IDENTIFICATION OF RICE (*Oryza sativa* L.) VARIETIES USING DUS DESCRIPTORS AND GENE-SPECIFIC MARKERS

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(GENETICS AND PLANT BREEDING)

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BY
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B.Sc. (Ag.)

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DECLARATION

I, **Ms. MONDEM BHARGAVI,** hereby declare that the thesis entitled **"IDENTIFICATION OF RICE (*Oryza sativa* L.) VARIETIES USING DUS DESCRIPTORS AND GENE-SPECIFIC MARKERS"** submitted to **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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CERTIFICATE

Ms. MONDEM BHARGAVI has satisfactorily prosecuted the course of research and that the thesis entitled **"IDENTIFICATION OF RICE (*Oryza sativa* L.) VARIETIES USING DUS DESCRIPTORS AND GENE-SPECIFIC MARKERS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any university.

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This is to certify that the thesis entitled **“IDENTIFICATION OF RICE (*Oryza sativa* L.) VARIETIES USING DUS DESCRIPTORS AND GENE-SPECIFIC MARKERS”** submitted in partial fulfilment of the requirements for the degree of **‘Master of Science in Agriculture’** of the Acharya N.G. Ranga Agricultural University, Lam, Guntur, is a record of the bonafide original research work carried out by **Ms. MONDEM BHARGAVI** under our guidance and supervision. The subject of the thesis has been approved by the student’s advisory committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

$\overline{X}$	: Grand mean
%	: per cent
<	: Less than
>	: Greater than
µl	: Microlitre
°C	: Degree centigrade
2-D	: 2 Dimensional
∞	: Infinity
ANOVA	: Analysis of Variance
CD	: Critical Difference
cm	: Centimeter
CV	: Coefficient of Variation
d.f	: Degrees of freedom
DNA	: Deoxyribose Nucleic Acid
dNTP	: Deoxyribose Nucleotide Tri Phosphate
DUS	: Distinctness, Uniformity, Stability
EDTA	: Ethylene Diamine Tetraacetic Acid
et al.,	: and other people
EtBr	: Ethidium Bromide
etc.	: And so forth
g	: Gram
GA	: Genetic Advance
GAM	: Genetic Advance as per cent of Mean
GCV	: Genotypic Coefficient of Variation
h^2 (bs)	: Heritability in broad sense
HCl	: Hydrogen Chloride
hr	: Hour
HRM	: High Resolution Melting
m	: Metre
M	: Molar

mg	:	Milligrams
min.	:	Minute
ml	:	Millilitre
mM	:	Milli molar
mm	:	Millimeter
NaCl	:	Sodium Chloride
ng	:	Nano grams
nm	:	Nanometer
NTSYS	:	Numerical Taxonomy and Multivariate Analysis System
OD	:	Optical Density
PBRs	:	Plant Breeders’ Rights
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic Coefficient of Variation
PIC	:	Polymorphism Information Content
pM	:	Pico molar
ppm	:	Parts Per Million
PPV and FR	:	Protection of Plant Varieties and Farmers’ Rights
psi	:	Pounds per square inch
PVP	:	Plant Variety Protection
PVP	:	Poly Vinyl Pyrrolidone
QR	:	Quick Response
QTL	:	Quantitative Trait Loci
rpm	:	Revolutions Per Minute
RT-PCR		Real Time- Polymerase Chain Reaction
sec.	:	Second
UPGMA	:	Unweighted Pair Group Method with Arithematic Mean
UPOV	:	International Union for the Protection of New Varieties of Plants
v/v	:	Volume/volume
via	:	Through
viz.,	:	Namely
w/v	:	Weight/volume

ABSTRACT

Author of the thesis	:	**M. BHARGAVI**
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The identification of varieties is a vital step during the breeding and registration process, seed production, trade and inspection in crops. The rapid identification and characterization of cultivars or varieties would therefore, provide valuable information for their introduction and genetic improvement. Plant cultivar or variety identification is a critical field as an ever increasing number of varieties are developed every year and the need to identify them unambiguously also increases. Hence, in the present study, an attempt was made to identify 52 rice varieties using 22 DUS descriptors and 25 gene-specific markers.

Out of 22 morphological DUS descriptors studied, four were monomorphic, nine were dimorphic and the remaining nine were polymorphic. Of these 18 traits (both dimorphic and polymorphic), only 10 were essential traits. The descriptor notes of these 10 traits were used for generating DUS 2-D barcode for identification of rice varieties. The 2-D barcode developed using these 10 DUS descriptors was able to distinguish 26 varieties and the remaining 26 genotypes were unable to be distinguished.

The analysis of variance demonstrated significant differences among 52 genotypes for all the traits studied. High PCV and GCV were recorded for the traits *viz.*, panicle number per plant, weight of 1000 fully developed grains and grain yield plant^{-1} indicating that large amount of variation was present among the genotypes for these characters. Whereas, low PCV and GCV were recorded for the traits *viz.*, grain length, decorticated grain length and length/ breadth ratio indicating that the variability for these characters among the genotypes was

meagre. The variability studies of the present investigation indicated that the estimates of PCV were slightly higher than the corresponding GCV for all the characters indicating that the characters were less influenced by the environment.

High heritability coupled with high genetic advance as per cent of mean was recorded for time of heading, stem length, panicle number per plant, weight of 1000 fully developed grains, decorticated grain width and grain yield plant^{-1} indicating that these traits were mostly under the control of additive gene action and direct selection of these traits would be effective for crop improvement. While high heritability coupled with moderate genetic advance as per cent of mean was observed for the traits *viz*., panicle length of main axis, time of maturity, grain length, grain width, decorticated grain length and length/breadth ratio.

Out of 25 gene-specific markers used for screening, 18 were polymorphic. The number of alleles of the polymorphic markers ranged from two to three with an average of 2.05 alleles per locus. The PIC values of these gene-specific markers ranged from 0.037 to 0.369 with an average of 0.212. Varietal specific fingerprints were generated using these 18 markers. The order of the alleles of these gene-specific markers were used for generating the fingerprints for all the 52 rice varieties. These fingerprints were able to distinguish 50 genotypes. BPT2295 and Jaya were the two varieties possessing similar DNA fingerprints (allele codes) and could not be distinguished using these allele codes.

Using DUS descriptors and gene-specific markers, it was demonstrated to distinguish 26 and 50 varieties respectively, when analysed separately. However, in combination of both DUS descriptors and gene-specific markers, it was possible to distinguish all the 52 rice varieties. These DUS and allele codes together were used to develop QR (Quick Response) codes using an online tool (available at www.barcode-generator.org) for their rapid identification.

The dendrogram developed by UPGMA using the polymorphism of gene-specific markers grouped all the 52 rice genotypes into seven clusters at 60 % similarity coefficient. Cluster I, II and III comprised of seven, 37 and four genotypes respectively. The genotypes *viz*., Varadhan, Kasturi, Udayagiri and Vandana formed into four distinct monogenic clusters (Cluster IV, V, VI and VII) indicating that they are genetically divergent from the remaining genotypes. Hierarchical clustering of the 52 genotypes based on morphological data using nearest neighbour methods based on squared Euclidean distance classified the genotypes into four major clusters. Cluster I comprised of 47 genotypes and Cluster II comprised of three genotypes and the remaining two genotypes, Vandana and Rasi formed into two distinct monogenic clusters, appearing to be the more diverse and may be useful as a source for variable characters for rice improvement.

In conclusion, in the present study, it was demonstrated that by the combination of DUS traits and molecular markers, it was possible to develop DNA fingerprints which were further converted into QR codes that can be applied for unambiguous identification of all rice varieties.

Chapter ~ I

Introduction

Chapter – I

INTRODUCTION

Rice (*Oryza sativa* L.) (2n = 24) is the most important cereal crop which serves as a staple food for over 60 per cent of the world's population (Singh and Singh, 2008). Approximately 90 per cent of rice grown on the planet is produced and consumed in Asia (Singh *et al.*, 2015). The cultivation of rice is more than any other crop in the world with cultivated area of 162.57 million hectares with production of 499.07 million metric tonnes in 2018-19 (USDA, 2020). India is the second largest producer of rice after China occupying 43.8 million hectare area with an average grain yield of 3.99 metric tonnes per hectare while the total production is 116.48 million metric tonnes in 2018-19 (USDA, 2020).

India, being the part of centre of origin, rice is bestowed with wide range of germplasm which encompasses landraces, obsolete cultivars, wild or weedy species, improved varieties and so forth, which led to the tremendous genetic diversity. The genus *Oryza* has 24 species, comprising of 22 wild species and two cultivated species *i.e.*, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). *Oryza sativa* has been further classified into five sub-species *viz.*, *japonica* (low amylose content, sticky, short grained and adapted to temperate regions), *indica* (high amylose content, non-sticky, long-grained and adapted to the tropics), the intermediate *javanica* is designated as *tropical japonica*, *aus* (deep water rice) and *aromatic* (Basmati, Jasmine, Joha etc.) (Garris *et al.*, 2005).

The varieties released from India have shown profound impact in the global rice production. After the initiation of All India Coordinated Rice Improvement Programme (AICRIP), the development and release of rice varieties have been increased tremendously; as of now more than 1200 varieties have been released for various agro-ecosystems in India. Development of new varieties is an expensive task which involves more skill, infrastructure and genetic resources and above all it is a time-consuming process which takes around ten years to release a variety through pedigree breeding, the most commonly used method of variety development. After development of a new variety by painstaking research by the breeders, it is necessary to register newly bred varieties. The protection of ownership of new varieties is of paramount importance to prevent their unauthorized utilization. There are different ways to protect a variety and provide exclusive rights to the breeder. Among them Plant Variety Protection (PVP) through International Union for the Protection of New Varieties of Plants (UPOV) provides Intellectual Property Rights (IPR) to Plant Breeders' Rights (PBR). According to UPOV's PVP system, new variety must satisfy three criteria to be eligible for protection. The first criterion is that the new variety must be Distinct (D) from any other Common Knowledge Varieties (CKVs) in at least one morphological trait. The second criterion is that the variety should be sufficiently Uniform (U) in relevant characteristics depending on reproduction system of the species and finally the distinctive traits should show Stable (S) expression after two independently growing cycles. Thus, the DUS test provides foundation for plant variety protection and to identify a new variety from already released varieties (also called as reference collection). In India, the plant variety protection is resolved through an authorized *sui generis* enactment of Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV & FRA), though it is through UPOV (International Union for the Protection of New Varieties of Plants) at international level.

Under PPV and FR Act, DUS testing is performed with morpho-physiological descriptors (Anonymous, 2007) which has been capable for distinguishing genetic material in different crops. However, this technique has now become less reliable for separation and recognition of commercial varieties (Rahman *et al*., 2009) as there might be minimal morphological variation among cultivars with related pedigrees which often creates ambiguity in identification. Besides, with ever increasing number of varieties every year, conducting DUS test is becoming increasingly expensive. In addition, majority of the characters utilized are multigenic or quantitative and their expression is altered by several environmental factors (Kumar *et al*., 2007) which ultimately makes DUS testing a tedious and most expensive procedure which demands large area of land and skilled manpower to formulate subjective decisions. These circumstances prompt us to find quick, stable and cost-effective strategies to assist DUS testing.

The wide accessibility and cost-effectiveness of DNA-based markers in recent years makes them appealing alternatives to investigate their utilization to complement the existing morphological based identification as they are free from environmental impacts (Noli *et al*., 2008) and they could be applied at any phase of plant development. Even UPOV also endorsed the utilization of molecular markers as the standard method of varietal identification in crops. The potentiality of molecular markers has been demonstrated in several aspects such as genetic purity testing during hybrid seed production, detection and quantification of the adulteration in Basmati rice (Vemireddy *et al*., 2007) etc. Additionally, Vemireddy *et al.* (2015) developed varietal specific allele profiles for major Indian rice varieties employing hypervariable microsatellite markers. Several molecular markers like SSRs (Simple Sequence Repeats) (Singh *et al*., 2004, Sarao *et al*., 2009 and Pourabed *et al*., 2015), EST-SSRs (Expressed Sequence Tag-derived Simple Sequence Repeats) (Bonow *et al*., 2009), RAPDs (Randomly Amplified Polymorphic DNAs) and ISSRs (Inter Simple Sequence Repeats) (Shukla *et al*., 2011) have been tested to complement

DUS testing in rice and they were found to be able to distinguish varieties unambiguously and establish level of uniformity and stability. However, these markers are neutral and found largely unlinked to any of the morphological traits. The usage of gene-specific markers is more reliable as they are firmly linked to the genes controlling the phenotypic expression. Although there are several QTLs and cloned genes identified in rice, very few gene-specific markers are available to assist DUS descriptors. As per the national guidelines for the conduct of DUS tests in rice, there are 62 DUS related traits (Rani *et al.*, 2006). Of them, gene-specific markers were identified for very few traits, especially grain related traits (Zhao *et al.*, 2015b). These merits facilitate to create exclusive varietal specific profiles or fingerprints from the molecular marker data (Rahman *et al.*, 2009; 2010). This cultivar-specific fingerprints could be an amazing asset to sustain the rights approved to the breeders.

Information on the genetic diversity within and among closely related crop varieties is fundamental for a rational use of genetic resources for progressive crop improvement. Assessment of the genetic diversity in plant varieties is basic for successful use of plant genetic resources in crop improvement. Morphological characters, both qualitative and quantitative have been utilized for evaluating diversity. Because of stage specific expression of characters and impact of environment, morphological diversity estimates are less reliable. Protein or isozyme marker studies are also affected by environment and uncover low polymorphism. Besides, now and then there might be minimal morphological diversity among cultivars with related pedigrees. Presently, the rapid advances in biotechnology methods allows easy examination of countless loci disseminated all through the genome of plants. Utilization of molecular markers in addition to morphological characters is viewed as most viable option for assessing the genetic diversity and also for varietal identification.

With this background, the present investigation entitled, “Identification of rice (*Oryza sativa* L.) varieties using DUS descriptors and gene-specific markers” was undertaken with the following objectives.

1. Study of morphological DUS descriptors in released rice varieties.
2. Development of varietal-specific DNA fingerprints using gene-specific markers.
3. Genetic diversity analysis using both DUS descriptors and gene-specific markers.

Chapter ~ II

Review of Literature

Chapter – II

REVIEW OF LITERATURE

In the present investigation, an attempt was made to identify the rice varieties employing DUS descriptors and molecular markers. Available literature, as of now, in line with the objectives of the present investigation is reviewed in this chapter.

2.1 NEED FOR THE IDENTIFICATION OF RICE VARIETIES

The identification of varieties is a vital step during the breeding and registration process, seed production, trade and inspection in crops. The rapid identification and characterization of cultivars or varieties would therefore, provide valuable information for their introduction and genetic improvement. Plant cultivar or variety identification is a critical field as more and more varieties or cultivars are developed, the need to identify them unambiguously also increases.

2.2 METHODS OF IDENTIFICATION OF RICE VARIETIES

The rice varieties are identified employing several conventional and advanced methods. Previously, physical appearance or cooking quality traits were used for cultivar identification. However, the DUS descriptors and molecular markers are being used widely to distinguish rice genotypes. Recently, rice varieties are being identified using artificial intelligence-based neural network and machine learning classifiers. Further, spectroscopy was also used for cultivar identification. The important strides in this aspect are described hereunder.

2.2.1 Physical, Cooking and Protein Characteristics of the Grain

Thind and Sogi (2005) used physical, cooking and protein characteristics of the grain to establish the criteria for identification of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice cultivars.

2.2.2 DUS Testing

India has a rich and wide range of genetic wealth of rice. It has been estimated from various surveys that nearly 50,000 accessions of rice are being grown in the country. A large number of new varieties are being released every year, but not all of these have been widely accepted, as many of them have been considered to be merely forms of acknowledged varieties. In order to release a new variety commercially, it is essential to register it, which largely relies on the DUS (Distinctness, Uniformity, and Stability) test (Anonymous, 2001). Therefore, DUS test has been established to be the foundation of plant variety protection and also to identify a new variety from reference collection (Sharma and Singh, 2018).

A plant variety qualifies for protection when it meets the following essential criteria.

(i) **Distinctness** - means that a variety is clearly distinguishable by at least one essential characteristic from any other variety, whose existence is a matter of common knowledge. An essential characteristic is a heritable trait which is determined by one or more genes or the heritable determinants that contribute to the principle features, performance or value of plant variety (Rani *et al.*, 2006).

(ii) **Uniformity-** implies that the variety should be sufficiently uniform in its essential characteristics if subjected to variation that may be expected from the particular features of its propagation (Rani *et al.*, 2006).

(iii) **Stability-** A variety is deemed to be stable when its characteristics remain unchanged after repeated propagation (Rani *et al.*, 2006).

(iv) **Novelty** - According to section 15 of the Act (PPV and FRA), "Novelty" means commercial novelty *i.e.,* the propagating or harvested material of the variety should not have been sold or otherwise disposed off earlier than one year in India, from the date of filing of application of registration or outside India, not earlier than six years in the case of trees or wines or not earlier than four years in any other case (new in commercial sense) and a unique and unambiguous denomination (name of the new variety).

Being a signatory to the General Agreement on Trade and Tariffs (GAAT), Government of India has enacted its *sui generis* system, Protection of Plant Varieties and Farmers' Right Act (PPV and FRA), 2001 for protection of plant varieties based on Distinctness, Uniformity and Stability (DUS) test, apart from novelty, as the existing UPOV models of plant variety protection was not suitable for Indian requirements.

The process of variety identification includes several steps such as identification of a variety, confirmation of the variety, distinctness of the variety from all other reference collections, purity of the variety and characterization of the variety which enumerates its full descriptors. The concept of distinctness, uniformity and stability are thus fundamental to the characterization of a variety as a unique creation. Registration is allowed for three types of plant varieties, new varieties bred by breeders, extant varieties and farmers' varieties subject to their fulfilling the conditions of Distinctness, Uniformity and Stability and Novelty in case of breeder's variety. The first step to implement our PPV and FR Act provisions is formulation of National Test Guidelines for conducting DUS tests. The new variety should pass legal examinations to be commercialized and receive the certificate for the Plant Breeders' Rights, a part of which consists of DUS tests according to morphological characteristics.

The new varieties will be subjected to DUS testing by PPV and FR Authority located in New Delhi. The PPV and FR Authority had developed and published in its journal, guidelines for DUS test for each crop (Anonymous, 2007) for the registration and protection of newly developed varieties from unauthorized utilization. The tests are envisaged to generate comprehensive data on the attributes of a variety so as to compare these with the varieties of common knowledge or similar existing varieties. These shall be based on multi-location field trials conducted for at least two crop seasons (Anonymous, 2007) at a minimum location, along with special chemical laboratory tests (Section 29, PPV and FR rules).

As per the 1991 Act of UPOV, protection granted for the new variety authorizes the breeder with the exclusive right to commercially exploit the variety by direct sale or by licensing to others for sale. Nevertheless, the UPOV Act, 1991 also provides for the protection of Essentially Derived Varieties (EDV) in which the breeder of an Essentially Derived Variety (EDV) needs to obtain authorization of the breeder of the original variety for commercial exploitation of the new variety. Farmers' rights were available to the farmers in the UPOV Act, 1978, but UPOV Act 1991 deprives farmers of their rights to reuse their produce as a seed.

