7 (Xing *et al*., 2014)
- It encodes a CCT (CO, CO-LIKE AND TIMING OF CAB1) domain protein (Xue *et al*., 2008)

Why is this study important?

- *Ghd7* QTL has been cloned and sequenced in several other rice germplasms belonging to indica and japonica varieties (Xing *et al*., 2014)
- None of the studies have been conducted on *Ghd7* for Sri Lankan rice germplasm
- This study focuses on improving the yield potential in Sri Lankan rice germplasm using DNA markers linked to the *Ghd7* QTL

OBJECTIVES

1. Screening of the 12 rice genotypes (belonging to high, moderate, low yielding) for vegetative, flowering and harvesting measurements in both the *Maha* and *Yala* season

2. To detect the genetic polymorphism of *Ghd7* locus among 12 rice genotypes using 12 DNA markers linked to *Ghd7* QTL

3. To study of marker-trait association

METHODOLOGY

Morphometric analysis

PLANT CULTIVATION

12 rice genotypes were grown under greenhouse conditions in *Yala* and *Maha* seasons

High

- At 362, Bg 300, Bg 366, Bg 90-2

Moderate

- At 307, Bg 310, Bg 352, Bw 367

Low

- Bg 250, Bw 272-6b, *Pachchaperumal*, *Suwadhal*

Morphometric analysis

PLANT CULTIVATION

12 rice genotypes were grown under greenhouse conditions in *Yala* and *Maha* seasons

Morphological data collection

Vegetative, flowering and harvesting measurements were taken at regular time intervals

Table 1 Morphological parameters used for the phenotypic screening

Measurement type	Measurement
Vegetative	Plant height (PH), Culm length (CL), Number of tillers (NT), Leaf blade length (LBL) & width (LBW)
Flowering	Heading date, Flag leaf length (FLL), Flag leaf width (FLW)
Harvesting	Days to harvest, yield, Seed number (SN), Seed length (SL), Seed width , Seed weight , 100 seed weight (100 SW), 100 endosperm weight (100 EW), Endosperm length (EL), Endosperm width (EW)

Morphometric analysis

PLANT CULTIVATION

12 rice genotypes were grown under greenhouse conditions in *Yala* and *Maha* seasons