2.2.2.1 Identification of varieties using morphological DUS descriptors

Plant morphological characters have been recognized as the universally undisputed descriptors for DUS testing and varietal characterization of crop varieties. Use of morphological descriptors in sequential fashion is useful and convenient to discriminate different varieties (Patra *et al.*, 2010). The morphological DUS descriptors can be effectively used for identification and grouping of varieties and varieties satisfying the DUS criteria for these morphological descriptors could be registered under the PPV and FR Act for obtaining Plant Breeders' and Farmers' rights. There are 62 DUS descriptors (29 essential and 33 additional) in rice as per the national guidelines for the conduct of tests for Distinctness, Uniformity and Stability. Out of 62 DUS traits, the 29 essential traits should be examined and included in the description of varieties, which would be useful for identification of rice genotypes. A brief resume of work done in India and abroad with reference to this is discussed below:

Patra *et al.* (2010) assessed 60 morphological DUS descriptors of 18 Basmati rice varieties. Among these, 26 were monomorphic, 11 were dimorphic and seven were polymorphic indicating their potential for varietal characterization and distinctiveness.

Joshi *et al*. (2011) characterized 20 indigenous aromatic rice varieties using 60 morphological DUS descriptors. Out of these, 46 traits were visually assessed and 14 were measurable traits. Among 46 morphological characters, 24 were monomorphic, 17 were dimorphic and five were polymorphic.

Chakravorty and Ghosh (2012) characterized 51 landraces of rice using 46 agro-morphological traits following DUS test. Out of 51 varieties studied, 27 were found to be distinctive on the basis of 22 essential and 24 additional characters.

Characterization of 65 landraces of rice was done by Rao *et al.* (2013) using 43 DUS descriptors. Out of 65 landraces, 32 were found to be distinctive on the basis of 22 essential and 24 additional characters.

Characterization of 20 landraces of rice was done by Sinha and Mishra (2013) using DUS test. Out of these 20 investigated varieties, 11 varieties were distinctive for five essential and 18 additional characters.

Varietal identification of four rice varieties from Chhattisgarh was carried out through DUS Characterization by Tiwari *et al.* (2013) and stated that the morphological DUS descriptors could be effectively used for identification and grouping of varieties.

Mondal *et al.* (2014) characterized 21 rice varieties using 60 DUS descriptors (46 qualitative and 14 quantitative). Time to 50% heading, decorticated grain shape, colour of lemma and palea were the descriptors showing more discrimination. Eight qualitative and eight quantitative traits exhibited uniformity as determined by the recommended level.

DUS testing of 66 rice varieties was done by Raghavendra *et al.* (2014) using 27 morphological descriptors. This study demonstrated the effective use of DUS testing in varietal identification.

Patel and Srivastava (2015) characterized the aromatic rice varieties of Chhattisgarh using 45 morphological DUS descriptors. All the 45 unique morphological traits exhibited variability except ligule colour. The traits like grain size, grain shape and plant height contributed towards more diversity.

Singh *et al.* (2015) assessed 23 morphological DUS traits in 20 rice (10 mega varieties and 10 landraces) varieties. Among the 23 DUS characters, six showed no variation and found distinctive among all the cultivars. Maximum variability was recorded with respect to panicle length of main axis, spikelet colour of tip of lemma and panicle attitude of branches among all the cultivars.

Sanyal and Joshi (2016) characterized 61 extant varieties of Basmati and non-Basmati groups of rice using 55 DUS descriptors. Their study revealed that six characters were monomorphic, nine were dimorphic and 40 were polymorphic.

DUS characterization of 35 landraces of rice was carried out by Kalyan *et al.* (2017) using 29 DUS (agro-morphological) characters. Out of 35 landraces, 22 cultivars were found to be distinctive.

Komala *et al.* (2017) characterized eight rice genotypes using 56 morphological DUS descriptors. Among 56 characters studied, the variation was observed in 37 characters.

Umarani *et al.* (2017a) characterized 70 landraces of rice using 14 DUS characters. Out of 14 characters, stem anthocyanin colouration of node was dimorphic, three were trimorphic, six were tetramorphic, lemma anthocyanin colouration of apex and amylose content showed five states of expression. Based on decorticated grain colour six groups were made.

Vigneshwari *et al.* (2017) characterized 13 commercially cultivated rice varieties of Tamil Nadu using 31 morphological traits. The level of discrimination was high in quantitative grain characters compared to qualitative characters.

Kumar *et al.* (2018) characterized 55 rice genotypes following the DUS guidelines. Significant variation was observed for the characters like coleoptile colour, intensity of leaf green colour, culm attitude, flag leaf attitude, stem length, lemma and palea colour, grain length and test weight.

Manjunatha *et al.* (2018) assessed 25 DUS descriptors to characterize 60 landraces of rice including the aromatic genotypes collected from different parts of Wayanad, Kerala. Out of these, three characteristics were monomorphic, seven were dimorphic, six were trimorphic, seven were tetramorphic, decorticated grain shape showed five states of expression and lemma and palea colour recorded six states of expression.

Rawte and Saxena (2018) used 19 qualitative and 11 quantitative agro-morphological traits to characterize 100 rice accessions. The descriptors offering the most discrimination were time to 50% heading, time to maturity days, 1000 grain weight, stem length, grain length and width.

Sharma and Singh (2018) used 55 agro-morphological traits to characterize 136 farmers' varieties of rice following DUS test, only 66 FVs were found to be distinctive.

Aravind *et al.* (2019) used 24 DUS descriptors to characterize 45 rice mutant lines in the background of ADT 37 and ADT (R) 45. Out of these, 12 were visually assessed qualitative traits. Shape of ligule, colour of ligule, stigma colour, sterile lemma colour, panicle secondary branching and panicle attitude were monomorphic, culm attitude, panicle exertion, flag leaf attitude, presence of awns and distribution of awns were dimorphic and the grain shape was polymorphic.

Banshidhar *et al.* (2019) worked on DUS characterisation of advanced recombinant lines of Kalanamak rice for 47 visually assessed characters and 15 quantitative characters. Out of these characters studied, 27 were monomorphic, 18 were dimorphic and two were polymorphic.

The agro-morphological characterization of aromatic *Harinakhuri* rice landrace was done by Ghosh *et al.* (2019). The lemma and palea of matured grain was straw coloured with purple spot at tip and sterile lemma in purple colour.

Gour *et al.* (2019) characterized 83 indigenous rice lines based on 31 morphological DUS descriptors. Basal leaf sheath colour, colour of tip of lemma, lemma and palea colour showed highest variation in germplasm.

2.2.3 Varietal Identification using Molecular Markers

The variety identification serves the important goals such as mitigating legal claims, confirming Intellectual Property Rights and maintenance of genetic purity (Patra *et al*., 2010). The current plant variety identification for their protection relies on morphological description of plant varieties. However, establishing identity of varieties based on the DUS descriptors is quite problematic and difficult due to their limited number, stage specificity and environmental influence which complicate their evaluation (Nuel *et al*., 2001; Cooke and Reeves, 2003; Singh *et al*., 2004). In contrast, DNA or molecular markers are free from the environmental effects and could be applied at different stages of plant growth (Noli *et al*., 2008).

Table 2.1. List of previous studies on molecular characterization of rice genotypes

Marker system	DUS test	Key message	References
RAPDs	D	RAPD analysis could be used to differentiate four non-aromatic varieties from nine aromatic rice varieties	Baishya *et al.* (2000)
RAPDs	D	The rice genotypes could be distinguished from each other using RAPD analysis	Choudhury *et al.* (2001)
STMSs	D	A set of four STMS markers (RM206, RM216, RM258 and RM263) differentiated 11 hybrids from each other. These markers could be used as referral markers for unambiguous identification and protection of these hybrids	Nandakumar *et al.* (2004)
SSRs	D	13 rice cultivars (four commercial traditional Basmati, six cross-bred Basmati and three non-Basmati varieties) were evaluated using 35 SSRs. SSR analysis generated polymorphism sufficient to differentiate all the 13 rice genotypes	Pal *et al.* (2004)
SSRs	D, U, S	Mapped SSRs of 12 rice linkage groups could differentiate all 23 aromatic varieties. No variation was seen in the analysis of 20 individuals of all seed classes of "Pusa Basmati 1" using 55 SSRs, hence suggesting SSRs for uniformity and stability tests	Singh *et al.* (2004)
SSRs	D	Suggested the usage of SSR markers in genotype identification, monitoring purity and adulteration and plant variety protection	Siwach *et al.* (2004)
SSRs	D	The DNA fingerprinting helped to distinctly identify and characterize nine varieties using 18 different RM primers	Chakravarthi and Naravaneni (2006)
SSRs	D	Microsatellite markers could efficiently identify indigenous non- Basmati aromatic rice genotypes and support Intellectual Property Protection	Joshi and Behara (2007)

RAPDs : Randomly Amplified Polymorphic DNAs; **STMSs :** Sequence Tagged Microsatellite Sites; **SSRs :** Simple Sequence Repeats;
D : Distinctness; **U :** Uniformity; **S :** Stability.

Cont…

Table 2.1. Contd…

Marker system	DUS test	Key message	References
SSRs	D	A high degree of polymorphism obtained among eight rice genotypes using 14 SSRs suggested that SSRs would also be useful in variety fingerprinting and identification	Malky *et al.* (2007)
EST-SSRs	D	A small set of 5 EST-SSRs distinguished 37 rice varieties in Brazil based on their subspecies *i.e.*, *indica* or *japonica*. SSRs can efficiently complement morphological descriptors	Bonow *et al*. (2009)
SSRs	D	SSRs separated rice varieties into Basmati and non-Basmati group	Sarao *et al.* (2009)
SSRs	D	The microsatellite markers employed for DNA fingerprinting were able to differentiate the parental lines of respective hybrids included under study and also useful for variety identification	Tamilkumar *et al.* (2009)
Total protein, Isozymes, RAPDs	D	Biochemical and molecular markers were presented to potentially complement morpho-physiological descriptors which failed distinguishing a variety	Patra and Chawla (2010)
SSRs	D	Identified three varietal specific alleles, RM-11/147, RM-151/289, RM-153/178 for BR-11, Badshabhog and BR-19 cultivars using SSRs and suggested that a meaningful data could be obtained through DNA fingerprinting using SSRs	Rahman *et al.* (2010)
SNPs	D	Identified 205 cultivars of Japan by using 18 SNP markers. The 205 cultivars were individually distinguished from the others using a combination of upto six markers. Hence, suggested SNPs for the identification of Japanese commercial rice cultivars	Sato *et al.* (2010)

SSRs : Simple Sequence Repeats; **EST-SSRs :** Expressed Sequence Tag-derived Simple Sequence Repeats; **RAPDs :** Randomly Amplified Polymorphic DNAs; **SNP :** Single Nucleotide Polymorphism; **D :** Distinctness; **U :** Uniformity; **S :** Stability.

Cont…

Table 2.1. Cont…

Marker system	DUS test	Key message	References
RAPDs, ISSRs	D	ISSRs were more successful in distinguishing indigenous local strains of rice than RAPDs	Shukla *et al*. (2011)
SSRs	D	The highly polymorphic SSRs could be used for quick and reliable detection of specific genotype	Singh and Singh (2012)
SSRs	D	SSRs can efficiently complement morphological descriptors as they are useful for identification of closely related cultivars through fingerprinting	Zhu *et al.* (2012)
SSRs and AFLPs	D	SSR markers are better than AFLPs for varietal identification as SSRs are co-dominant and multiallelic markers in contrast to the nature of AFLP markers	Rabey *et al.* (2013)
ISSRs	D	The primer AM-8 might be the best marker for identification of aromatic rice varieties	Samal *et al.* (2014)
SSRs	D	A set of SSRs was defined to allow the identification and certification of *O. sativa* varieties including INIA and foreign varieties. Thus, SSRs could be a good complement to the existing agro-morphological data when varieties are closely related	Becerra *et al.* (2015)
SSRs	D	A combination of five polymorphic SSRs were successful in differentiating 40 varieties, suggesting these SSRs as useful set for complementing DUS tests	Pourabed *et al*. (2015)
SSRs, ISSRs and RAPDs	D	The use of a greater number of markers would be efficient to characterize the three varieties *i.e.*, Basmati, non-Basmati and aerobic rice varieties. SSR primer, RM-263 is highly informative in DNA fingerprinting based decoding since it recorded high PIC value (0.995)	Singh and Sengar (2015)

RAPDs : Randomly Amplified Polymorphic DNAs; **ISSRs :** Inter Simple Sequence Repeats; **SSRs :** Simple Sequence Repeats; **AFLPs :** Amplified fragment length polymorphism; **PIC :** Polymorphism Information Content; **D :** Distinctness; **U :** Uniformity; **S :** Stability.

Cont…

Table 2.1. Cont…

Marker system	DUS test	Key message	References
hypervariable microsatellite (hvRM) markers	D	Four of the eight hvRM markers *viz*., RM22250, RM13584, RM24260 and RM5844 located in genes could be of value in DUS testing by supplementing the morphological descriptors	Vemireddy *et al.* (2015)
SSRs	D	The SSRs (RM206, RM21, RM55, RM258 and RM433) were found robust enough to distinguish the duplicate and similar named rice germplasms for their higher PIC values (> 0.86)	Ahmed *et al.* (2016)
SSRs	D	The allelic diversity revealed by 45 SSR primers was sufficient enough to distinguish the tested 120 *aus* rice genotypes through DNA fingerprinting	Islam *et al.* (2017)
Gene-specific or linked markers (DEP1-1)	D	The primers DEP1-1 amplified a 1235bp fragment in the erect panicle variety and 1860bp fragment in the non-erect panicle variety. Thus, this primer can be used for identification of varieties having dense and erect panicle	Li *et al.* (2017)
SSRs	D	The average similarity index of 0.65 for morphological data and 0.35 for SSR data apparently defines the advantage of SSR over morphology in unambiguous and quick identification of closely related varieties	Vigneshwari *et al.* (2017)
SSRs	D	69 SSR markers were used for their molecular characterization and discrimination of 24 entries of rice. The molecular markers showing PIC value >0.5 can be used to distinguish NILs from their respective parents apart from gene linked markers of targeted trait	Reddy and Rani (2018)

SSRs : Simple Sequence Repeats; **NILs :** Near Isogenic Lines; **PIC :** Polymorphism Information Content; **D :** Distinctness; **U :** Uniformity; **S :** Stability;

The increased availability and cost-efficiency of DNA-based markers in recent years makes them an attractive option to explore their use to supplement or even ultimately replace the existing morphological and protein-based approaches (Jamali *et al.*, 2019). Among the DNA based markers, microsatellite or Simple Sequence Repeat (SSR) markers were mostly used by many of the scientists to identify the rice genotypes. However, employment of gene-specific markers is more reliable in identification of a variety as these markers are tightly linked to the gene controlling the phenotypic expression.

DNA fingerprinting or profiling developed by using molecular markers to describe the combined use of several single locus detection systems is being used as a versatile tool for characterization of rice genotypes. Owing to these reasons, the International Union for the Protection of New Varieties of Plants (UPOV), an intergovernmental organization whose system of plant variety protection is intended to encourage innovation in the field of plant breeding (Jordens, 2005) has called for the adoption of a DNA-based system that will enable examiners to deploy trait-specific DNA markers in DUS testing (Jamali *et al*., 2019).

Soybean barcode system was developed by Sohn *et al*. (2017) using InDel makers, each of which is specific to a dense variation block (dVB). The polymorphism of the markers was assessed in 147 soybean cultivars and the soybean barcode system allowed a clear distinction among soybean cultivars. “Singang” and “Sowon” were identified by using 27 InDel markers.

Singh *et al*. (2019) developed a user friendly server based tool, VIS*Ta* (Variety Identification System of *Triticum aestivum*) (http://webtom.cabgrid.res.in/vista) using 54 SNPs and 36 DUS features. This tool is having dispute resolution of sovereignty and access benefit sharing (ABS) issues. This model approach can be used in other crops with pan-global level management of crop germplasm in endeavour of crop productivity.

2.2.4 Identification of Rice Varieties using Neural Network

Liu *et al*. (2005) used a digital image analysis algorithm based on colour and morphological features to identify rice seeds of six varieties (ey7954, syz3, xs11, xy5968, xy9308 and z903). Seven colour and 14 morphological features were used for discriminant analysis. 240 kernels were used as the training data set and 60 kernels as the test data set in the neural network to identify rice seed. When the model was tested on the test data set, the identification accuracies were 90.00%, 88.00%, 95.00%, 82.00%, 74.00% and 80.00% for ey7954, syz3, xs11, xy5968, xy9308 and z903 respectively.

2.2.5 Identification by Artificial Intelligence-based Machine Learning Classifiers

Dheer and Singh (2019) carried out a study with simple classifier models like Linear Discriminate Analysis, Logistic Regression, K-Nearest Neighbour's (KNN) and Naive-Bayes method using eight different rice varieties with 100 samples each. KNN out performed over the other methods with an accuracy, precision and recall of 99.16%, 99.12% and 99.12% respectively. The reported results suggest that this method can provide an accurate solution to the rice varieties for their classification and identification problems.

2.2.6 Raman Spectroscopic Identification of Rice Varieties

Sha *et al*. (2020) analysed 72 rice genotypes (28 *indica* rice, 25 *japonica* rice and 19 sticky rice) using a portable laser Raman spectrometer. This method combined PCA, window analysis and hierarchical cluster analysis with support vector machine which can be used as an effective feature extraction method to improve the efficiency for identification of rice varieties.

2.3 VARIABILITY, HERITABILITY AND GENETIC ADVANCE

The relative estimates of variability and their heritable components for yield contributing characters in the germplasm are of immense value in any breeding programme in achieving higher yields. The phenotype of quantitative trait is a combined product of genotype, environment and their interaction. Therefore, the success of any breeding activity depends on the extent of genetic variability available for utilization in crop improvement.

Heritability in broad sense refers to the genetic variation present in the population in relation to the total observed variance. Consistency in the performance of selection in succeeding generations depends on the magnitude of heritable variation present in relation to observed variation. Basic information on heritability is a prerequisite for planning any breeding programme. High heritability indicates that it should be easy to conduct effective selection for the trait. Genetic advance refers to the improvement in the mean genotypic value of the selected plants over the base population**.** Johnson *et al.* (1955) reported that though the heritable estimates give useful indication of relative values of selection based on phenotypic expression. The genetic gain should also be considered to arrive at a more reliable conclusion.

Shanthi and Singh (2001) evaluated 16 rice mutants to study the variability. High GCV and PCV were recorded for number of grains per panicle and grain yield plant^{-1}. High heritability coupled with high genetic advance as per cent of mean was recorded for plant height, number of grains per plant and 1000 grain weight.

The extent of genetic variation in 16 parents and 48 hybrids was studied by Subbaiah *et al.* (2011) for yield and its components and 25 quality characters. High GCV and PCV were recorded for harvest index, total number of productive tillers per plant and gelatinization temperature in parents.

Babu *et al*. (2012) carried out a study of genetic parameters for yield, yield attributing, quality and nutritional characters in 21 rice hybrids. The characters *viz.,* number of filled grains per panicle, number of chaffy grains per panicle and iron content exhibited high GCV and PCV. The characters *viz.,* number of filled grains per panicle and water uptake exhibited high heritability coupled with high genetic advance.