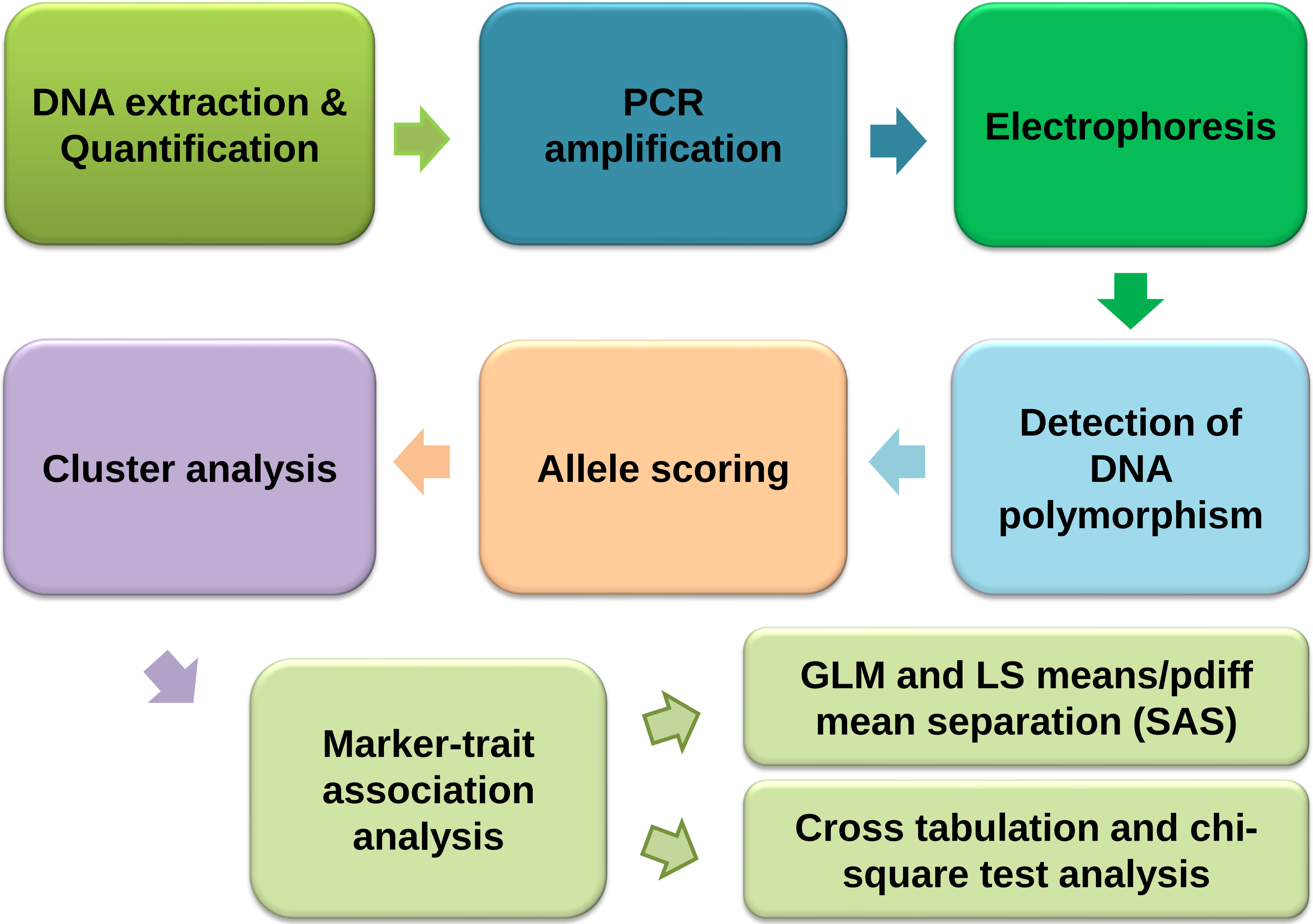
Morphological data collection

Vegetative, flowering and harvesting measurements were taken at regular time intervals

DATA ANALYSIS

Data were analyzed by General Linear Model (GLM) and LS means/pdiff mean separation procedures Using SAS 9.1.3

Genetic analysis



DNA extraction & Quantification

PCR amplification

Electrophoresis

Detection of DNA polymorphism

Allele scoring

Cluster analysis

Marker-trait association analysis

GLM and LS means/pdiff mean separation (SAS)

Cross tabulation and chi-square test analysis

Table 2 DNA markers used for the phenotypic screening

DNA marker	Annealing T (°C)	Reference
K20	55	Chin *et al*., (2010)
Seq1-2	53	Lu *et al*., (2012)
Seq7-8	54	
RM1135	58	Gramene QTL database, (2017)
RM5499	57	
RM5346	56	
RM5436	57	
G7rq	59	Xue *et al*., (2008)
Hd3a	56	
LHY	56	
PRR	58	
GI	57	

RESULTS
&
DISCUSSION

Morphometric analysis

- **PH**
  Significantly different
  Pachchaperumal (*Maha*)
  Suwadhal (*Yala*) ($P<0.05$)

- Newly improved varieties have been developed with a better resistance to lodging (IRRI, 1983)

- The results are corroborated with Saito *et al*., (2006)

- **NT & LBW**
  Not significantly different
  ($P<0.05$)

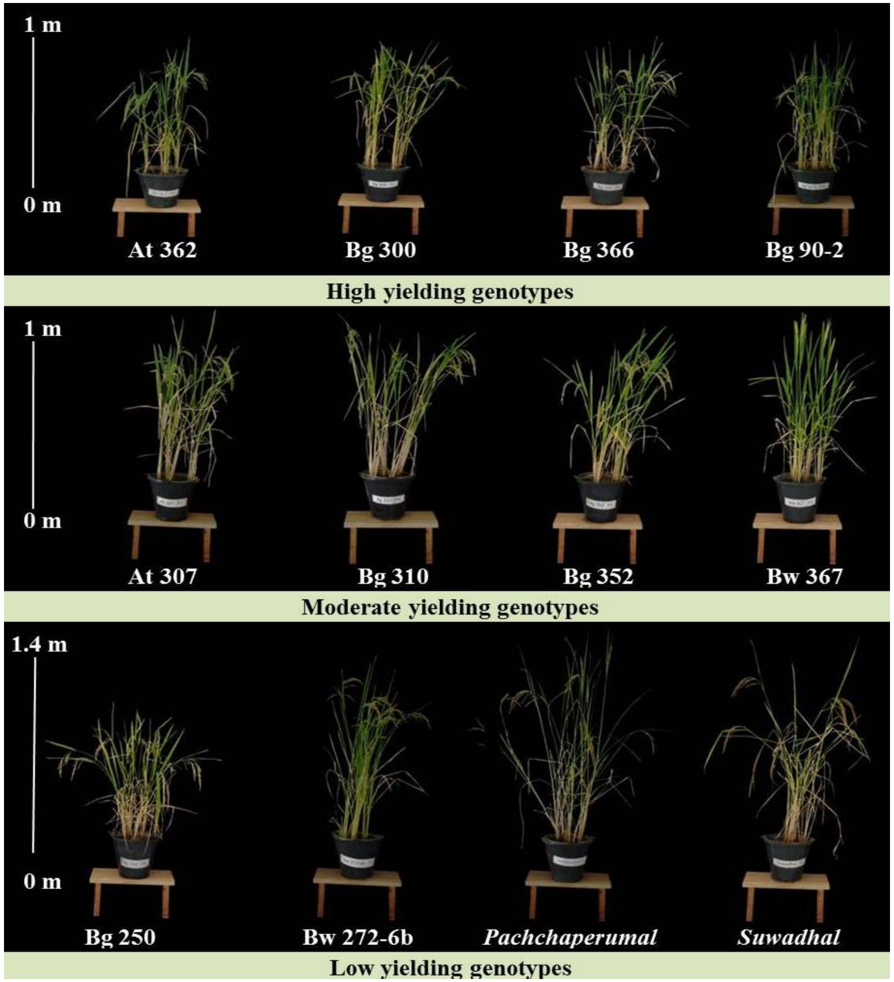


Figure1 Morphological appearances of rice genotypes at the flowering stage in *Maha* season under greenhouse conditions