Pratap *et al.* (2012) evaluated 100 high yielding rice genotypes for variability studies. High estimates of heritability, genetic advance, genotypic and phenotypic coefficients of variation were recorded for panicle hill^{-1}, flag leaf area and grain yield hill^{-1}.

Aditya and Bhartiya (2013) evaluated 18 rice genotypes for genetic parameter studies of 15 quantitative traits. The estimates of GCV and PCV were highest for grain yield plot^{-1}. The broad sense heritability was highest for plant height.

Gangashetty *et al*. (2013) used 42 rice genotypes to know the extent of genetic variability. High GCV and PCV were observed for plant height, number of tillers per plant, number of productive tillers per plant, panicle weight, grain length, test weight, iron and zinc content and grain yield plant^{-1}. High heritability with high genetic advance was observed for all traits except days to 50 per cent flowering.

Veni *et al*. (2013) assessed 70 rice genotypes for genetic variability. High GCV and PCV were recorded for water uptake (26.28), grain yield plant^{-1} (26.18) and test weight (20.35). High heritability coupled with high genetic advance as per of mean was observed for grain yield plant^{-1}, water uptake, test weight and productive tillers per plant.

Dhurai *et al.* (2014) conducted genetic variability studies in 32 rice varieties. The higher magnitude of PCV and GCV was recorded for number of grains per panicle and grain yield plant^{-1}. High heritability coupled with high genetic advance as per cent mean was recorded for plant height, number of effective tillers per plant, panicle length, number of grains per panicle, kernel elongation ratio, kernel length after cooking, 1000-grain weight, harvest index and grain yield plant^{-1}.

Islam *et al.* (2015) evaluated 23 rice genotypes for genetic variability. High GCV and PCV were recorded for plant height, number of filled grains per panicle, days to 50% flowering, 1000 grain weight, grain width and grain yield.

Senapati and Kumar (2015) evaluated 22 Recombinant Inbred Lines (RIL's) for genetic variability, heritability and genetic advance. High PCV and GCV were observed for grain yield plant^{-1}, 1000 grain weight, L/B ratio, grain breadth and panicle weight. High heritability coupled with moderate to high genetic advance as per cent of mean was recorded for plant height, panicle weight, grain length, grain L/B ratio, 1000 grain weight and yield plant^{-1}.

Devi *et al.* (2016) assessed PCV, GCV, heritability and genetic advance in 27 rice genotypes. Higher estimates of PCV and GCV were observed for yield plant^{-1} and filled seeds per panicle. High heritability in broad sense and high genetic advance as percent of mean were exhibited by effective tillers, plant height, flag leaf length, filled seeds per panicle, test weight, yield plant^{-1}, head rice recovery and length/breadth ratio.

The extent of genetic variation in 36 rice genotypes was studied by Abebe *et al.* (2017). High to medium heritability coupled with high GCV and high genetic advance as percentage of means were exhibited for plant height and grain yield.

Bhinda *et al.* (2017) studied genetic variability for in 42 Advanced Breeding Lines of rice for 15 characters. Alkali spreading value exhibited highest GCV (19.44%) and PCV (21.07%) followed by filled grains per panicle (GCV 14.83% and PCV 19.90%). High heritability coupled with high genetic advance was reported for gel-consistency, plant height, filled grains per panicle, number of grains per panicle and days to 50% flowering.

Umarani *et al*. (2017b) carried out an experiment to assess the variability, heritability and genetic advance in 70 landraces of rice for 16 agro-morphological and grain quality parameters. Among all the characters, total number of grains per panicle exhibited highest PCV and GCV, whereas lowest PCV and GCV were recorded for spikelet fertility.

Chuchert *et al*. (2018) evaluated 22 rice genotypes for genetic variability in nine traits. High phenotypic and genetic coefficients of variation were observed for yield plant^{-1}, number of panicles per plant, and number of spikelets per panicle. High broad-sense heritability was recorded for yield plant^{-1}.

Gyawali *et al*. (2018) evaluated seven rice genotypes for genetic variability. Genotypic and phenotypic coefficients of variation were high for effective tillers per m^2, 1000 grain weight, grain yield, harvest index. The highest heritability was recorded by the character, anthesis days (0.97) and genetic advance was found highest for effective tillers per m^2 (33.80).

Ranjith *et al*. (2018) assessed GCV, PCV, heritability and genetic advance in 30 rice genotypes using 12 characters. High PCV and GCV were observed for fertile grains, flag leaf area and tillers per hill. High heritability coupled with high genetic advance was recorded for plant height.

Singh and Verma (2018) estimated genetic variability, heritability and genetic advance for 15 characters. High genotypic and phenotypic coefficients of variation were observed for flag leaf area and panicle bearing tillers per plant. High heritability coupled with high genetic advance as per cent of mean was observed for grains per panicle, panicle bearing tillers per plant, spikelet per panicle and plant height.

Aravind *et al*. (2019) evaluated 45 mutant lines for genetic variability of 12 characters. High GCV and PCV were observed for number of productive tillers and single plant yield. The traits *viz.*, number of productive tillers, leaf blade width, 1000 grain weight, LB ratio and single plant yield has high heritability along with genetic advance as percent of mean.

Rahangdale *et al.* (2019) evaluated 67 rice genotypes for 28 morphological and quality traits. High GCV and PCV were obtained for number of spikelets per panicle, spikelet density, grain yield plant^{-1}, fertile spikelets per panicle, biological yield plant^{-1} and panicle weight plant^{-1}. High heritability accompanied with high genetic advance was observed for the traits 1000 grain weight (g), head rice recovery (%), spikelet fertility (%), harvest index (%), number of spikelets per panicle, fertile spikelets per panicle, spikelet density, grain yield plant^{-1} (g), panicle weight plant^{-1} (g), number of tillers per plant and number of productive tillers per plant.

Sao *et al*. (2019) observed high PCV and GCV for number of filled grains per panicle, number of unfilled grains per panicle, total spikelets per panicle, grain weight per plant, panicle weight and harvest index using 47 rice genotypes. High heritability with high genetic advance as percent mean was observed for all yield attributing traits like grain weight per plant, harvest index %, number of filled grains per panicle, panicle weight.

2.4 GENETIC DIVERSITY ANALYSIS USING BOTH DUS DESCRIPTORS AND MOLECULAR MARKERS

The analysis of genetic variation within and among elite breeding materials is of fundamental interest to plant breeders. Diversity based on phenological and morphological characters usually varies with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. Molecular markers in addition to morphological characters have proven to be powerful tool in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers have been widely used in genetic diversity studies *viz*., SSRs (Simple Sequence Repeats) (McCouch *et al*., 2002; Garris *et al*., 2005; Lu *et al*., 2005; Chuang *et al*., 2011; Islam *et al*., 2011; Kanawapee *et al*., 2011), RAPDs (Random Amplified Polymorphic DNAs) (Barooah and Sarma, 2004), STMSs (Sequence Tagged Microsatellite Sites) (Nandakumar *et al*., 2004), SNPs (Single Nucleotide Polymorphisms) (Singh *et al*., 2013) providing greater confidence for the assessment of genetic diversity and relationships. Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars. A brief resume of work done in India and abroad with reference to this is discussed below.

Nandakumar *et al.* (2004) used 10 Sequence Tagged Microsatellite Site (STMS) markers for fingerprinting 11 rice hybrids and their parental lines and performed cluster analysis based on Jaccard’s similarity coefficient using UPGMA. The genetic similarity between the hybrids ranged from 0.33 to 0.92 with an average similarity index of 0.63 and the hybrids were grouped into three clusters.

Genetic relationship was assessed among 24 rice genotypes by UPGMA clustering using 50 SSRs by Siwach *et al.* (2004). This study revealed that three traditional Basmati varieties were closely related.

Chakravarthi and Naravaneni (2006) worked on the genetic diversity analysis of 15 elite rice genotypes using 30 SSR primers. Cluster analysis grouped the rice genotypes into 10 classes in which japonica types DH- 1 (Azucena) and Moroborekan clustered separately from *indica* types.

Jalaluddin *et al.* (2007) analysed genetic diversity of 22 *indica* rice cultivars through DNA fingerprinting using five microsatellite markers (RM1, RM3, RM168, RM226 and OSR19). The highest genetic diversity

(GD), 0.6336 was detected in locus RM168 and the lowest (0.2496) on locus OSR19. Average GD was 0.4446, indicating a moderate level of genetic diversity existing among the tested cultivars.

Joshi and Behara (2007) analysed genetic diversity of 38 traditional indigenous non-Basmati aromatic rice cultivars using 12 microsatellite marker pairs. Polymorphism Information Content (PIC) ranged between 0.00 to 0.83. A dendrogram based on cluster analysis grouped 38 genotypes into three major groups effectively differentiating the slender aromatic rice cultivars from the short bold and long bold aromatic cultivars.

Joshi *et al*. (2007) characterized 19 rice varieties considering 52 DUS traits. High difference between PCV and GCV values for panicle number per plant shows high environmental effect. The varieties were grouped into different clusters based on the eight grouping characters of National DUS testing guidelines.

Herrera *et al*. (2008) studied the genetic relationship of some Venezuelan rice cultivars by SSR markers and found low genetic diversity among them.

Joshi *et al*. (2011) studied 20 indigenous aromatic rice varieties using 60 morphological DUS descriptors. On the basis of grouping characteristics seven varieties showed unique profiles.

Hossain *et al.* (2012) determined the allelic diversity and relationship of 12 elite aromatic rice cultivars using 24 SSR markers. The genetic distance-based results seen in the unrooted neighbour-joining tree and UPGMA clustering revealed five genetic groups in which closely related genotypes were clustered together.

Singh and Singh (2012) have done diversity analysis of 45 rice genotypes for grain quality characters using 45 SSR markers. In the dendrogram constructed by SSR markers, all genotypes were grouped into two major and nearly equal clusters having 22 and 23 genotypes at 50 per cent similarity coefficient.

Zhu *et al.* (2012) used 32 SSRs to identify *indica* and *japonica* subspecies. The UPGMA cluster analysis separated the 48 cultivars into two major groups. The first major cluster consisted of the 34 *indica* cultivars, and the remaining 14 *japonica* cultivars formed the second major cluster. This study revealed the presence of narrow genetic diversity among the 48 main commercial rice cultivars.

Alice *et al.* (2013) evaluated 195 rice accessions for diversity pattern among them. The genotypes were grouped into 10 clusters. The inter-cluster distance is higher than intra-cluster indicating wide genetic diversity among the genotypes. The highest inter-cluster distance was observed between cluster VI and VII.

Choudhary *et al.* (2013) performed genetic diversity studies using 64 SSRs in 100 rice genotypes. Of 64 rice SSR primers studied, 52 showed polymorphism. A total of 184 alleles was identified averaging 3.63 alleles per locus. Cluster analysis grouped the 100 genotypes into five clusters.

Rabey *et al.* (2013) used AFLP and SSR markers to study the genetic diversity of eight rice cultivars. The Polymorphism Information Content value for the two types of assays was 0.825 and 0.967 for SSR and AFLP markers respectively. Cluster analysis was used to address relationship between the rice cultivars based on the SSRs and AFLP data.

Genetic relatedness among 375 rice varieties was studied by Singh *et al.* (2013) utilizing an unrooted tree. The genotypes were grouped into three major clusters with both 36 SSR and 36 SNP markers.

Raghavendra *et al.* (2014) worked on DUS testing and genetic diversity analysis of 66 rice varieties using 27 morphological descriptors. Genetic similarities among genotypes varied from 0.11 to 1.0 with an average of 0.36. The cluster analysis grouped all the genotypes into two major clusters at 0.21 level of genetic similarity.

Roy and Sharma (2014) worked on assessment of genetic diversity in 84 rice cultivars of West Bengal through DUS characterization and a dendrogram constructed showed genetic diversity among 84 cultivars by grouping them into five major clusters. All the descriptors evaluated in this study have showed that there is enough genetic diversity among cultivars.

Samal *et al.* (2014) characterized 48 traditional aromatic rice varieties using 24 ISSR markers and morphological markers. The genetic diversity was evaluated using UPGMA cluster dendrogram which identified two clusters with a similarity coefficient of 53%.

The genetic diversity and phylogenetic relationship of six improved African lowland rice varieties *viz*., BW-348-1, FARO-44, FARO-57, NERICA-L-19, NERICA-L-34 and WITA-4 was investigated using 129 SSR primers distributed on 12 chromosomes of rice by Adegbaju *et al*. (2015). The SSR based dendrogram generated clustered two genotypes BW348-1 and FARO-44 together and three genotypes FARO-57, NERICA-L-19 and WITA-4 together.

Genetic clustering of 16 rice varieties was done by Becerra *et al.* (2015) using 54 SSRs with Roger coefficient which determined the separation of *japonica* and *indica* rice. The cluster analysis indicated that varieties were grouped according to their grain type and pedigree.

Kunusoth *et al.* (2015) worked on genetic diversity assessment of 24 elite Indian rice varieties using 24 agro-morphological traits and 86 SSR markers. Genetic diversity assessment based on SSR markers displayed genetic similarity coefficients and grouped the varieties into five major clusters.

Singh and Sengar (2015) have done DNA fingerprinting using SSR, ISSR and RAPD markers to evaluate the genetic divergence of 30 rice varieties. The dendrogram developed for aroma and quality traits by UPGMA showed that the genotypes with common phylogeny and geographical orientation tend to cluster together.

Ahmed *et al.* (2016) evaluated 31 rice genotypes of Bangladesh to assess the genetic variation for the agro-morphological and physico-chemical traits. The cluster analysis grouped the genotypes into four clusters. The dendrogram from UPGMA clustering also classified the genotypes into four groups.

Mahalingam *et al.* (2016) estimated genetic diversity among 51 restorer and five CMS lines in hybrid rice breeding. Cluster analysis using NTSYS generated dendrogram divided all the 56 parental lines into 13 different clusters with 80 per cent coefficient of similarity.

Islam *et al.* (2017) worked on the allelic diversity and the relationship among 120 *aus* rice landraces through DNA fingerprinting using microsatellite (SSR) markers. The genetic distance-based results obtained in the unrooted neighbour-joining tree clustering revealed nine genetic groups.

Vigneshwari *et al.* (2017) characterized 13 rice varieties of Tamil Nadu using 31 morphological traits and more than 200 SSR markers. Out these SSR markers, 88 showed polymorphism. Cluster analysis based on morphological characters grouped the varieties of medium and long slender grain type in one cluster and short bold grain type into another cluster at about 65 per cent similarity level. The cluster analysis of SSR markers grouped the varieties into four major clusters at a similar level of 35 per cent.

Aljumaili *et al.* (2018) worked on genetic diversity among 53 aromatic rice accessions using 32 SSR markers. The dendrogram based on UPGMA and Nei's genetic distance classified these 53 rice accessions into 10 clusters.

Reddy and Rani (2018) have done molecular and morphological characterization of 24 Near Isogenic Lines (NILs) of rice developed for major abiotic stresses. Results of molecular characterization showed genetic diversity among 24 entries. Cluster analysis grouped 24 entries into two distinct clusters at similarity coefficient of 33 per cent.

Chapter ~ III

Material and Methods

Chapter – III

MATERIAL AND METHODS

The details of the plant material used and methodology adopted in the present study entitled, “Identification of rice (*Oryza sativa* L.) varieties using DUS descriptors and gene-specific markers” are briefly described in this chapter.

3.1 MATERIAL

In all, 52 rice varieties released from various research stations in India were included in the present investigation (Table 3.1.).

3.2 METHODS

3.2.1 DUS Characterization

3.2.1.1 Experimental site

The present research was conducted at Wetland Farm, S.V. Agricultural College, Acharya N.G. Ranga Agricultural University, Tirupati, Andhra Pradesh, located at geographical co-ordinates of 13°54’ N latitude and 79°54’ E longitude and 182.9 m altitude.

3.2.1.2 Experimental design and layout

The experiment was conducted using Randomized Block Design (RBD) with three replications with spacing of 20 × 15 cm with row length of 1.5 m during *kharif,* 2019. Crop was maintained by employing standard cultural and management practices. The experimental field of research work is shown in Plate 1.

3.2.1.3 Characterization of rice genotypes for DUS descriptors

Morphological characters of 52 rice genotypes were studied for 22 DUS descriptors. Observations on DUS descriptors were recorded at different

Table 3.1. List of rice genotypes included in the present investigation

S. No.	Variety	Parentage	Released from
1	Bhadrakali	Phalguna × IR-36	RARS, Warangal, Telangana
2	BPT1235	Sabarmati × WGL12708	ARS, Bapatla, Andhra Pradesh
3	BPT2295	BPT 1768 × NLR 33641	ARS, Bapatla, Andhra Pradesh
4	BPT5204	GEB-24 × T(N)1 × Mahsuri	ARS, Bapatla, Andhra Pradesh
5	DRR Dhan 38	BPT5204 × KMR-3	IIRR, Hyderabad, Telangana
6	Govind	IR20 × IR24	GPUAT, Uttarakhand
7	IR64	IR-5857-33- 2-1 × IR-2061-465- 1-5-5	IRRI, Manila, Philippines
8	Jaya	TN1 × T-141	IIRR, Hyderabad, Telangana
9	JGL17004	WGL14377 × JGL3855	RARS, Jagtial, Telangana
10	JGL3844	(BPT 5204 × ARC 5984) × Kavya	RARS, Jagtial, Telangana
11	Kasturi	Basmati-370 × CRR-88-17- 1-5	IIRR, Hyderabad, Telangana
12	Kavya	WGA-27120 × WGL-17672 × Mahsuri × Surekha	RARS, Warangal, Telangana
13	Krishna hamsa	Rasi × Fine Gora	IIRR, Hyderabad, Telangana
14	MTU1001	MTU5249 × MTU7014	RARS, Maruteru, Andhra Pradesh
15	MTU1010	Krishnaveni × IR-64	RARS, Maruteru, Andhra Pradesh
16	MTU1121	BPT5204 × MTU BB 8- 24-1	RARS, Maruteru, Andhra Pradesh
17	MTU1140	MTU5249 × PLA8572	RARS, Maruteru, Andhra Pradesh

RARS : Regional Agricultural Research Station; **ARS :** Agricultural Research Station; **IIRR :** Indian Institute of Rice Research;
GPUAT : Govind Ballabh Pant University of Agriculture and Technology; **IRRI :** International Rice Research Institute.

Cont…

Table 3.1. Cont…

S. No.	Variety	Parentage	Released from
18	MTU1153	MTU1010 × MTU1081	RARS, Maruteru, Andhra Pradesh
19	MTU1155	MTU1001 × Annadha	RARS, Maruteru, Andhra Pradesh
20	MTU1156	MTU1010 × MTU1081	RARS, Maruteru, Andhra Pradesh
21	MTU1172	MTU7029 × MTU1064	RARS, Maruteru, Andhra Pradesh
22	MTU1190	MTU1081 × Swarna Sub1	RARS, Maruteru, Andhra Pradesh
23	MTU3626	IR-8 × MTU-3	RARS, Maruteru, Andhra Pradesh
24	N22	A selection from Rajbhog	ZRS, Nagina, Uttar Pradesh
25	Nidhi	Sona × ARC-14529	IIRR, Hyderabad, Telangana
26	NLR34449	IR72 × BPT5204	ARS, Nellore, Andhra Pradesh
27	Pankaj	Peta × Tongkai Rotan	ARI, Rajendra Agricultural University, Pusa, Bihar
28	Pothana	IR-579 × WGL-12708	RARS, Warangal, Telangana
29	PR106	(IR-8 × Peta 5) × Bella Patna	RRRI, Ludhiana, Punjab
30	Pusa Basmati-1	Pusa-150 × Karnal Local	Rajendra Agricultural University, Pusa, Bihar
31	PUSA1121	Pusa614-1-2 × Pusa614-2-4-3	Indian Agricultural Research Institute, New Delhi
32	Rajeswari	T-90 × IR-8	RRS, Indira Gandhi Agricultural University, Raipur
33	Rasi	TN1 × Co-29	IIRR, Hyderabad, Telangana
34	RP Bio -226	Samba Mahsuri (BPT 5204)*4 × SS 1113	IIRR, Hyderabad, Telangana
35	Shobini	Early Samba × RNR 19994	Rice Research Centre, Rajendranagar, Telangana

RARS : Regional Agricultural Research Station; **ZRS :** Zonal Research Station; **IIRR :** Indian Institute of Rice Research; **RRS :** Rice Research Station; **ARS :** Agricultural Research Station; **ARI :** Agricultural Research Institute; **RRRI :** Regional Rice Research Institute.

Cont…

Table 3.1. Cont…

S. No.	Variety	Parentage	Released from
36	Shobini	Early Samba × RNR 19994	Rice Research Centre, Rajendranagar, Telangana
37	Siddhi	BPT-5204 × ARC-5984 × 45Kavya × Kavya × BPT-5204	RARS, Warangal, Telangana
38	Sona Mahsuri	Sona × Mahsuri	ARS, Bapatla, Andhra Pradesh
39	Sonasali	Sona × Manoharsali	IIRR, Hyderabad, Telangana
40	Swarna sub1	Swarna × FR13A	NRRI, Cuttack, Odisha
41	Tellahamsa	HR-12 × T(N)1	Rice Research Centre, Rajendranagar, Telangana
42	Triguna	Swarna Dhan × RP-1579-38	IIRR, Hyderabad, Telangana
43	Udayagiri	IRAT-138 × IR-13543-66	Odisha University of Agriculture and Technology, Bhubaneswar, Odisha
44	Vajram	Sowbhagya × ARC-6650	RARS, Maruteru, Andhra Pradesh
45	Vandana	C-22 × Kalakeri	NRRI, Cuttack, Odisha
46	Varalu	Erramallelu × CR 544-1-2	RARS, Warangal, Telangana
47	Varadhan	(Swarna × 9314) × BR 827-36	IIRR, Hyderabad, Telangana
48	Vasumati	PR109 × Pak Basmati	IIRR, Hyderabad, Telangana
49	Vikramarya	RPW 6-13 × PTB 2	IIRR, Hyderabad, Telangana
50	Warangal Samba	BPT 5204 × ARC 5566 × BPT 3291	RARS, Warangal, Telangana
51	WGL32100	Divya × BPT 5204	RARS, Warangal, Telangana
52	Saket-4	TKM-6 × IR-8	NRRI, Cuttack, Odisha

RARS : Regional Agricultural Research Station; **ARS :** Agricultural Research Station; **IIRR :** Indian Institute of Rice Research; **NRRI :** National Rice Research Institute.

Vegetative stage

Grain filling stage

Plate 1. Experimental field view

growth stages as per DUS test guidelines on rice issued by PPV&FR Authority (Anonymous, 2007). Four types of assessments were followed for recording the DUS characters (Table 3.2.).

3.2.1.4 Recording of observations

Visual observations were recorded on single plant basis in each genotype at appropriate growth stages according to Rani *et al.* (2006) on different morphological characters. Observations for grain yield and other measurable characters were recorded on five randomly selected plants for each entry in each replication. The mean data was estimated for each character per replication. The data collected was subjected to statistical analysis. The method of recording data for each trait is described below.

3.2.1.4.1 Basal leaf sheath colour

Basal leaf sheath may show various colourations *viz.*, green, light purple, purple lines and uniform purple.

3.2.1.4.2 Leaf auricles

The leaf may show either presence or absence of auricles.

3.2.1.4.3 Leaf collar

The leaf may show either presence or absence of collar.

3.2.1.4.4 Leaf ligule

The leaf may show either presence or absence of ligule.

3.2.1.4.5 Shape of leaf ligule

The leaf ligule may show various shapes *viz.*, truncate, acute and split.

3.2.1.4.6 Colour of leaf ligule

The leaf ligule may show various colourations *viz.*, white, light purple and purple.

3.2.1.4.7 Time of heading (50% of plants with panicles) (days)

The total number of days taken from the date of sowing to the complete exertion of 50 per cent of panicles in each genotype was recorded as days to 50 per cent flowering and classified the genotypes as very early (<71), early (71-90), medium (91-110), late (111-130) and very late (>131).

3.2.1.4.8 Lemma anthocyanin colouration of area below apex

The lemma may either show presence or absence of anthocyanin colouration of area below apex.

3.2.1.4.9 Stem length (excluding panicle; excluding floating rice) (cm)

The length of the stem was measured from the base of the stem to the panicle base at milk development stage and expressed in centimetre (cm) and further the genotypes were as classified very short (<91 cm), short (91-110 cm), medium (111-130 cm), long (131-150 cm) and very long (>150 cm).

3.2.1.4.10 Panicle length of main axis (cm)

The length of panicles from each plant was measured in centimetres from the base of the panicle to the tip of the last spikelet and classified the genotypes as very short (<16 cm), short (16-20 cm), medium (21-25 cm), long (26-30 cm) and very long (>30 cm) accordingly.

3.2.1.4.11 Panicle number per plant

The total number of ear bearing tillers was counted on each plant at the time of maturity and categorised the genotypes as few (<11), medium (11-20) and many (>20) accordingly.

3.2.1.4.12 Panicle awns

The panicles may show either presence or absence of awns.

3.2.1.4.13 Panicle distribution of awns

The panicles may show either the distribution of awns at the tip only or upper half or whole length.

3.2.1.4.14 Presence of panicle secondary branching

The panicles may either show presence or absence of secondary branches.

3.2.1.4.15 Panicle secondary branching

The panicles may either show either weak or strong or clustered type of secondary branches.

3.2.1.4.16 Time of maturity (days)

The total number of days taken from the date of sowing to the stage when 85 per cent of grains turn straw colour and classified the genotypes as very early (<100), early (101-120), medium (121-140), late (141-160) and very late (>160) accordingly.

3.2.1.4.17 Weight of 1000 fully developed grains (g)

The weight of 1000 filled grains selected randomly in each plant was weighed and expressed in grams and the genotypes were classified as very low (<15 g), low (15-20 g), medium (21-25 g), high (26-30 g) and very high (>30 g).

3.2.1.4.18 Grain length and width (mm)

Ten fully developed grains of each variety with both ends intact were selected per replication and the length and width of the grains were measured using digital Vernier callipers in millimetres. The average of length and width of grains were taken in millimetres separately and classified them according to the national guidelines for conduct of tests for DUS in rice.

Table 3.2. DUS characteristics recorded in rice genotypes

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
1	Basal leaf: sheath colour	Green	1	Booting stage	VS
		Light purple	2		
		Purple lines	3		
		Uniform purple	4		
2	Leaf: auricles	Absent	1	Booting stage	VS
		Present	9		
3	Leaf: collar	Absent	1	Booting stage	VS
		Present	9		
4	Leaf: ligule	Absent	1	Booting stage	VS
		Present	9		
5	Leaf: shape of ligule	Truncate	1	Booting stage	VS
		Acute	2		
		Split	3		
6	Leaf: colour of ligule	White	1	Booting stage	VS
		Light purple	2		
		Purple	3		
7	Time of heading (50% of plants with panicles) (days)	Very early (<71)	1	Half of inflorescence emerged	VG
		Early (71-90)	3		
		Medium (91-110)	5		
		Late (111-130)	7		
		Very late (>131)	9		
8	Lemma: anthocyanin colouration of area below apex	Absent	1	Anthesis half way	VS
		Weak	3		
		Medium	5		
		Strong	7		
		Very strong	9		
9	Stem: length (excluding panicle; excluding floating rice)	Very short (<91 cm)	1	Milk development stage	MS
		Short (91-110 cm)	3		
		Medium (111-130 cm)	5		
		Long (131-150 cm)	7		
		Very long (>150 cm)	9		
10	Panicle: length of main axis	Very short (<16 cm)	1	Dough development to Ripening stage	MS
		Short (16-20 cm)	3		
		Medium (21-25 cm)	5		
		Long (26-30 cm)	7		
		Very long (>30 cm)	9		
11	Panicle: number per plant	Few (<11)	3	Dough development to Ripening stage	MS
		Medium (11-20)	5		
		Many (>20)	7		
12	Panicle: awns	Absent	1	Ripening stage	VG
		Present	9		
13	Panicle: distribution of awns	Tip only	1	Ripening stage	VS
		Upper half only	3		
		Whole length	5		

Cont…

Table 3.2. Cont…

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
14	Panicle: presence of secondary branching	Absent	1	Ripening stage	VG
		Present	9		
15	Panicle: secondary branching	Weak	1	Ripening stage	VG
		Strong	2		
		Clustered	3		
16	Time of maturity (days)	Very early (<100)	1	Ripening stage	VG
		Early (101-120)	3		
		Medium (121-140)	5		
		Late (141-160)	7		
		Very late (>160)	9		
17	Grain: weight of 1000 fully developed grains	Very low (<15 g)	1	Caryopsis hard	MG
		Low (15-20 g)	3		
		Medium (21-25 g)	5		
		High (26-30 g)	7		
		Very high (>30 g)	9		
18	Grain: length	Very short (<6.0 mm)	1	Caryopsis hard	MS
		Short (6.1-8.5 mm)	3		
		Medium (8.6-10.5 mm)	5		
		Long (10.6-12.5 mm)	7		
		Very long (>12.5 mm)	9		
19	Grain: width	Very narrow (<2.0 mm)	1	Caryopsis hard	MS
		Narrow (2.1-2.5 mm)	3		
		Medium (2.6-3.0 mm)	5		
		Broad (3.1-3.5 mm)	7		
		Very broad (>3.5 mm)	9		
20	Decorticated grain: length	Short	1	Caryopsis hard	MS
		Medium	3		
		Long	5		
		Long* (Long for Basmati type)	7		
		Extra Long	9		
21	Decorticated grain: width	Narrow (<2.0 mm)	3	Caryopsis hard	MS
		Medium (2.0-2.5 mm)	5		
		Broad (>2.5 mm)	7		
22	Decorticated grain: shape (in lateral view)	Short slender	1	Caryopsis hard	MS
		Short bold	2		
		Medium slender	3		
		Long bold	4		
		Long slender	5		
		Long slender* (For Basmati type)			
		Extra-long slender	6		

VS : Visual assessment by observation of individual plant or parts of plants.
VG : Visual assessment by a single observation of a group of plants or parts of plants.
MS : Measurement of a number of individual plants or parts of plants.
MG : Measurement by a single observation of a group of plants or parts of plant.

3.2.1.4.19 Decorticated grain length and width (mm)

After dehusking, ten fully developed rice kernels of each variety with both ends intact were selected per replication and the length and width of the kernels were measured using digital Vernier callipers in millimetres. The average of length and width of kernels were taken in millimetres separately and classified them according to the national guidelines for conduct of tests for DUS in rice.

3.2.1.4.20 Decorticated grain shape (in lateral view)

Average of the kernel length and kernel breadth was taken and length/ breadth ratio was calculated. Based on kernel length and kernel L/B ratio, grain type was assigned as per DUS guidelines (As referred in Rice Research in India, ICAR Publication, 1985).

State	Kernel length (mm)	Length/breadth ratio
Short Slender	< 6.0	> 3.0
Short Bold	< 6.0	< 2.5
Medium Slender	< 6.0	2.5 – 3.0
Long Slender	> 6.0	> 3.0
Long Bold	> 6.0	< 3.0
Extra Long Slender	> 7.5	> 3.0
Basmati type	> 6.61	> 3.0

3.2.1.4.21 Grain yield plant^{-1} (g)

Weight of total grain yield of five plants was recorded in grams and the mean yield plant^{-1} was calculated after harvest.

3.2.1.5 Statistical analysis

The mean data in respect of various DUS characters were subjected to the ANOVA (Analysis of Variance) using INDOSTAT (9.2).

3.2.1.5.1 Analysis of variance

The analysis of variance for each character was calculated as per the standard statistical procedure given by Panse and Sukhatme (1961).

$$Y_{ij} = \mu + b_i + t_j + e_{ij}$$

where,

Y_{ij} : Performance of the j^{th} genotype in the i^{th} block

μ : General mean

b_i : True effect of i^{th} block

t_j : True effect of j^{th} genotype

e_{ij} : Random error associates with j^{th} genotype and i^{th} block

The structure of analysis of variance was as follows:

ANOVA Table for Randomized Block Design

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F ratio
Replications	(r – 1)	RSS	M_r = RSS / (r-1)	$\frac{M_r}{M_e}$
Treatments	(t – 1)	TrSS	M_t = Trss / (t-1)	$\frac{M_t}{M_e}$
Error	(r – 1) (t – 1)	ESS	M_e = ESS / (r-1) (t-1)	-
Total	(rt – 1)	TSS		

where,

r : Number of replications

t : Number of treatments

M_r : Mean sum of squares of replication

M_t : Mean sum of squares of treatment

M_e : Mean sum of squares of error

Test of significance for each character was carried out against the corresponding error degrees of freedom using ‘F’ table values given by Fisher and Yates (1963).

3.2.1.5.2 Estimation of genetic parameters

Phenotypic and genotypic variance

Phenotypic and genotypic variances were estimated using the following formula,

$$\text{Genotypic variance } (\sigma^2_g) = \frac{M_t - M_e}{r}$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + M_e = \frac{M_t - M_e}{r} + M_e$$

Coefficient of variation

The genotypic and phenotypic coefficients of variation were calculated using the formula (Burton, 1952).

a. Genotypic Coefficient of Variation (GCV)

$$\text{GCV} = \frac{\text{Genotypic standard deviation } (\sigma_g)}{\text{General mean } (\bar{X})} \times 100$$

b. Phenotypic Coefficient of Variation (PCV)

$$\text{PCV} = \frac{\text{Phenotypic standard deviation } (\sigma_g)}{\text{General mean } (\bar{X})} \times 100$$

The GCV and PCV values were classified as described by Sivasubramanian and Madhavanmenon (1973).

Classification	**GCV/ PCV**
Low	Less than 10%
Moderate	10 – 20%
High	More than 20%

Heritability

Heritability in broad-sense was computed as the ratio of genetic variance to the total phenotypic variance suggested by Johnson *et al.* (1955) and expressed as percentage.

$$\text{Heritability in broad-sense } (h^2_{bs}) = \frac{\text{Genotypic variance } (\sigma^2_g)}{\text{Phenotypic variance } (\sigma^2_p)} \times 100$$

Heritability in broad sense was categorized as per the classification given by Johnson *et al.* (1955).

Classification	**Heritability**
Low	Less than 30%
Moderate	30 – 60%
High	More than 60%

Genetic Advance

Genetic advance was calculated based on formula given by Johnson *et al.* (1955).

$$\text{Genetic Advance (GA)} = K \times \sigma_p \times h^2$$

where,

K : Selection differential at 5% selection intensity (2.06)

σ_p : Phenotypic standard deviation

h^2 : Heritability in broad sense

Genetic advance as per cent of mean (GAM)

$$\text{GAM} = \frac{\text{GA}}{\overline{X}} \times 100$$

where,

GA : Genetic advance

$\overline{X}$: Grand mean of the character

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

Classification	**GAM**
Low	Less than 10%
Moderate	10 – 20%
High	More than 20%

3.2.2 Molecular Characterization

3.2.2.1 Isolation of genomic DNA

The genomic DNA was isolated from leaves of 20-25 days old seedlings using Cetyl Trimethyl Ammonium Bromide (CTAB) method developed by Murray and Thompson (1980). The extraction buffer was prepared by using the chemicals and stock solutions as per the procedure given below.

3.2.2.2 Reagent stock solutions for genomic DNA extraction

Various reagents and stock solutions used for DNA extraction are given below:

a. 1 M Tris HCl (pH 8.0)

12.11 g Tris was dissolved in 80 ml distilled water and pH was adjusted to 8.0 by adding 1N HCl. Final volume was made up to 100 ml.

b. 0.5 M EDTA (pH 8.0)

18.6 g EDTA was dissolved in 80 ml distilled water by adding NaOH pellets simultaneously till it completely dissolves. Final volume was made up to 100 ml.

c. 5 M NaCl

29.2 g NaCl was dissolved in 80 ml distilled water and the final volume was made up to 100 ml.

d. CTAB extraction buffer: for 100 ml

2.0 per cent (w/v) CTAB (stock 20%) : 10 ml

100 mM Tris Cl (1 M stock, pH 8.0) : 10 ml

20 mM EDTA (0.5 M stock, pH 8.0) : 4 ml

140 mM NaCl (stock 5 M) : 28 ml

3.0 per cent PVP : 3 g

Sterile distilled water was added to make up the total volume to 100 ml then pH was adjusted to 8.0 and autoclaved at 121^{o}C temperature, 15 psi pressure for 20 minutes.

e. Choloform: Isoamyl alcohol (24:1):

The solution was prepared by mixing 24 parts of chloroform and 1 part of isoamylalcohol and stored at 4^{o}C in an amber coloured bottle.

f. Ethanol 70 per cent (v/v)

70 ml of ethanol was mixed with 30 ml distilled water to make 100 ml of 70% of ethanol and stored at room temperature.

3.2.2.3 Protocol of genomic DNA isolation

1. Healthy leaf samples of 2-3 cm size (approximately 0.5 g) were taken from 20-25 days old seedlings and cut into small pieces and placed in a small sized (11 cm diameter) alcohol sterilized porcelain mortar.

2. About 400 µl of extraction buffer (100 mM Tris HCl, pH 8.0; 20 mM EDTA; 140 mM NaCl and 2% CTAB) was added to the leaf sample and was ground with the help of alcohol sterilized porcelain pestle until it was completely homogenized.

3. Another 300 µl of extraction buffer was added to the mortar containing the homogenized leaf sample. Using a micropipette of 1 ml capacity (the tip was cut at the bottom using a sterile scissor), the entire contents from the mortar were transferred to a fresh 1.5 ml capacity micro centrifuge tube.

4. These tubes were incubated at 65°C in a hot water bath (KEMI, KWB-220) for 45 minutes. The contents were mixed gently inverting the tubes for every 10-15 minutes.

5. The tubes were cooled to room temperature and 400-500 µl chloroform: isoamylalcohol (24:1) was added to each tube. The contents were mixed by inverting the tubes several times.

6. The tubes were centrifuged (Eppendorf, mod. 5424R) at 10,000 rpm for about 10 minutes.

7. After centrifugation, the upper clear aqueous supernatant was aspirated from the tube using micropipette without disturbing the intermediate layer and transferred to a fresh 1.5 ml microcentrifuge tube.

8. The steps 5-7 were repeated 2 times to ensure that there was no protein contamination left.

9. To this clear supernatant, an equal volume of 500-600 µl chilled isopropanol was added to each tube and the contents were mixed by inverting the tubes several times and the tubes were kept at -20°C for 1 hour.