Morphometric analysis

- **Grain yield**

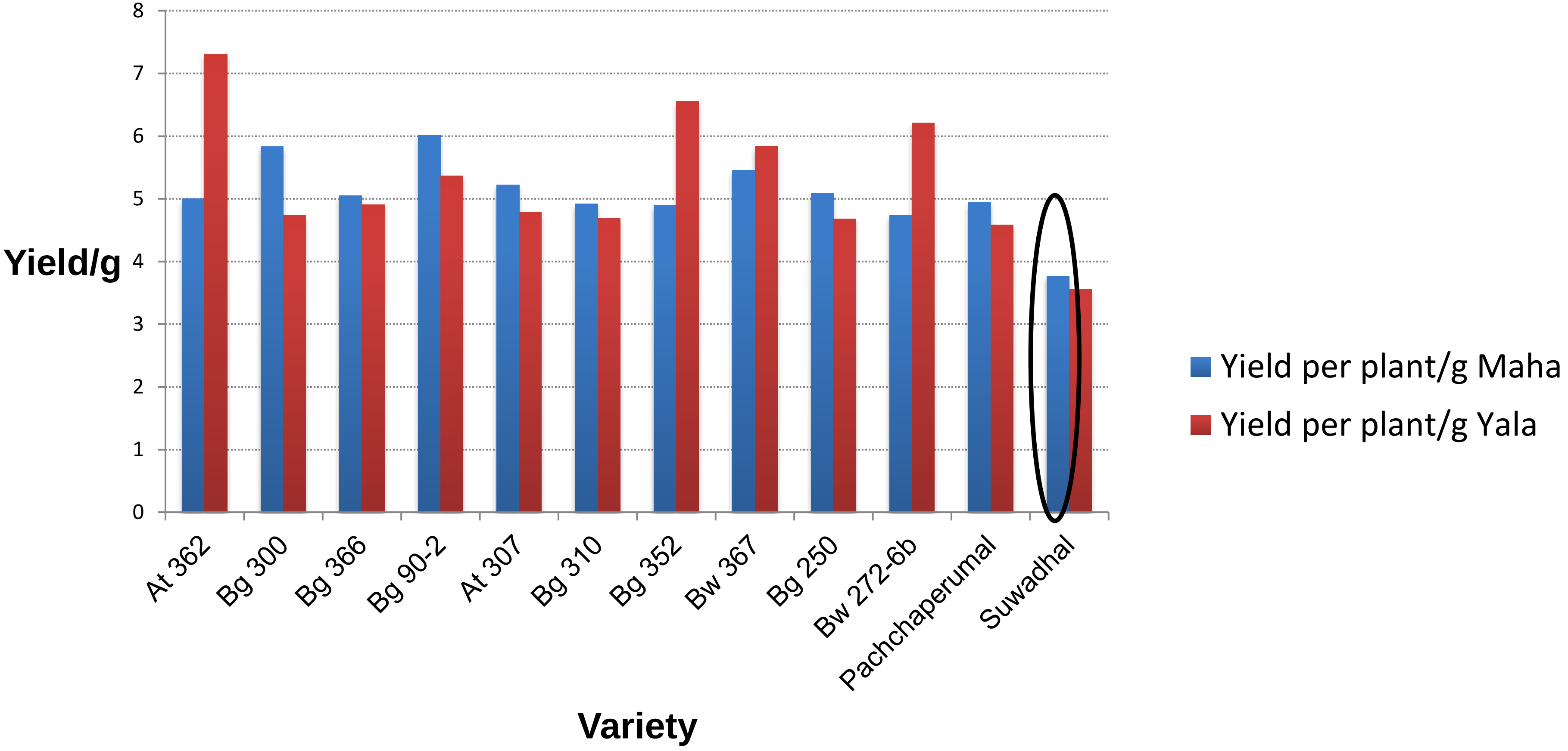


Figure 2 Variation of the grain yield of rice genotypes in both *Maha* and *Yala* season

- *Maha*: *Suwadhal* recorded a low yield
- *Yala* : Not significantly different (*P*<0.05)

Morphometric analysis

- All the harvested measurements except **SN** & **yield** were significantly different among the rice genotypes ($P<0.05$)

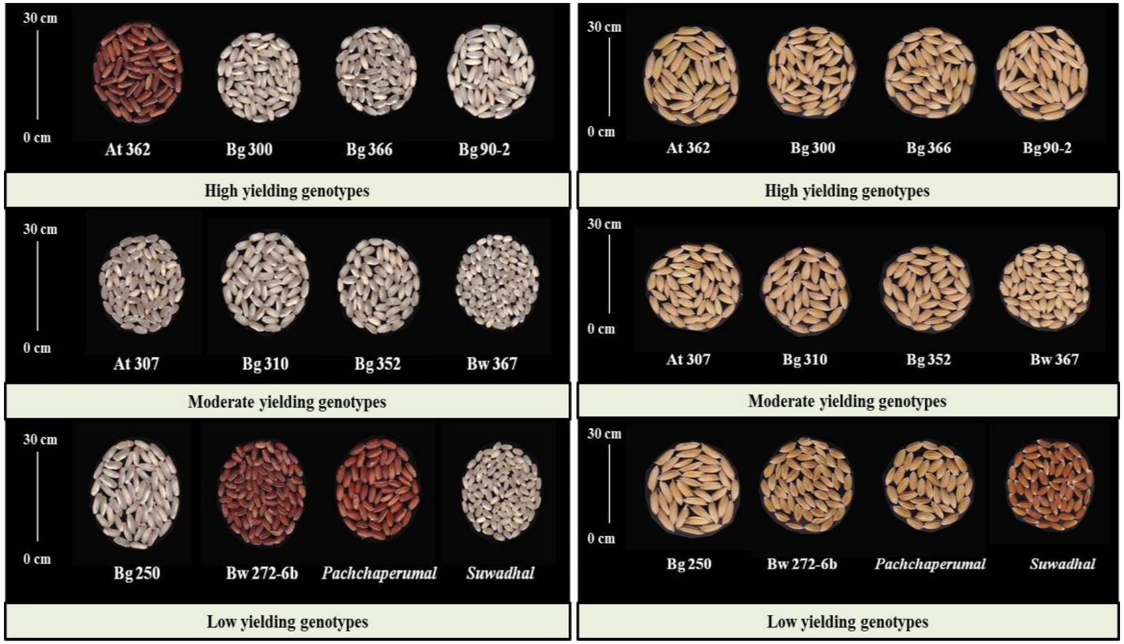


Figure 3 Morphological variance of rice seeds (left) and endosperms (right) of 12 rice genotypes in *Yala* season under greenhouse conditions

- Grain yield with respect to agronomical traits (number of grains, 100 GW, PH, NT, days to flowering, days to mature) have been reported in other studies (Kalyan *et al*., 2017)