10. The samples were then mixed gently and centrifuged at 13,000 rpm for 15 minutes at 4°C and the supernatant was drained gently without disturbing the DNA pellet at the bottom of the tubes.

11. About 300 µl of 70% ethanol was added in to the pellet and the centrifuge tube was tapped in order to dispense the pellet in ethanol. The contents were centrifuged at 12,000 rpm for 5 minutes at 4ºC. The supernatant was drained and 70% ethanol washing was repeated again.
12. After centrifugation, the supernatant was decanted. The pellet was air dried for 15-20 minutes at room temperature. After complete drying of the pellet, the pellet was dissolved in 100 µl sterilized distilled water.
13. The dissolved DNA was stored at -20ºC after proper labelling.

3.2.2.4 Quantification of DNA

The isolated DNA was quantified using Nanodrop (ND-1000, Thermo Scientific, Nanodrop Technologies, U.S.A).

1. The upper and lower pedestals of lifted sampling arm of Nanodrop were wiped by using sterilized distilled water and 2µl of distilled water was loaded into hole located on the lower pedestal.
2. The upper arm was closed and the operating software in the attached computer was initiated and the instrument was calibrated to zero. Then sampling arm was lifted and loaded water was wiped out by a paper towel.
3. Then 1 or 2µl of genomic DNA sample was loaded in the same manner and the reading was noted under the 260/280 nm spectral ratio and the curve given by the software was observed. After taking the readings, both the arms of the instrument were soft wiped by a clean paper towel. Then DNA was diluted to 50 ng $µl^{-1}$ based on the reading provided by the machine.
4. The ratio of OD_{260} / OD_{280} provides an estimate of purity of nucleic acid. Pure DNA preparations have OD_{260} / OD_{280} value of 1.8-2.0.

3.2.2.5 Template DNA amplification in PCR

Two microliters of diluted template DNA (50 ng µl^{-1}) from each genotype was added into separate PCR tubes (0.2 ml) and kept at 4°C until the other components were added. Then 8 µl of master mixture including PCR components was added into each properly labelled PCR tubes. The master mixture for PCR amplification was prepared as given below. The reaction was carried out in a total reaction volume of 10 µl.

Composition of the PCR mixture and ingredients concentration

Components	Stock concentration	Working concentration	Volume (µl)
PCR buffer with Mg^{2+}	10X	1X	1
dNTPs	1 mM	0.1 mM	0.5
Forward primer	100 pM µl^{-1}	5 pM µl^{-1}	0.5
Reverse primer	100 pM µl^{-1}	5 pM µl^{-1}	0.5
Taq Polymerase enzyme	5 U µl^{-1}	0.5 U µl^{-1}	0.1
Autoclaved double distilled water	-	-	5.4
Template DNA	-	50 ng µl^{-1}	2
Total			10 µl

dNTPs

The dNTPs used in the PCR reaction were purchased from Bangalore Genei pvt. Ltd., India.

DNA polymerase

Taq DNA polymerase is a thermostable enzyme which has 5’ to 3’ polymerase and 3’ to 5’ exonuclease activity with 72ºC as optimum temperature. The enzyme (5 U µl^{-1}) was obtained from Bangalore Genei pvt. Ltd., India.

Primer

Twenty-five gene-specific primers were used for amplification and the DNA fingerprinting was carried out using the primers mentioned in Table 3.3.

Table 3.3. List of primers included under study

S. No.	Primer	Primer sequence	Annealing Temp.	Trait	Gene	Chromosome number	Reference
1	DEP 1-1	F-TTTCGGTGGATCGGGTAT	58°C	Grain number	*DEP1*	9	Li *et al*. (2017)
		R-CGTGACACCGCTAATCGT					
2	Dep1S9	F-TGGACACTTGTTATCTTCTCAT	55°C	Grain number	*DEP1*	9	Ngangkham *et al*. (2018)
		R-AACTGGAAGTTTGTAACACTCA					
3	Ehd1 -2	F-TGAAATATGTTGCAAATGGC	57°C	Early heading date	*Ehd1*	10	
		R-GCAGATGACTGGCAACTTGC					
4	Ehd1 -3	F-AACTGATGCAGAAATACAGG	57°C	Early heading date	*Ehd1*	10	
		R-TAGAAATTCCAAAAACATGG					
5	GLW7	F-CCCGCCTTTATATCCCTTTC	52°C	Grain length and grain weight	GLW7	7	Si *et al*. (2016)
		R-CTGGAGGAGGGTGGAGAGAG					
6	GW8Pro2	F-CAAGGGAAGCAGAAACCCAGCAA	63°C	Grain width	*GW8*	8	Wang *et al*. (2012)
		R- GCAGCCGATGACGACGATACCG					
7	OsSnb-2	F-CGTTGTATTTTGCTCCATCGTTTG	59°C	Grain size	*OsSNB*	7	Ma *et al*. (2019)
		R-CTATGCTTTGTTTGACTTGACTCTG					
8	Hd1 InDel-2	F-CGGGTACAATTCGTACTACG	55°C	Heading date	*Hd1*	6	
		R-ATCTGTGTAAGCACTGACGC					
9	Hd3a	F-TTTTGCAAGATCGATGGCCG	59°C	Heading date	*Hd3*	6	
		R- TCGTTAGCTAGTTGTAGACC					
10	HY2-4	F-TTGATACTCGTCTTCGGATAGC	57°C	Grain number	*GN2*	2	Chen *et al*. (2017)
		R- GACTGACCTGACACACAAGGT					
11	LRR DEL	F-GTGGATCGAGAGAAGACAAG	55°C	Gall midge resistance	*Gm4*	8	Suvendhu *et al*. (2014)
		R- CTTGAGGACGATATTCAAGC					
12	PAY1SP6	F-TTGGATGAAAGGGAGATTTT	53°C	Grain yield	*PAY1*	8	Zhao *et al*. (2015a)
		R- GTCAAAGAACAGCACACCAG					

F : Forward; **R :** Reverse

Cont…

Table 3.3. Cont…

S. No.	Primer	Primer sequence	Annealing Temp.	Trait	Gene	Chromosome number	Reference
13	RGS 1	F-TCCACCTGCAGATTTCTTCC R-GCTGGTCTTGCACATCTCTCT	55°C	Grain length	*GS3*	3	Wang *et al.* (2011)
14	RM 202	F-TGGAACACCCATAGACAA R- TGGCAAGTGGTATTCTTC	55°C	Kernel length	*KL*	11	Agrama *et al*. (2007)
15	RM 208	F-AGTACCACCACCATTCTCG R-TCCATTGGCCATCAGTTCTCG	55°C	Blast resistant	*Pi39*, *Pi-b and Pid1(t)*	2	Agrama *et al*. (2007)
16	RM228	F-CTGGCCATTAGTCCTTGG R-GCTTGCGGCTCTGCTTAC	55°C	Grain yield and Length width ratio	GY and LWR	10	Agrama *et al*. (2007)
17	RM 5499	F-TGGAGTACGACGTGATCGTG R- CAGAAACGGGAGGGGATC	55°C	Grain length	*Ghd7*	7	Zhao *et al*. (2015b)
18	RM 7289	F-GGCCCACGACTTAATAGACATCG F-GGCAATATGATATGACCAGCAC	51°C	Panicle length	*LP1*	9	Liu *et al*. (2016)
19	RMw-513	F-GTATTTGTTTGTCGCATTC R- TAGGACCATAGATGTGAGTTA	51°C	Grain width and grain length	*gw5*	5	Wan *et al*. (2008)
20	S5-1	F-GGCAATATGATATGACCAGCAC R- ATGTAGCGAAGACAAAGGGAGT	53°C	Hybrid sterility	*S5*	6	Li *et al*. (2017)
21	S5803-5	F-CCATGACAATCGATGCTGAA R- TGGTTGTGAAGGTGATTGCT	56°C	Erect panicle	*EP3*	2	Piao *et al.* (2009)
22	sd1-h	F-GACTCAACAGGCCCTCCAAA R- CCACGCGGTTATTGCAAGTT	59°C	Short and thick culm	*sd-1*	1	Monna *et al.* (2002)
23	SPIKE-indel3	F-GGAGAGACATGGACGGCT R- TGGTGGCGATCATGCTGC	58°C	Grain number per panicle	*SPIKE*	8	Kim *et al*. (2016)
24	Sub1	F-AAAACAATGGTACCATACGAGAC R- GCCTATCAATGCGTGCTCTT	56°C	Submergence tolerance	Sub1	1	Pradhan *et al*. (2015)
25	RM17242	F-GGCAGCATGAACACTTAT R- TCTAACCTAGGCCTGGAT	58°C	Awn length	*LABA1*	4	Hua *et al*. (2015)

F : Forward; **R :** Reverse

PCR assay buffer

The PCR assay buffer was supplied as 10X solution.

The master mix (minus DNA template) was prepared to reduce pipetting error. The final volume of master mix was calculated based on the number of samples with 10% extra volume. The master mix was vortexed for 5 sec. for mixing of the components. Subsequently, 8 µl of master mix was added into each PCR tube having 2 µl of template DNA to make the final volume to 10 µl. Tubes were covered properly by its cap and placed in a programmable thermal cycler (Eppendorf, Germany) for the reaction to take place as given below.

Temperature profile used for PCR amplification using rice gene-specific markers

Steps	Activity	Temperature (°C)	Duration	Cycles
1.	Initial denaturation	94	5 min.	1
2.	Denaturation	94	30 sec.	34
3.	Annealing	(55-65) based on primer	30 sec.	34
4.	Extension	72	1 min.	34
5.	Final Extension	72	10 min.	1
6.	Storage	4	∞	-

3.2.2.6 Agarose gel electrophoresis

Agarose gel electrophoresis technique is simple, rapid to perform and also capable of resolving fragments of DNA.

Reagents/ stock solutions for agarose gel electrophoresis

a) Ethidium bromide solution

10 mg ml^{-1} of ethidium bromide solution was prepared by dissolving 50 mg of ethidium bromide in 5 ml of distilled water.

b) Running buffer (10X TBE)

The running buffer was prepared by dissolving 108 g of Tris base in 500 ml double distilled water, to which 55 g of Boric acid and 9.3 g of EDTA were added and mixed well by continuous stirring on magnetic stirrer. Then the solution was made up to 1000 ml using double distilled water. Later, the solution was sterilized by autoclaving at 121°C temperature, 15 psi pressure for 20 minutes. Later, 0.5X TBE was prepared by diluting 50 ml of 10X TBE to 950 ml by the addition of sterilized distilled water.

c) Gel loading dye (6X): 10 ml

Bromophenol blue : 25 mg

Xylene cyanol FF : 25 mg

Glycerol : 3.3 ml

Double distilled water : 6.7 ml

6.7 ml of double distilled water was added and an aliquot was prepared and stored at -20°C.

Procedure

1. The open ends of a clean, dry gel casting tray supplied with the electrophoresis apparatus were sealed with tape so as to form a mould. The mould was set on a horizontal section of a bench.

2. Agarose gel was prepared by dissolving appropriate amount to make 3% of agarose in 0.5X TBE buffer. Agarose is dissolved by heating the solution at 100°C. Then it is allowed to cool and EtBr (0.5 µg ml^{-1}) was added and mixed well. The melted agarose was poured into gel mould. A comb with adequate number of wells was inserted to make the wells.

3. After complete setting of the gel, the comb was removed carefully, the tape was removed and the gel was mounted in an electrophoresis tank.
4. The gel loading dye, bromophenol blue was mixed with the DNA samples in 1:5 ratio and loaded in the wells with a micropipette.
5. Electrophoresis was done at 100V for 1-2 hrs.
6. The gel was visualized in gel documentation system (Alpha Innotech Cooperation, U.S.A) and the banding pattern of the gel picture was scored visually.

Reproducibility of PCR assay was tested by performing duplicate reactions at different times by using identical genotypes and primer combinations and only reproducible bands was recorded.

3.2.2.7 Data analysis and detection of genetic diversity

The amplified products for marker analysis were scored visually based on the presence (taken as '1') or absence (taken as '0') of band for each primer. Each marker fragment was treated as a unit character and only clear and unambiguous bands were scored. The PIC was calculated using GeneCalc (Bio-informatics tool). For accessions being used in this study, QR (Quick Response) codes were generated using online tool (available at www.barcode-generator.org).

The molecular data was used to generate Jaccard's coefficient of similarity for expressed marker bands. The Jaccard's coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). The computer package NTSYS-pc (Rohlf, 1998) was used for cluster analysis and Hierarchical Clustering analysis for morphological characters was performed by SPSS 2.0.

Chapter ~ IV

Results & Discussion

Chapter – IV

RESULTS AND DISCUSSION

Identification of rice varieties is vital for protecting Plant Breeders' Rights and also during the trade and seed production. There are two strategies commonly used for cultivar identification such as morphological descriptors and molecular or DNA markers. Traditionally, morphological descriptors are used for testing Distinctness, Uniformity and Stability (DUS), as they are less expensive and easy to measure without having sophisticated laboratory facilities. However, limited number, stage-specific and inconsistent expression due to the environmental influence, renders DUS descriptors unsuitable to exploit extensively for rapid identification of cultivars. Furthermore, they failed to distinguish large number of varieties or breeding lines with slight variation in agronomic traits- a common phenomenon in recently released varieties. These problems of the morphological descriptors can be solved efficiently by molecular markers as they are stable, abundant with high discriminating power, although expensive. In the present investigation, we have developed fingerprints for 52 rice varieties released from various research stations in India employing both DUS descriptors and molecular markers.

The results obtained from the above investigation are discussed under the following headings.

4.1 Morphological characterization of rice varieties

4.2 Analysis of variance and genetic variability studies

4.3 Identification of rice varieties using molecular markers

4.4 Genetic divergence

4.1 MORPHOLOGICAL CHARACTERIZATION OF RICE VARIETIES

4.1.1 DUS Descriptors in Rice Varieties

In rice, in total, 62 DUS descriptors have been notified by the Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) in India for conducting DUS test. In the present study, 52 rice varieties were characterized using 22 DUS descriptors. The varietal-specific DUS traits (22 traits) of 52 rice varieties recorded in the present study as per DUS guidelines are presented in the Table 4.1. DUS descriptors related to leaf and panicle is presented in Plate 1, grain and decorticated grain in Plate 2.

4.1.1.1 Basal leaf sheath colour

This character was found to be dimorphic and exhibited two variations among 52 genotypes *viz*., green (51) and only one genotype (Varadhan) exhibited purple lines. Similar results were reported by Umarani *et al.* (2017a), Kumar *et al*. (2018) and Gour *et al*. (2019).

4.1.1.2 Leaf auricles

Auricles are hinge like structures that function to project the leaf away from the vertical axis of the plant. Among the 52 genotypes studied, 51 genotypes exhibited presence of leaf auricle and only one genotype (Vikramarya) exhibited absence of leaf auricle. This finding was also confirmed by Sanyal and Joshi (2016).

4.1.1.3 Leaf collar

There was no variation in the genotypes for this character. All the genotypes (100%) included in the present study showed presence of leaf collar. This result was in agreement with the findings of Kumar *et al*. (2018) and Gour *et al*. (2019).

4.1.1.4 Leaf ligule

The ligule appears to be a continuation of the leaf sheath and encircles or clasps the stem as does the leaf sheath. Traditionally, it has been assumed in protecting the culm and leaves that it encloses from the entry of water, dust and harmful spores. There was no variation in the genotypes for this character. All the genotypes showed presence of leaf ligule. Similar findings were reported by Sanyal and Joshi (2016), Kumar *et al*. (2018) and Gour *et al*. (2019).

4.1.1.5 Shape of leaf ligule

All the genotypes included under this study reported split shape of leaf ligule. Similar results were reported by Kumar *et al*. (2018) and Gour *et al*. (2019).

4.1.1.6 Colour of leaf ligule

Only one genotype (Varadhan) exhibited purple coloured ligule and the remaining 51 genotypes exhibited white coloured ligule. These results were in accordance with Komala *et al*. (2017), Kumar *et al*. (2018) and Gour *et al*. (2019).

4.1.1.7 Time of heading (50% of plants with panicles) (days)

The experimental material was categorized into four classes based on this trait. The time of heading of four genotypes (N22, Udayagiri, Vandana and Varalu) was very early with less than 71 days duration, five genotypes were of early heading type with the duration ranging from 71-90 days, 10 genotypes were found to be late with 111-130 days to heading and the remaining 33 genotypes were found to be medium with 91-110 days to heading. Similar results were reported by Kalyan *et al*. (2017), Umarani *et al.* (2017a), Rawte and Saxena (2018) and Aravind *et al*. (2019).

4.1.1.8 Lemma anthocyanin colouration of area below apex

This character was found to be dimorphic and exhibited two variations. Among the 52 genotypes studied, 51 genotypes showed absence of anthocyanin colouration and only one genotype (Varadhan) showed presence of anthocyanin colouration of area below apex of lemma. Sanyal and Joshi (2016), Kumar *et al.* (2018), Manjunatha *et al.* (2018) and Gour *et al.* (2019) have also reported the similar results pertaining to this trait.

4.1.1.9 Stem length (excluding panicle; excluding floating rice) (cm)

Based on the stem length, the experimental material was grouped into three classes. Very short stem length (< 91 cm) was recorded by six genotypes (Govind, NLR34449, Pankaj, Udayagiri, Varalu and Saket-4), 15 genotypes were found to have medium stem length ranging from 110-130 cm. And the remaining 31 genotypes recorded short stem length ranging from 91-110 cm. These results were in accordance with Kalyan *et al.* (2017), Umarani *et al.* (2017a), Rawte and Saxena (2018) and Aravind *et al.* (2019).

4.1.1.10 Panicle length of main axis (cm)

According to panicle length of main axis, the genotypes were categorized into three groups, dominated by 36 genotypes with medium sized panicles (21-25 cm), 12 genotypes had long panicles (26-30 cm) and four genotypes (JGL17004, NLR34449, Pankaj and Rajeswari) had short panicles (16-20 cm). Similar results pertaining to this trait were reported by Kalyan *et al.* (2017), Umarani *et al.* (2017a), Rawte and Saxena (2018) and Aravind *et al.* (2019).

4.1.1.11 Panicle number per plant

The experimental material in the present study was dimorphic for this character. Out of 52 genotypes, 14 genotypes (BPT2295, Govind, IR64, JGL17004, JGL3844, Kavya, MTU1121, MTU1140, MTU1153, MTU1155, MTU1156, Varalu, Varadhan and WGL32100) possessed fewer panicles per plant (<11) and the remaining 38 genotypes possessed medium number (11-20) of panicles. These results were in accordance with the findings of Kalyan *et al.* (2017), Umarani *et al.* (2017a), Rawte and Saxena (2018) and Aravind *et al*. (2019).

4.1.1.12 Panicle awns

Among the 52 genotypes, only six genotypes (DRR Dhan 38, Kasturi, Nidhi, Pusa Basmati-1, PUSA1121 and Varadhan) possessed awns and the remaining 46 genotypes were awn-less. This result was in accordance with the findings of Sanyal and Joshi (2016), Kumar *et al.* (2018), Manjunatha *et al*. (2018) and Gour *et al*. (2019).

4.1.1.13 Panicle distribution of awns

Out of 52, only six genotypes possessed awns. Out of these six, only one genotype (PUSA1121) possessed awns at tip only and the remaining five genotypes (DRR Dhan 38, Kasturi, Nidhi, Pusa Basmati-1 and Varadhan) possessed awns throughout the whole length of panicle. Sanyal and Joshi (2016), Kumar *et al.* (2018), Manjunatha *et al*. (2018) and Gour *et al*. (2019) have also reported the similar results.

4.1.1.14 Presence of panicle secondary branching

All the genotypes showed presence of secondary branching of the panicles. There was no variation in the genotypes with respect to this character. Similar results were reported by Sanyal and Joshi (2016) and Manjunatha *et al*. (2018).

4.1.1.15 Panicle secondary branching

The experimental material in the present study was dimorphic for this character. Out of 52 genotypes, 39 genotypes possessed strong secondary branches and 13 genotypes possessed weak secondary branches. This finding was also reported by Manjunatha *et al*. (2018) and Ghosh *et al*. (2019).

4.1.1.16 Time of maturity (days)

The genotypes in the present study were categorized into four classes based on this character. The time of maturity of four genotypes (N22, Udayagiri, Vandana and Varalu) was very early with less than 100 days duration, five genotypes were of early maturing type with the duration ranging from 101-120 days, 10 genotypes were found to be late with 141-160 days to maturity and the remaining 33 genotypes were of medium duration (121-140) to days to maturity. These results were in accordance with Kalyan *et al.* (2017), Umarani *et al.* (2017a), Rawte and Saxena (2018) and Aravind *et al.* (2019).

4.1.1.17 Weight of 1000 fully developed grains (g)

Based on this character, the genotypes in the present study were categorized into four classes. Very low 1000 grain weight (<15 g) was recorded by five genotypes (BPT1235, NLR34449, Rasi, RP Bio-226 and Siddhi), 15 genotypes recorded low 1000 grain weight ranging from 15-20 g and seven genotypes recorded high 1000 grain weight ranging from 26-30 g and the remaining 25 genotypes recorded medium 1000 grain weight ranging from 21-25 g. Mondal *et al.* (2014) and Komala *et al.* (2017) recorded the similar results.

4.1.1.18 Grain length (mm)

This trait was observed to be dimorphic. Out of 52 genotypes, 19 genotypes had medium sized grains (8.6-10.5 mm) and the remaining 33 genotypes had short grains (6.1-8.5 mm). Similar results were reported by Mondal *et al.* (2014) and Komala *et al.* (2017).

4.1.1.19 Grain width (mm)

The genotypes in the present study were categorized into three classes with respect to width of the grain. 27 genotypes were narrow (2.1-2.5 mm), 22 genotypes had medium grain width (2.6-3.0 mm) and remaining three genotypes (Govind, MTU3626 and Varadhan) had broad grain width (3.1-3.5 mm). These results were in agreement with the findings of Komala *et al.* (2017).

4.1.1.20 Decorticated grain length (mm)

In the present study, decorticated grain length of 52 genotypes was categorized into three groups. 21 genotypes were short (< 6.0 mm), 21 genotypes were long (6.0-6.6 mm) and 10 genotypes were long for Basmati type (> 6.61 mm). Mondal *et al*. (2014), Komala *et al*. (2017) and Umarani *et al*. (2017a) have recorded the similar results.

4.1.1.21 Decorticated grain width (mm)

The expression of this character among the genotypes was trimorphic *viz*., narrow (<2.0 mm) in 14 genotypes (BPT1235, BPT5204, JGL17004, JGL3844, Krishna hamsa, Nidhi, NLR34449, PUSA1121, Rasi, RP Bio-226, Shonini, Siddhi, Vikramarya and WGL32100), broad (> 2.5 mm) in three genotypes (Jaya, MTU3626 and Udayagiri) and medium (2.0-2.5 mm) in the remaining 35 genotypes. Similar results were reported by Mondal *et al*. (2014), Komala *et al*. (2017) and Umarani *et al*. (2017a).

4.1.1.22 Decorticated grain shape (in lateral view)

In the present study, decorticated grain shape of the genotypes was categorized into three groups *viz*., short slender (21), long slender (21) and long slender for Basmati type (10). The results obtained were similar with Mondal *et al*. (2014), Komala *et al*. (2017) and Umarani *et al*. (2017a).

Of 22 morphologically assessed DUS descriptors, four were monomorphic, nine were dimorphic and the remaining nine were polymorphic. The above mentioned results are summarised in the Table 4.2 and Table 4.3.

4.1.2 Identification of Rice Varieties using DUS Descriptors

In the present study, 22 DUS descriptors were recorded in 52 released rice varieties. Of them, four were monomorphic, nine were dimorphic and the remaining nine were polymorphic. Earlier, Sanyal and Joshi (2016) recorded

Table 4.1. DUS descriptors of 52 rice varieties used in the study

S. No.	Variety	BLSC	LA	LC	LL	SLL	CLL	TH	LABA	SL	PLMA	PNPP
1	Bhadrakali	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
2	BPT1235	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
3	BPT2295	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Few
4	BPT5204	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Medium
5	DRR Dhan 38	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Long	Medium
6	Govind	Green	Present	Present	Present	Split	White	Medium	Absent	Very short	Medium	Few
7	IR64	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Few
8	Jaya	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Long	Medium
9	JGL17004	Green	Present	Present	Present	Split	White	Early	Absent	Short	Short	Few
10	JGL3844	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Few
11	Kasturi	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Medium
12	Kavya	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Few
13	Krishna hamsa	Green	Present	Present	Present	Split	White	Early	Absent	Short	Long	Medium
14	MTU1001	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Medium
15	MTU1010	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
16	MTU1121	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Few
17	MTU1140	Green	Present	Present	Present	Split	White	Late	Absent	Medium	Medium	Few
18	MTU1153	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Few
19	MTU1155	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Long	Few
20	MTU1156	Green	Present	Present	Present	Split	White	Early	Absent	Short	Medium	Few
21	MTU1172	Green	Present	Present	Present	Split	White	Late	Absent	Medium	Long	Medium
22	MTU1190	Green	Present	Present	Present	Split	White	Late	Absent	Medium	Long	Medium
23	MTU3626	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Medium
24	N22	Green	Present	Present	Present	Split	White	Very early	Absent	Short	Long	Medium
25	Nidhi	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium

BSLC : Basal Leaf Sheath Colour; **LA :** Leaf Auricles; **LC :** Leaf Collar; **LL :** Leaf Ligule; **SLL :** Shape of Leaf Ligule; **CLL :** Colour of Leaf Ligule; **TH :** Time of Heading; **LABA :** Lemma Anthocyanin colouration of area Below Apex; **SL :** Stem Length; **PLMA :** Panicle Length of Main Axis; **PNPP :** Panicle Number Per Plant.

Cont…

Table 4.1. Cont…

S. No.	Variety	BLSC	LA	LC	LL	SLL	CLL	TH	LABA	SL	PLMA	PNPP
26	NLR34449	Green	Present	Present	Present	Split	White	Medium	Absent	Very short	Short	Medium
27	Pankaj	Green	Present	Present	Present	Split	White	Medium	Absent	Very short	Short	Medium
28	Pothana	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Long	Medium
29	PR106	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
30	Pusa Basmati-1	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Long	Medium
31	PUSA1121	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Medium	Medium
32	Rajeswari	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Short	Medium
33	Rasi	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
34	RP Bio -226	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
35	Sasyasree	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
36	Shobini	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Long	Medium
37	Siddhi	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Medium
38	Sona Mahsuri	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Medium
39	Sonasali	Green	Present	Present	Present	Split	White	Medium	Absent	Medium	Long	Medium
40	Swarna sub1	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Medium
41	Tellahamsa	Green	Present	Present	Present	Split	White	Early	Absent	Short	Medium	Medium
42	Triguna	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Long	Medium
43	Udayagiri	Green	Present	Present	Present	Split	White	Very early	Absent	Very short	Medium	Medium
44	Vajram	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
45	Vandana	Green	Present	Present	Present	Split	White	Very early	Absent	Medium	Medium	Medium
46	Varalu	Green	Present	Present	Present	Split	White	Very early	Absent	Very short	Medium	Few
47	Varadhan	Purple lines	Present	Present	Present	Split	Purple	Medium	Strong	Short	Medium	Few
48	Vasumati	Green	Present	Present	Present	Split	White	Late	Absent	Short	Medium	Medium
49	Vikramarya	Green	Absent	Present	Present	Split	White	Medium	Absent	Short	Medium	Medium
50	Warangal Samba	Green	Present	Present	Present	Split	White	Late	Absent	Medium	Medium	Medium
51	WGL32100	Green	Present	Present	Present	Split	White	Medium	Absent	Short	Medium	Few
52	Saket-4	Green	Present	Present	Present	Split	White	Early	Absent	Very short	Medium	Medium

BSLC : Basal Leaf Sheath Colour; **LA :** Leaf Auricles; **LC :** Leaf Collar; **LL :** Leaf Ligule; **SLL :** Shape of Leaf Ligule; **CLL :** Colour of Leaf Ligule; **TH :** Time of Heading, **LABA :** Lemma Anthocyanin colouration of area Below Apex; **SL :** Stem Length; **PLMA :** Panicle Length of Main Axis; **PNPP :** Panicle Number Per Plant

Cont…

Table 4.1. Cont…

S. No.	Variety	PA	PDA	PPSB	PSB	TM	WTG	GL	GW	DGL	DGW	DGS
1	Bhadrakali	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long*	Medium	Long slender*
2	BPT1235	Absent	-	Present	Strong	Medium	Very low	Short	Narrow	Short	Narrow	Short slender
3	BPT2295	Absent	-	Present	Strong	Late	Medium	Short	Medium	Short	Medium	Short slender
4	BPT5204	Absent	-	Present	Strong	Late	Low	Short	Narrow	Short	Narrow	Short slender
5	DRR Dhan 38	Present	Whole length	Present	Weak	Medium	Medium	Medium	Narrow	Long	Medium	Long slender
6	Govind	Absent	-	Present	Weak	Medium	High	Medium	Broad	Long	Medium	Long slender
7	IR64	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long*	Medium	Long slender*
8	Jaya	Absent	-	Present	Strong	Medium	High	Short	Medium	Long	Broad	Long slender
9	JGL17004	Absent		Present	Strong	Early	Low	Short	Narrow	Short	Narrow	Short slender
10	JGL3844	Absent	-	Present	Strong	Medium	Low	Short	Narrow	Short	Narrow	Short slender
11	Kasturi	Present	Whole length	Present	Weak	Medium	Medium	Short	Medium	Long	Medium	Long slender
12	Kavya	Absent	-	Present	Strong	Medium	Medium	Short	Narrow	Long	Medium	Long slender
13	Krishna hamsa	Absent	-	Present	Weak	Early	Medium	Medium	Narrow	Long*	Narrow	Long slender*
14	MTU1001	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long	Medium	Long slender
15	MTU1010	Absent	-	Present	Strong	Medium	Medium	Medium	Narrow	Long	Medium	Long slender
16	MTU1121	Absent	-	Present	Strong	Medium	Medium	Short	Narrow	Long	Medium	Long slender
17	MTU1140	Absent	-	Present	Strong	Late	Low	Short	Narrow	Short	Medium	Short slender
18	MTU1153	Absent	-	Present	Strong	Medium	Medium	Short	Medium	Long*	Medium	Long slender*
19	MTU1155	Absent	-	Present	Strong	Medium	Medium	Short	Medium	Long	Medium	Long slender
20	MTU1156	Absent	-	Present	Strong	Early	Medium	Medium	Medium	Long	Medium	Long slender
21	MTU1172	Absent	-	Present	Strong	Late	Medium	Short	Narrow	Short	Medium	Short slender
22	MTU1190	Absent	-	Present	Strong	Late	Low	Short	Narrow	Short	Medium	Short slender
23	MTU3626	Absent	-	Present	Strong	Medium	High	Medium	Broad	Long*	Broad	Long slender*
24	N22	Absent	-	Present	Weak	Very early	Medium	Short	Medium	Short	Medium	Short slender
25	Nidhi	Present	Whole length	Present	Strong	Medium	Low	Medium	Narrow	Long*	Narrow	Long slender*

PA : Panicle Awns; **PDA :** Panicle Distribution of Awns; **PPSB :** Presence of Panicle Secondary Branching; **PSB :** Panicle Secondary Branching; **TM :** Time of Maturity; **WTG :** Weight of 1000 fully developed Grains; **GL :** Grain Length; **GW :** Grain Width; **DGL :** Decorticated Grain Length; **DGW :** Decorticated Grain Width; **DGS :** Decorticated Grain Shape (in lateral view).

Cont…

Table 4.1. Cont…

S. No.	Variety	PA	PDA	PPSB	PSB	TM	WTG	GL	GW	DGL	DGW	DGS
26	NLR34449	Absent	-	Present	Strong	Medium	Very low	Short	Narrow	Short	Narrow	Short slender
27	Pankaj	Absent	-	Present	Weak	Medium	Medium	Short	Medium	Long	Medium	Long slender
28	Pothana	Absent	-	Present	Strong	Medium	Low	Short	Narrow	Short	Medium	Short slender
29	PR106	Absent	-	Present	Strong	Medium	Low	Medium	Narrow	Long	Medium	Long slender
30	Pusa Basmati-1	Present	Whole length	Present	Weak	Medium	Medium	Medium	Narrow	Long*	Medium	Long slender*
31	PUSA1121	Present	Tip only	Present	Weak	Medium	High	Short	Medium	Long	Narrow	Long slender
32	Rajeswari	Absent	-	Present	Weak	Medium	Medium	Short	Narrow	Long	Medium	Long slender
33	Rasi	Absent	-	Present	Strong	Medium	Very low	Short	Narrow	Short	Narrow	Short slender
34	RP Bio -226	Absent	-	Present	Strong	Medium	Very low	Short	Narrow	Short	Narrow	Short slender
35	Sasyasree	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long	Medium	Long slender
36	Shobini	Absent	-	Present	Strong	Medium	Low	Medium	Narrow	Long	Narrow	Long slender
37	Siddhi	Absent	-	Present	Strong	Late	Very low	Short	Narrow	Short	Narrow	Short slender
38	Sona Mahsuri	Absent	-	Present	Strong	Late	Low	Short	Medium	Short	Medium	Short slender
39	Sonasali	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long*	Medium	Long slender*
40	Swarna sub1	Absent	-	Present	Strong	Late	Low	Short	Medium	Short	Medium	Short slender
41	Tellahamsa	Absent	-	Present	Weak	Early	Medium	Medium	Medium	Long	Medium	Long slender
42	Triguna	Absent	-	Present	Weak	Medium	High	Medium	Medium	Long*	Medium	Long slender*
43	Udayagiri	Absent	-	Present	Weak	Very early	Medium	Short	Medium	Short	Broad	Short slender
44	Vajram	Absent	-	Present	Strong	Medium	Medium	Medium	Medium	Long*	Medium	Long slender*
45	Vandana	Absent	-	Present	Weak	Very early	Medium	Short	Narrow	Long	Medium	Long slender
46	Varalu	Absent	-	Present	Strong	Very early	Low	Medium	Narrow	Long	Medium	Long slender
47	Varadhan	Present	Whole length	Present	Strong	Medium	High	Short	Broad	Long	Medium	Long slender
48	Vasumati	Absent	-	Present	Strong	Late	Low	Short	Medium	Short	Medium	Short slender
49	Vikramarya	Absent	-	Present	Strong	Medium	High	Short	Narrow	Short	Narrow	Short slender
50	Warangal Samba	Absent	-	Present	Strong	Late	Low	Short	Narrow	Short	Medium	Short slender
51	WGL32100	Absent	-	Present	Strong	Medium	Low	Short	Narrow	Short	Narrow	Short slender
52	Saket-4	Absent	-	Present	Strong	Early	Medium	Short	Medium	Long	Medium	Long slender

PA : Panicle Awns; **PDA :** Panicle Distribution of Awns; **PPSB :** Presence of Panicle Secondary Branching; **PSB :** Panicle Secondary Branching; **TM :** Time of Maturity; **WTG :** Weight of 1000 fully developed Grains; **GL :** Grain Length; **GW :** Grain Width; **DGL :** Decorticated Grain Length; **DGW :** Decorticated Grain Width; **DGS :** Decorticated Grain Shape (in lateral view).

Table 4.2. List of monomorphic, dimorphic and polymorphic DUS descriptors observed in the present study

S. No.	Monomorphic	Dimorphic	Polymorphic
1	Leaf ligule	Basal leaf sheath colour	Time of heading (50% of plants with panicles)
2	Leaf collar	Leaf auricles	Stem length (excluding panicle; excluding floating rice)
3	Shape of leaf ligule	Colour of leaf ligule	Panicle length of main axis
4	Presence of panicle secondary branching	Lemma anthocyanin colouration of area below apex	Time of maturity
5		Panicle number per plant	Weight of 1000 fully developed grains
6		Panicle awns	Grain width
7		Panicle distribution of awns	Decorticated grain length
8		Panicle secondary branching	Decorticated grain width
9		Grain length	Decorticated grain shape (in lateral view)

Table 4.3. Frequency distribution of 52 rice varieties for various DUS traits

S. No.	Characteristics	States	No. of accessions	Frequency (%)
1	Basal leaf sheath colour	Green	51	98
		Purple lines	1	2
2	Leaf auricles	Absent	1	2
		Present	51	98
3	Leaf collar	Present	52	100
4	Leaf ligule	Present	52	100
5	Shape of leaf ligule	Split	52	100
6	Colour of leaf ligule	White	51	98
		Purple	1	2
7	Time of heading (50% of plants with panicles) (days)	Very early (<71)	4	8
		Early (71-90)	5	10
		Medium (91-110)	33	63
		Late (111-130)	10	19
8	Lemma anthocyanin colouration of area below apex	Absent	51	98
		Strong	1	2
9	Stem length (excluding panicle; excluding floating rice)	Very short (<91 cm)	6	11
		Short (91-110 cm)	31	60
		Medium (111-130 cm)	15	29
10	Panicle length of main axis	Short (16-20 cm)	4	8
		Medium (21-25 cm)	36	69
		Long (26-30 cm)	12	23
11	Panicle number per plant	Few (<11)	14	27
		Medium (11-20)	38	73
12	Panicle awns	Present	46	88
		Absent	6	12
13	Panicle distribution of awns	Tip only	1	2
		Whole length	5	10
14	Panicle presence of secondary branching	Present	52	100

Cont…

Table 4.3. Cont…

S. No.	Characteristics	States	No. of accessions	Frequency (%)
15	Panicle secondary branching	Weak	13	25
		Strong	39	75
16	Time of maturity (days)	Very early (<100)	4	8
		Early (101-120)	5	10
		Medium (121-140)	33	63
		Late (141-160)	10	19
17.	weight of 1000 fully developed grains	Very low (<15 g)	5	10
		Low (15-20 g)	15	29
		Medium (21-25 g)	25	48
		High (26-30 g)	7	13
18	Grain length	Short (6.1-8.5 mm)	33	63
		Medium (8.6-10.5 mm)	19	37
19	Grain width	Narrow (2.1-2.5 mm)	27	52
		Medium (2.6-3.0 mm)	22	42
		Broad (3.1-3.5 mm)	3	6
20	Decorticated grain length	Short	21	40
		Long	21	40
		Long* (Long for Basmati type)	10	20
21	Decorticated grain width	Narrow (<2.0 mm)	14	27
		Medium (2.0-2.5 mm)	35	67
		Broad (>2.5 mm)	3	6
22	Decorticated grain shape (in lateral view)	Short slender	21	40
		Long slender	21	40
		Long slender* (For Basmati type)	10	20