Morphometric analysis

- The effect of seasonal changes on crop yield was evaluated in this study
- Similar studies have previously been conducted in Sri Lanka (Weerakoon *et al*., 2011; De costa *et al*., 2003) and in worldwide (Welch *et al*., 2010; Zhang *et al*., 2010)
- Different rice genotypes can be categorized based on the phenotypic evaluation
- Based on the results, the morphological parameters except the yield, SN, LBW and NT could be used to distinguish among the genotypes

Morphometric analysis

- Morphological variability would not be desirable in selecting the genotypes with high yield
  - The yield is highly determined by environmental factors

- The applicability of phenotypic evaluation is limited due to,
  - less accuracy
  - high cost
  - space and time consuming nature

Genetic analysis

1. DNA polymorphism of *Ghd7* locus

2. Haplotype detection of *Ghd7* locus

3. Cluster analysis

4. Marker-trait association analysis

   - Association between DNA markers and yield traits

   - Association between DNA markers and yield class

1. DNA polymorphism of *Ghd7* locus

- *RM5346*, *RM5436*, *Seq1-2*, *Seq7-8*, *G7rq* and *GI* were detected with polymorphism

- The highest polymorphism
  - *GI*
  - *Seq1-2*

- 20 alleles were detected with 1-3 alleles per locus

- The results reveal a genetic diversity across the 12 rice genotypes

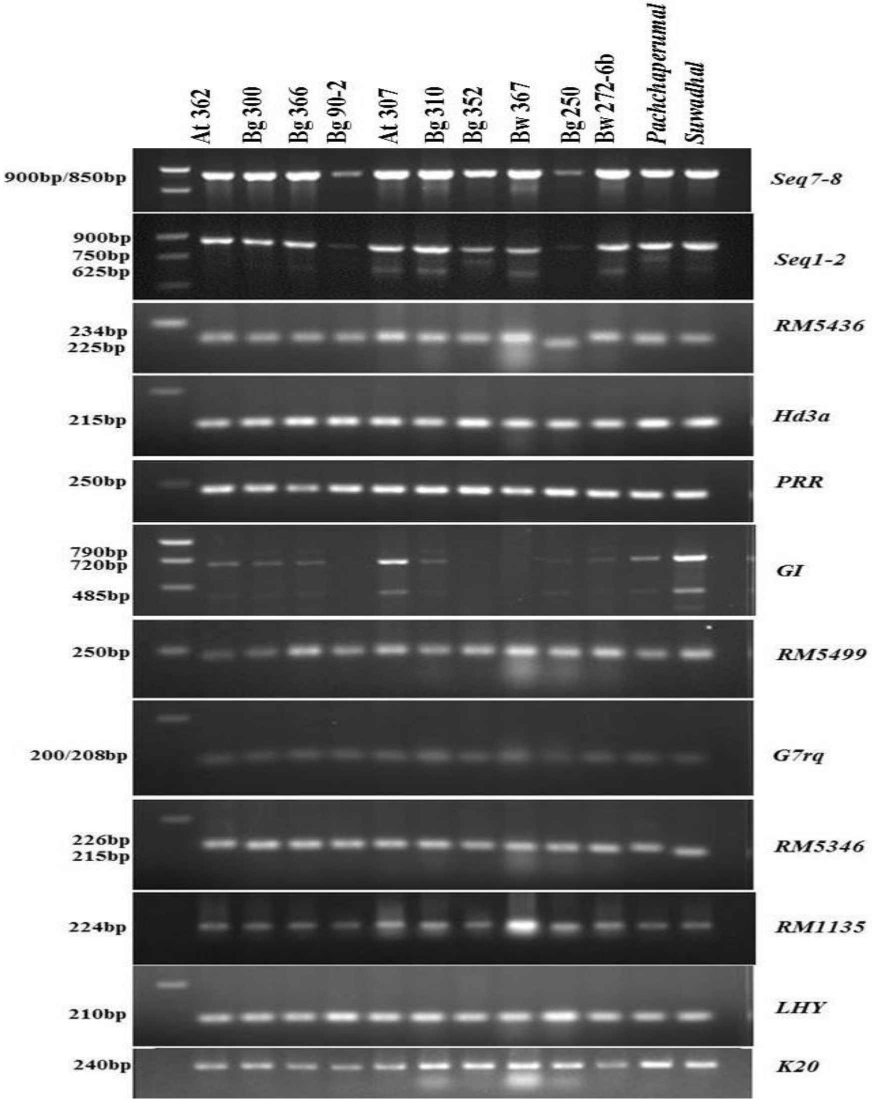


Figure 4 The DNA polymorphism of 12 markers linked to *Ghd7* QTL in 12 rice genotypes

2. Haplotype detection of *Ghd7* locus

- The haplotypes were detected using the haplotype of Bg 90-2 using as the reference haplotype

- 10 marker haplotypes were detected for 12 rice genotypes

- Bg 366, Bg 310 and Bw 272-6b were belonged to the same haplotype

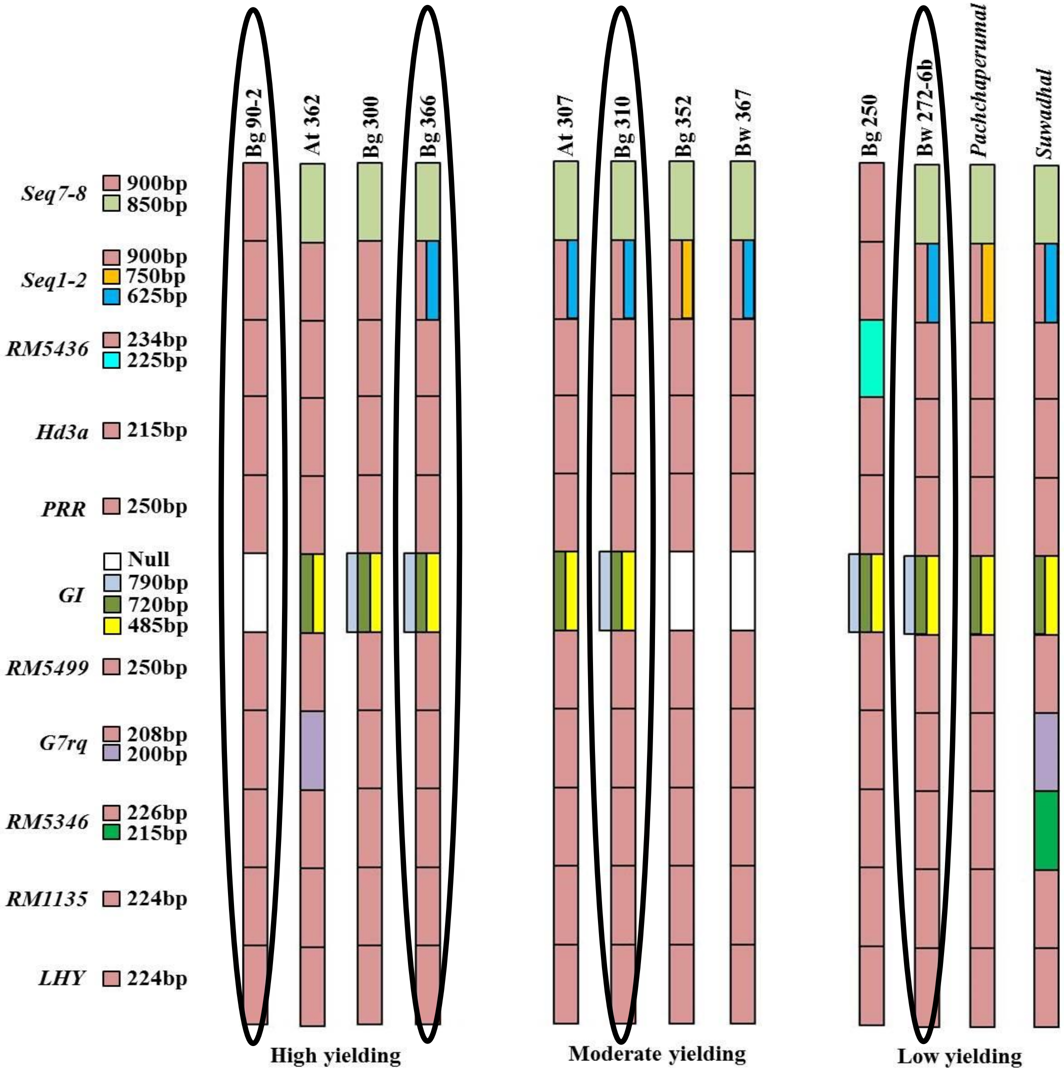


Figure 5 *Ghd7* linked marker haplotypes of 12 rice genotypes.