Long slender* : for Basmati type

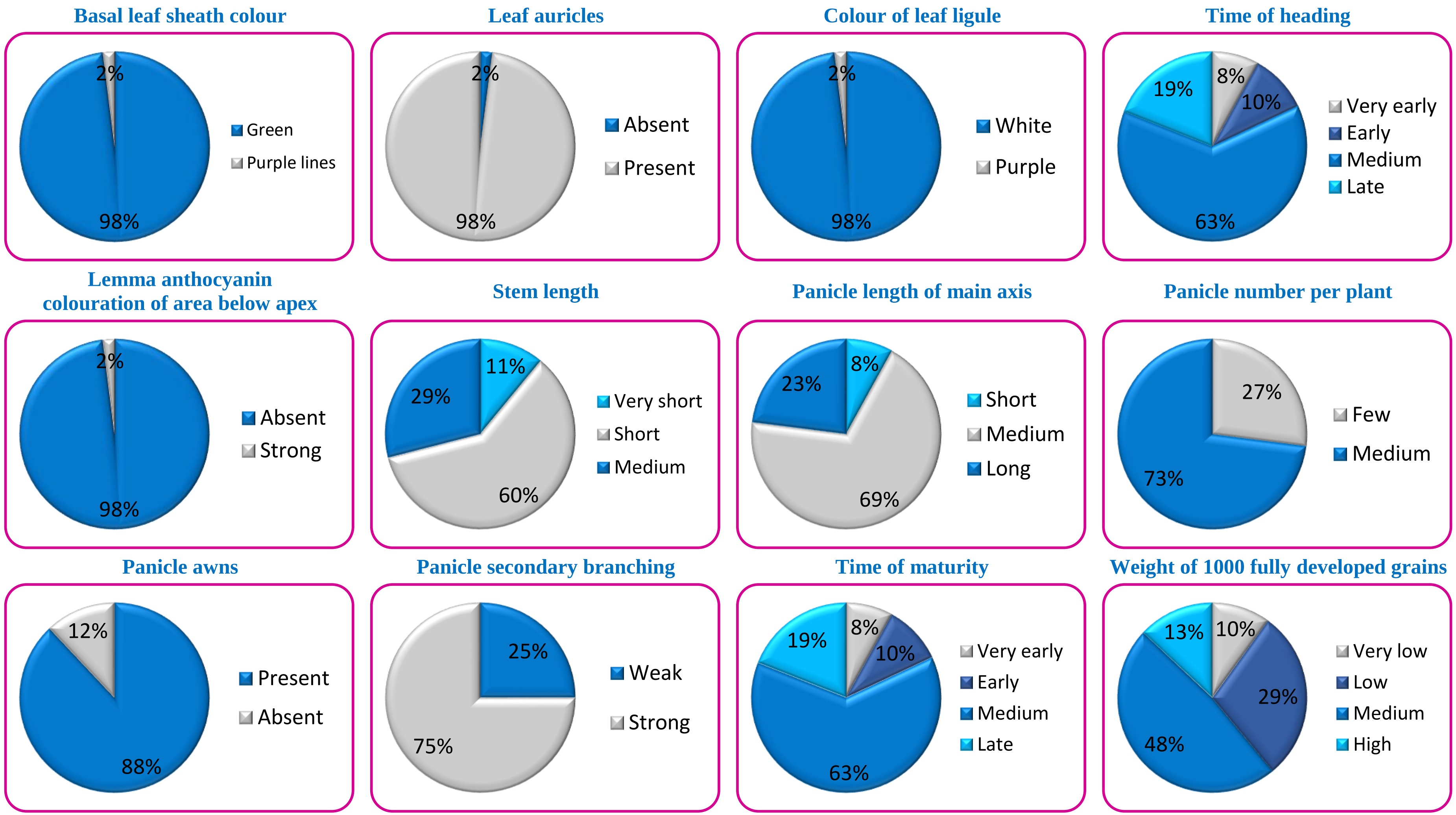
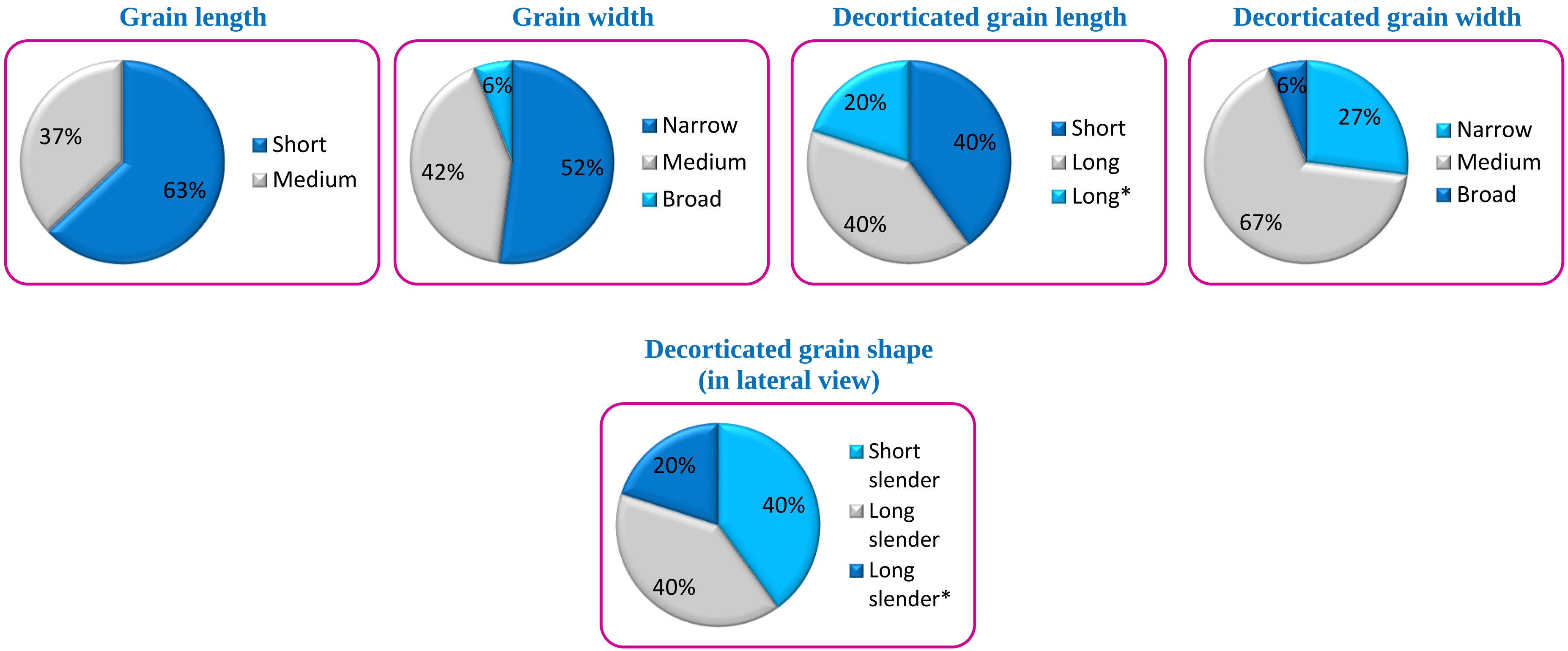
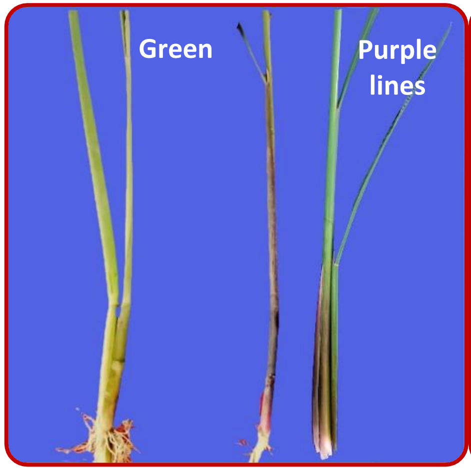


Fig. 4.1. Frequency distribution of 52 rice varieties for various DUS traits

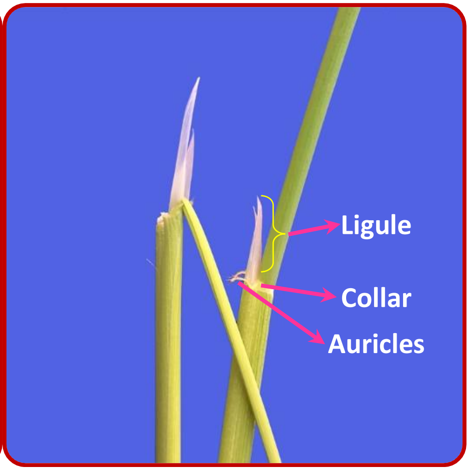
Cont…

Fig. 4.1 Cont…

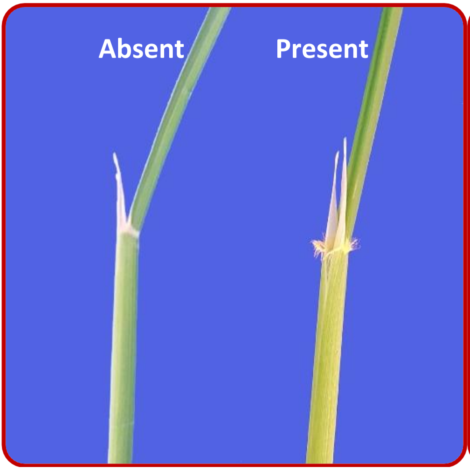

Grain length
37%
63%
Short
Medium
Grain width
6%
42%
52%
Narrow
Medium
Broad
Decorticated grain length
20%
40%
40%
Short
Long
Long*
Decorticated grain width
6%
27%
67%
Narrow
Medium
Broad
Decorticated grain shape
(in lateral view)
20%
40%
40%
Short slender
Long slender
Long slender*



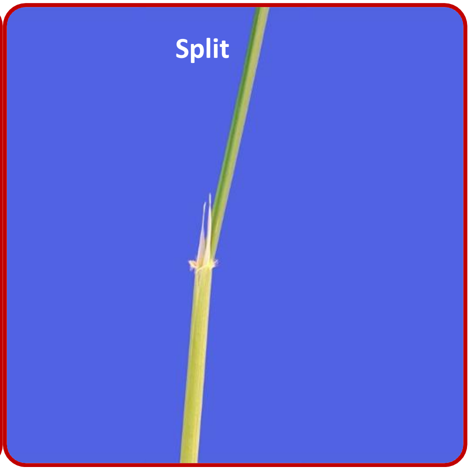
Basal leaf sheath colour



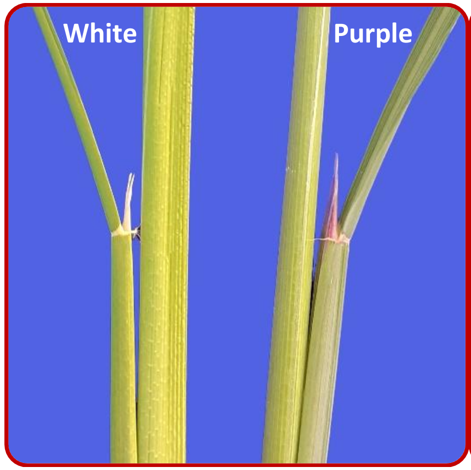
Presence of leaf ligule and collar



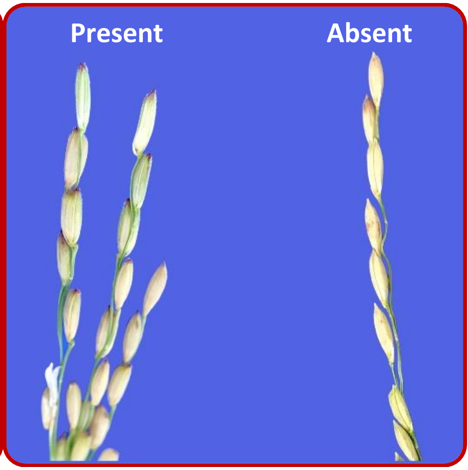
Leaf auricles



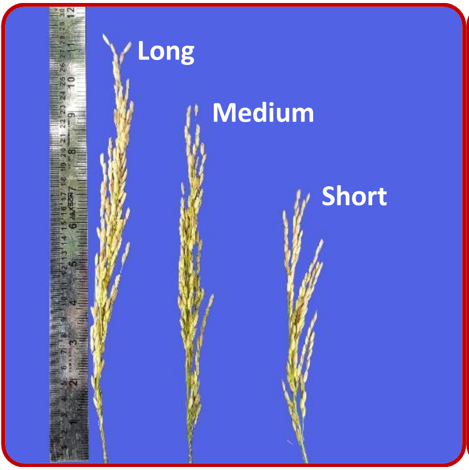
Shape of leaf ligule



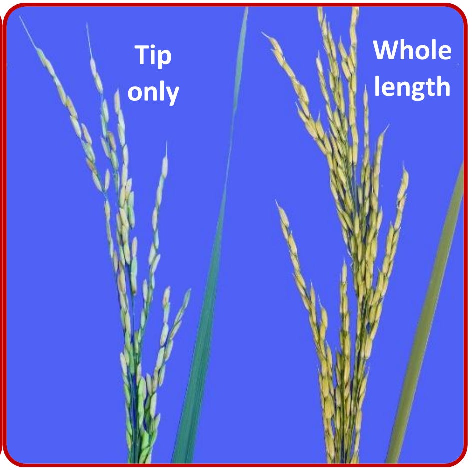
Colour of leaf ligule



Lemma anthocyanin colouration of area below apex

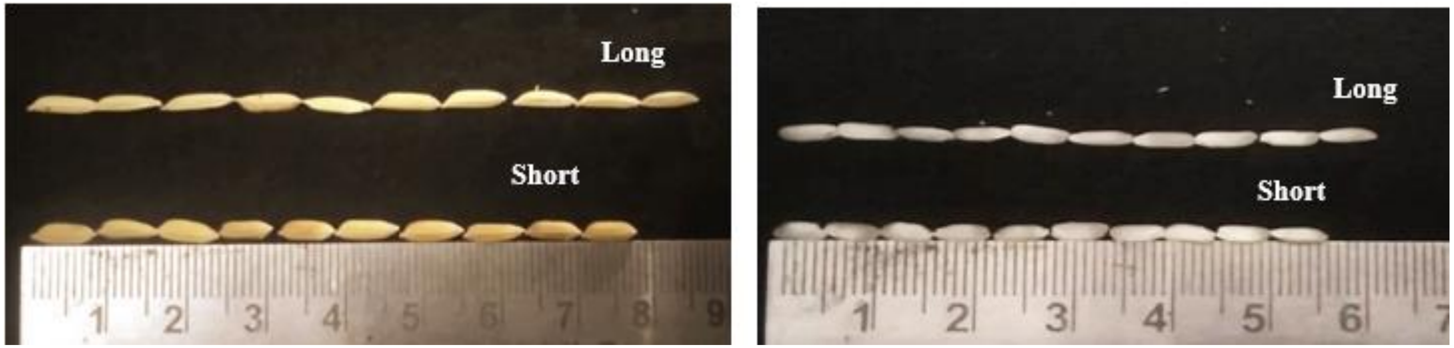


Panicle length of main axis



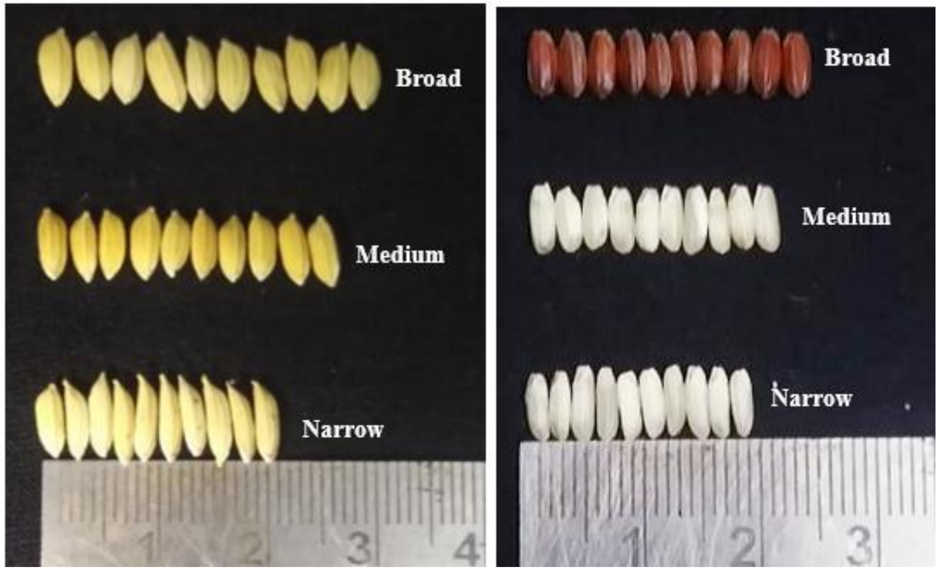
Panicle distribution of awns

Plate 2. DUS descriptors related to leaf and panicle



Grain length

Decorticated grain length

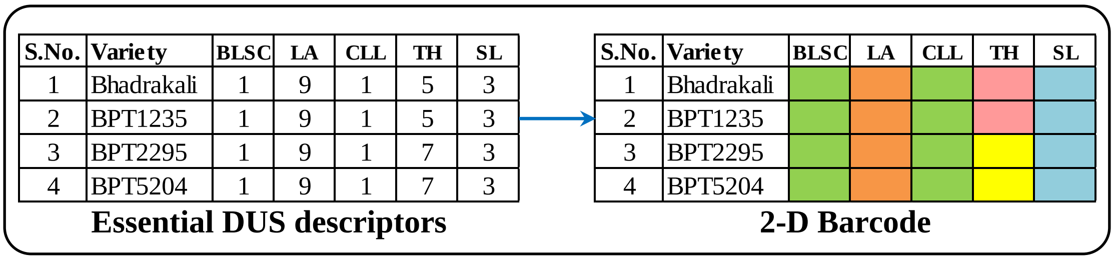


Grain width

Decorticated grain width

Plate 3. DUS descriptors related to grain and decorticated grain

40 polymorphic traits using Indian rice varieties. The number of polymorphic traits depends on the type of genotypes used. For instance, the study including wild species and landraces would exhibit more number of polymorphic traits than using released varieties alone. Of these 18 traits (dimorphic and polymorphic), time of heading, time of maturity and weight of 1000 fully developed grains were able to distinguish maximum number of varieties. Of these 18 polymorphic traits, 10 were essential traits. Identification of rice varieties using DUS descriptors was done by converting the descriptor notes of the essential traits into 2-D barcode. This 2-D barcode was generated by denoting different descriptor notes of the essential DUS descriptors in different colours as given in Figure 4.2. These 10 essential traits were used for creating DUS 2-D barcode using the descriptor notes for all the 52 rice varieties (Figure 4.3). Using this DUS 2-D barcodes of the 10 traits, 26 varieties were unambiguously distinguished. However, this barcode system could not be able to distinguish the remaining 26 varieties. By including more DUS descriptors it would likely enable us to distinguish those varieties with similar barcodes.