3. Cluster analysis

- 3 main clusters could be detected at 17% molecular similarity coefficient
- They were subdivided into 7 clusters at 80% molecular similarity coefficient
- Bg 366, Bg 310 and Bw 272-6b genotypes showed 100% similarity
  - ➢ Conserved allelic pattern of *RM5436*, *RM5346*, *Seq7-8*, *G7rq* and *GI* markers

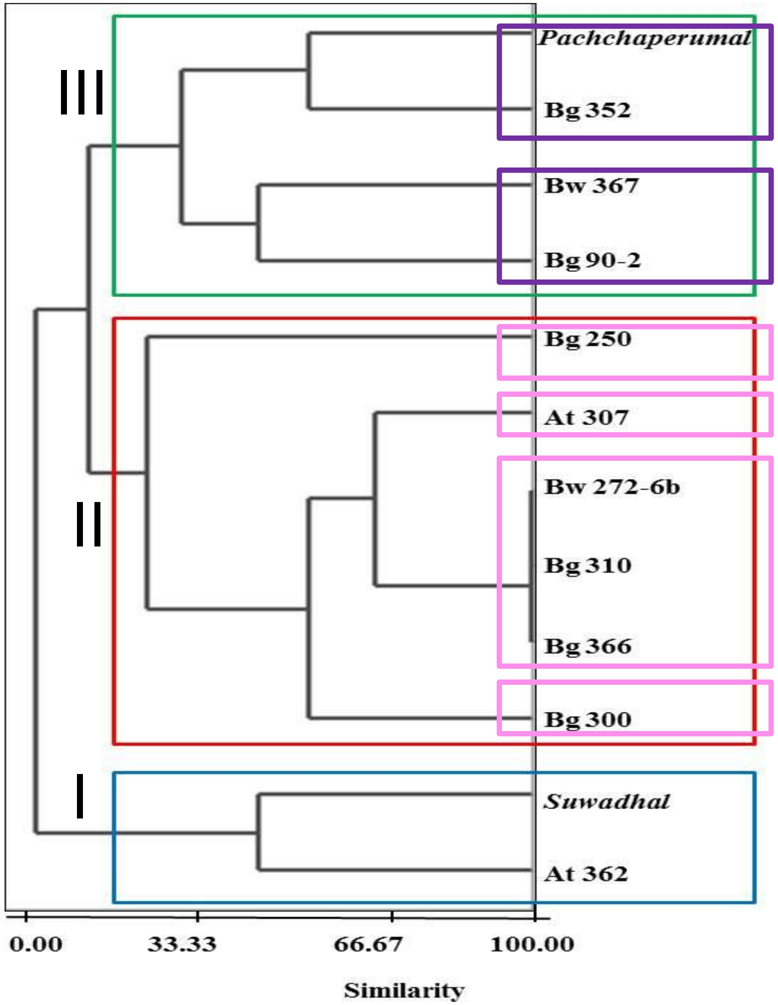


Figure 6 Dendrogram constructed for 12 rice genotypes using the algorithms of Complete Linkage and Euclidean distance in Minitab 17 (Minitab Inc.USA)

3. Cluster analysis

- *Pachchaperumal & Suwadhal* were grouped into 2 clusters

- Similar studies to evaluate the genetic variation using different SSR markers have been reported (Ming *et al*., 2010)

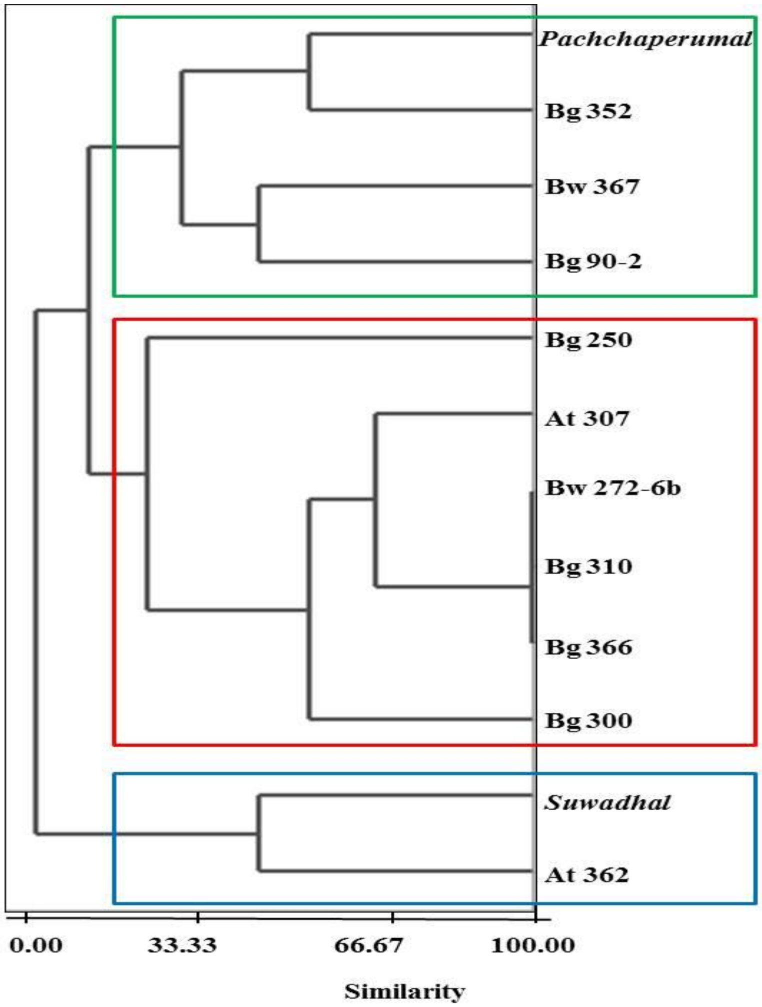


Figure 6 Dendrogram constructed for 12 rice genotypes using the algorithms of Complete Linkage and Euclidean distance in Minitab 17 (Minitab Inc.USA)

4. Marker-yield trait association analysis

Table 3 Marker-trait association for yield traits with DNA markers in two seasons

Marker	Allele (bp)	*Maha*	Number of Traits	*Yala*	Number of Traits
RM5436	234	S. Width	4	SN, S. weight, S. width, EW	**6**
	225	S. weight, SL, EL		SL, EL	
RM5346	226	Yield, S. weight, SL, S. width, EL, EW	**6**	Yield, S. weight, SL, S. width, EL, EW	**6**
Seq7-8	900	Yield, S. weight, **SL, EL**	**7**	**SL, EL**	5
	850	**SN, S. width, EW**		**SN, S. width, EW**	
Seq1-2	750	SN, S. weight, SL, EW	5	SN, S. width, EW	3
	625	S. Width		-	
G7rq	208	Yield, EL, S. width, EW	4	SL, S. width, EW	4
	200	-		S. Weight	
GI	790	S. weight, SL, S. width	4	-	1
	720	S. weight, SL, S. width		S. Weight	
	485	S. weight, SL, EL, S. width		S. Weight	

4. Marker-yield class association analysis

- None of the marker allele was significantly associated with the yield class ($P<0.05$)

- Cramer’s V-square revealed a much less strong relationship for all the associations (Value was closer to 0)

4. Marker-trait association analysis

- Marker assisted selection (MAS) is beneficial over the phenotypic selection:
  - Easiness in screening
  - Select the genotypes at the seedling stage
  - Distinguish between homozygotes & heterozygotes
  - Time & space saving (Acquaah, 2012)

- Selection of desirable genotypes for different agronomical traits based on molecular markers have been reported for many crop species (Zhou *et al*., 2003; Zhang *et al*., 2004; Liu *et al*., 2017)

4. Marker-trait association analysis

- The number of marker-trait associations are in contradiction to the previous studies (Borba *et al*., 2010)
- The presence of marker allele was coupled with higher trait values in most of the cases
- Increased states of 100 SW & 100 EW were coupled with the absence of 625bp allele of *Seq1-2* marker
- This result is inconsistent with the results of some studies (Chamikara *et al*., 2015)

CONCLUSIONS

- Phenotypic screening is not much effective in selecting the high yielding genotypes

- Genetic screening is more beneficial over the phenotypic selection

- The present study reflects a genetic polymorphism of *Ghd7* locus among the 12 rice genotypes

- All the six polymorphic markers could be used in MAS

- *Seq7-8* and *RM5346* would be more useful in MAS in future breeding programs

FUTURE DIRECTIONS

- The results facilitate marker-assisted backcrossing in future breeding programs

- The DNA markers could be used to detect the genetic diversity and similarity among rice genotypes

- The haplotype information would be useful for the development of novel varieties with specific allele combinations with the use of marker-assisted selection

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THANK YOU

Photo source: botany