S.No.	Variety	BLSC	LA	CLL	TH	SL
1	Bhadrakali	1	9	1	5	3
2	BPT1235	1	9	1	5	3
3	BPT2295	1	9	1	7	3
4	BPT5204	1	9	1	7	3

Essential DUS descriptors

S.No.	Variety	BLSC	LA	CLL	TH	SL
1	Bhadrakali	🟩	🟧	🟩	🟥	🟦
2	BPT1235	🟩	🟧	🟩	🟥	🟦
3	BPT2295	🟩	🟧	🟩	🟨	🟦
4	BPT5204	🟩	🟧	🟩	🟨	🟦

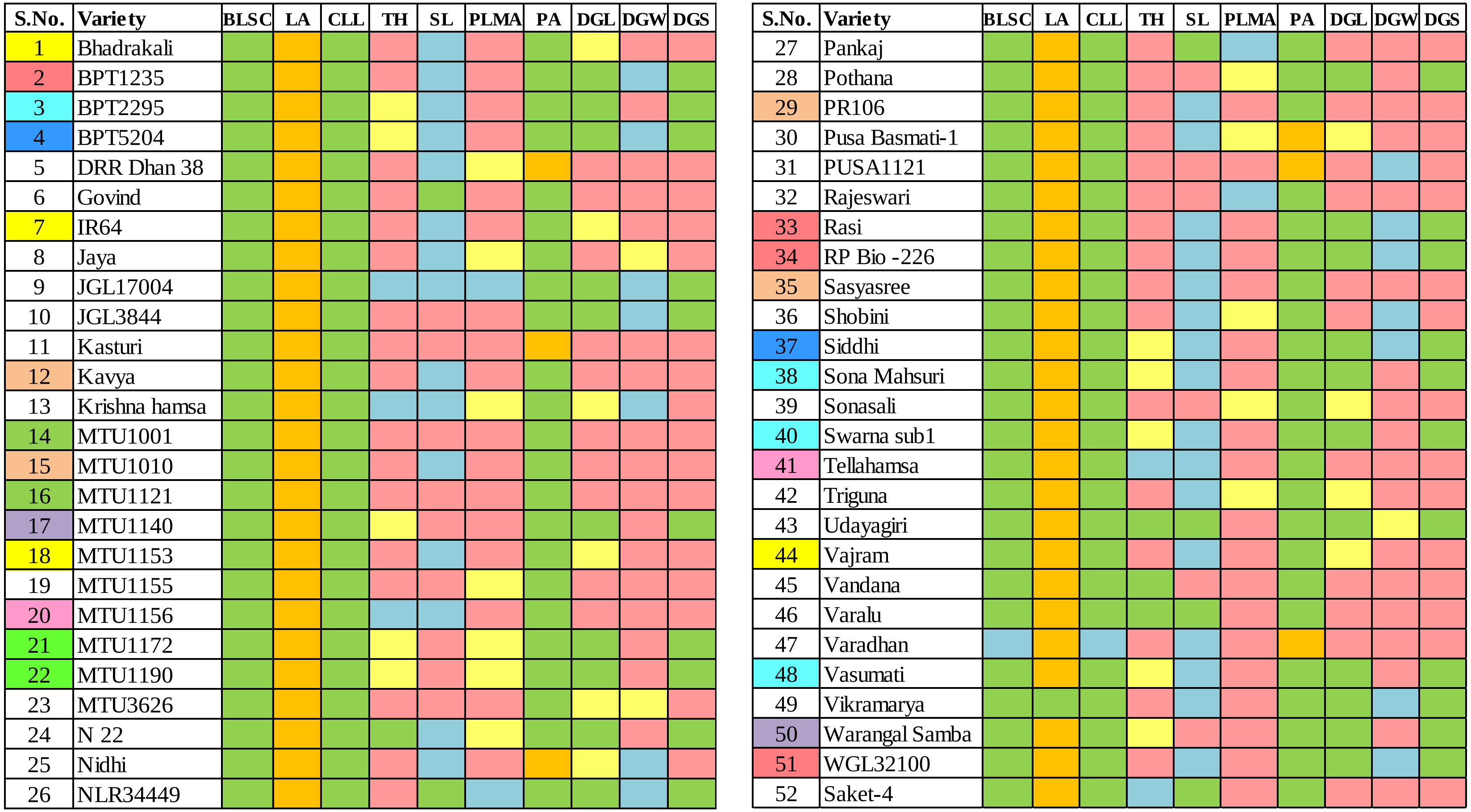
2-D Barcode

Fig. 4.2. Conversion of descriptor notes of the essential DUS descriptors into 2-D barcode

4.2 ANALYSIS OF VARIANCE AND GENETIC VARIABILITY STUDIES

4.2.1 Analysis of variance (ANOVA)

Analysis of variance for 12 characters including grain yield plant^{-1} revealed significant differences among the genotypes for these characters under study indicating the presence of greater variability among the genotypes. The details pertaining to ANOVA are furnished in Table 4.4.



S.No.	Variety	BLSC	LA	CLL	TH	SL	PLMA	PA	DGL	DGW	DGS
1	Bhadrakali	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟨	🟥	🟥
2	BPT1235	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟩	🟦	🟩
3	BPT2295	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟥	🟩
4	BPT5204	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟦	🟩
5	DRR Dhan 38	🟩	🟧	🟩	🟥	🟦	🟨	🟧	🟥	🟥	🟥
6	Govind	🟩	🟧	🟩	🟥	🟩	🟥	🟩	🟥	🟥	🟥
7	IR64	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟨	🟥	🟥
8	Jaya	🟩	🟧	🟩	🟥	🟦	🟨	🟩	🟥	🟨	🟥
9	JGL17004	🟩	🟧	🟩	🟦	🟦	🟦	🟩	🟩	🟦	🟩
10	JGL3844	🟩	🟧	🟩	🟥	🟥	🟥	🟩	🟩	🟦	🟩
11	Kasturi	🟩	🟧	🟩	🟥	🟥	🟥	🟧	🟥	🟥	🟥
12	Kavya	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟥	🟥	🟥
13	Krishna hamsa	🟩	🟧	🟩	🟦	🟦	🟨	🟩	🟨	🟦	🟥
14	MTU1001	🟩	🟧	🟩	🟥	🟥	🟥	🟩	🟥	🟥	🟥
15	MTU1010	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟥	🟥	🟥
16	MTU1121	🟩	🟧	🟩	🟥	🟥	🟥	🟩	🟥	🟥	🟥
17	MTU1140	🟩	🟧	🟩	🟨	🟥	🟥	🟩	🟩	🟥	🟩
18	MTU1153	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟨	🟥	🟥
19	MTU1155	🟩	🟧	🟩	🟥	🟥	🟨	🟩	🟥	🟥	🟥
20	MTU1156	🟩	🟧	🟩	🟦	🟦	🟥	🟩	🟥	🟥	🟥
21	MTU1172	🟩	🟧	🟩	🟨	🟥	🟨	🟩	🟩	🟥	🟩
22	MTU1190	🟩	🟧	🟩	🟨	🟥	🟨	🟩	🟩	🟥	🟩
23	MTU3626	🟩	🟧	🟩	🟥	🟥	🟥	🟩	🟨	🟨	🟥
24	N 22	🟩	🟧	🟩	🟩	🟦	🟨	🟩	🟩	🟥	🟩
25	Nidhi	🟩	🟧	🟩	🟥	🟦	🟥	🟧	🟨	🟦	🟥
26	NLR34449	🟩	🟧	🟩	🟥	🟩	🟦	🟩	🟩	🟦	🟩
27	Pankaj	🟩	🟧	🟩	🟥	🟩	🟦	🟩	🟥	🟥	🟥
28	Pothana	🟩	🟧	🟩	🟥	🟥	🟨	🟩	🟩	🟥	🟩
29	PR106	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟥	🟥	🟥
30	Pusa Basmati-1	🟩	🟧	🟩	🟥	🟦	🟨	🟧	🟨	🟥	🟥
31	PUSA1121	🟩	🟧	🟩	🟥	🟥	🟥	🟧	🟥	🟦	🟥
32	Rajeswari	🟩	🟧	🟩	🟥	🟥	🟦	🟩	🟥	🟥	🟥
33	Rasi	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟩	🟦	🟩
34	RP Bio -226	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟩	🟦	🟩
35	Sasyasree	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟥	🟥	🟥
36	Shobini	🟩	🟧	🟩	🟥	🟦	🟨	🟩	🟥	🟦	🟥
37	Siddhi	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟦	🟩
38	Sona Mahsuri	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟥	🟩
39	Sonasali	🟩	🟧	🟩	🟥	🟥	🟨	🟩	🟨	🟥	🟥
40	Swarna sub1	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟥	🟩
41	Tellahamsa	🟩	🟧	🟩	🟦	🟦	🟥	🟩	🟥	🟥	🟥
42	Triguna	🟩	🟧	🟩	🟥	🟦	🟨	🟩	🟨	🟥	🟥
43	Udayagiri	🟩	🟧	🟩	🟩	🟩	🟥	🟩	🟩	🟨	🟩
44	Vajram	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟨	🟥	🟥
45	Vandana	🟩	🟧	🟩	🟩	🟥	🟥	🟩	🟥	🟥	🟥
46	Varalu	🟩	🟧	🟩	🟩	🟩	🟥	🟩	🟥	🟥	🟥
47	Varadhan	🟦	🟧	🟦	🟥	🟦	🟥	🟧	🟥	🟥	🟥
48	Vasumati	🟩	🟧	🟩	🟨	🟦	🟥	🟩	🟩	🟥	🟩
49	Vikramarya	🟩	🟩	🟩	🟥	🟦	🟥	🟩	🟩	🟦	🟩
50	Warangal Samba	🟩	🟧	🟩	🟨	🟥	🟥	🟩	🟩	🟥	🟩
51	WGL32100	🟩	🟧	🟩	🟥	🟦	🟥	🟩	🟩	🟦	🟩
52	Saket-4	🟩	🟧	🟩	🟦	🟩	🟥	🟩	🟥	🟥	🟥

Fig. 4.3. Identification of rice varieties using 2-D barcode of DUS descriptors

Different descriptor notes of the essential DUS descriptors were denoted in different colours *i.e.*, 1 : Green; 3 : Blue; 5 : Pink; 7 : Yellow and 9 : Orange. BSLC : Basal Leaf Sheath Colour; **LA :** Leaf Auricles**, CLL :** Colour of Leaf Ligule; **TH :** Time of Heading; **SL :** Stem Length; **PLMA :** Panicle Length of Main Axis; **PA :** Panicle Awns; **DGL :** Decorticated Grain Length; **DGW :** Decorticated Grain Width; **DGS :** Decorticated Grain Shape (in lateral view). The varieties that are not distinguished from the remaining varieties using this set of DUS descriptors were highlighted in different colours (the varieties having similar DUS descriptors were represented in the same colour).

Table 4.4. Analysis of variance for grain yield and its component traits in 52 rice genotypes

S. No.	Character	Mean sum of squares for		
		Replications (df : 2)	Genotypes (df : 51)	Error (df : 102)
1	Time of heading (50% of plants with panicles) (days)	4.410	529.497**	6.783
2	Stem length (excluding panicle; excluding floating rice) (cm)	2.438	462.845**	39.382
3	Panicle length of main axis (cm)	0.517	17.692**	1.434
4	Panicle number per plant	3.000	21.860**	1.503
5	Time of maturity (days)	2.673	479.659**	5.549
6	Weight of 1000 fully developed grains (g)	0.458	54.707**	0.715
7	Grain length (mm)	0.003	1.316**	0.056
8	Grain width (mm)	0.007	0.202**	0.006
9	Decorticated grain length (mm)	0.002	0.783**	0.020
10	Decorticated grain width (mm)	0.001	0.139**	0.003
11	Length/breadth ratio	0.002	0.180**	0.018
12	Grain yield plant^{-1} (g)	155.579	735.304**	55.868

** : Significant at 1 per cent level

4.2.2 Mean performance of Rice Genotypes for Various Traits

The mean performance of 52 genotypes for grain yield plant^{-1} and its component traits are presented in the Table 4.5.

4.2.2.1 Time of heading (50% of plants with panicles) (days)

The number of days to 50% of plants with panicles ranged from 63.67 to 118.33 days with a general mean of 99.34 days. Among all the genotypes, Udayagiri (63.67 days) was found to be earliest while BPT2295 (118.33 days) was found to be late.

4.2.2.2 Stem length (excluding panicle; excluding floating rice) (cm)

For this trait, the genotypes exhibited a range of 75.93 to 124.73 cm with a general mean of 104.59 cm. The shortest genotype was Pankaj (75.93 cm) and the longest genotype was MTU1140 (124.73 cm).

4.2.2.3 Panicle length of main axis (cm)

The panicle length ranged from 18.73 to 28.60 cm with a general mean of 23.93 cm. The genotype JGL17004 had shorter panicle length of 18.73 cm while the genotype MTU1155 exhibited highest value of 28.60 cm which was almost 9.87 cm longer than JGL17004.

4.2.2.4 Panicle number per plant

The number of panicles per plant ranged from 6.67 (JGL3844) to 17.67 (Rasi) panicles with a general mean of 11.87.

4.2.2.5 Time of maturity (days)

The time of maturity was within the range of 97.00 to 150.67 days with a general mean of 127.88 days. Among all the genotypes, Vandana (97.00 days) was found to be earliest, while BPT2295 (150.67 days) was found to be late.

4.2.2.6 Weight of 1000 fully developed grains (g)

The general mean of weight of 1000 fully developed grains was 20.89 g and the values ranged from 13.00 to 29.77 g. Among the genotypes used, Govind (29.77 g) recorded highest 1000 grain weight followed by Vikramarya (28.20 g), Triguna (28.03 g), PUSA1121 (27.77 g) and Jaya (27.53 g) and the lowest 1000 grain weight was recorded for BPT1235 (13.00 g).

4.2.2.7 Grain length (mm)

The general mean of grain length was 8.35 mm and the values ranged from 7.27 to 9.67 mm. Among the genotypes used, PUSA1121 (9.67 mm) recorded the maximum grain length and the minimum grain length was recorded by Rasi (7.27 mm).

4.2.2.8 Grain width (mm)

The grain width was within the range of 2.13 to 3.17 mm with a general mean of 2.57 mm. The maximum grain width was recorded for Govind (3.17 mm) and the minimum grain width was recorded for Siddhi (2.13 mm).

4.2.2.9 Decorticated grain length (mm)

The decorticated grain length falls within the range of 5.30 to 7.40 mm with a general mean of 6.18 mm. The highest and lowest values for this trait were recorded by PUSA1121 (7.40 mm) and MTU1172 (5.30 mm) respectively.

4.2.2.10 Decorticated grain width (mm)

The decorticated grain width was within the range of 1.80 to 2.67 mm with a general mean of 2.13 mm. The genotype Udayagiri (2.67 mm) recorded maximum decorticated grain width and the genotypes, Shobini (1.80 mm) and RP Bio-226 (1.80 mm) recorded minimum decorticated grain width.

Table 4.5. Mean performance for grain yield and its component traits of 52 rice genotypes

S. No.	Variety	Time of heading (50% of plants with panicles) (days)	Stem length (excluding panicle; excluding floating rice) (cm)	Panicle		Time of maturity (days)	Weight of 1000 fully developed grains (g)	Grain		Decorticated grain			Grain Yield plant^{-1} (g)
				Length of main axis (cm)	Number per plant			Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length/ breadth ratio	
		1	2	3	4	5	6	7	8	9	10	11	12
1	Bhadrakali	104.33	98.40	23.00	11.67	131.00	24.30	8.97	2.80	7.10	2.30	4.34	53.46
2	BPT1235	105.00	97.80	22.77	12.00	128.33	13.00	7.53	2.37	5.47	1.90	3.87	36.31
3	BPT2295	118.33	98.37	23.20	8.33	150.67	21.80	8.20	2.70	5.77	2.30	3.50	32.58
4	BPT5204	113.33	98.20	22.57	13.00	143.67	15.83	7.67	2.33	5.33	1.83	3.70	45.02
5	DRR Dhan 38	104.33	102.03	27.50	13.00	129.33	21.97	9.23	2.40	6.33	2.00	4.01	47.05
6	Govind	101.33	85.87	23.13	7.00	126.67	29.77	8.20	3.17	6.33	2.37	3.71	25.36
7	IR64	104.00	98.30	22.50	8.00	130.00	23.70	9.37	2.63	7.30	2.20	4.37	30.86
8	Jaya	104.00	100.27	27.53	12.67	128.33	27.53	8.27	3.00	6.30	2.60	3.47	74.80
9	JGL17004	84.67	99.83	18.73	8.67	117.00	16.27	8.27	2.23	5.77	1.90	3.82	24.09
10	JGL3844	94.67	123.23	22.63	6.67	122.33	17.83	8.20	2.23	5.77	1.87	3.86	26.54
11	Kasturi	93.00	121.67	22.23	14.00	122.33	22.03	8.40	2.80	6.40	2.30	3.82	25.58
12	Kavya	108.00	100.73	23.60	7.00	134.00	21.67	8.27	2.47	6.10	2.00	3.87	38.59
13	Krishna hamsa	86.33	98.97	27.43	11.33	119.00	21.57	9.07	2.50	6.90	1.90	4.28	40.80
14	MTU1001	108.67	119.30	22.40	12.67	134.33	22.73	8.73	2.77	6.33	2.20	3.86	55.87
15	MTU1010	94.00	103.73	23.77	12.67	122.67	24.87	8.77	2.50	6.37	2.02	3.70	65.99
16	MTU1121	101.00	118.50	24.00	9.00	127.00	21.37	8.10	2.50	6.30	2.13	3.96	65.34
17	MTU1140	116.00	124.73	23.40	7.67	147.00	20.07	8.07	2.47	5.53	2.00	3.49	34.59
18	MTU1153	99.00	100.67	23.67	9.67	122.67	23.43	8.33	2.70	6.83	2.17	4.18	43.80

Cont…

Table 4.5. Cont…

S. No.	Variety	Time of heading (50% of plants with panicles) (days)	Stem length (excluding panicle; excluding floating rice) (cm)	Panicle		Time of maturity (days)	Weight of 1000 fully developed grains (g)	Grain		Decorticated grain			Grain Yield plant^{-1} (g)
				Length of main axis (cm)	Number per plant			Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length/ breadth ratio	
		1	2	3	4	5	6	7	8	9	10	11	12
19	MTU1155	107.33	118.30	28.60	7.33	137.67	21.43	7.67	2.80	6.10	2.30	3.67	30.14
20	MTU1156	85.67	103.83	23.50	7.00	118.00	23.53	8.70	2.70	6.37	2.27	3.83	34.41
21	MTU1172	114.67	120.63	27.47	13.33	144.33	22.93	7.53	2.47	5.30	2.02	3.50	57.33
22	MTU1190	114.67	124.30	28.40	12.33	143.67	16.30	7.57	2.43	5.77	2.00	3.64	56.07
23	MTU3626	103.33	123.70	23.57	11.67	134.33	26.50	8.83	3.17	6.23	2.63	4.00	77.59
24	N22	67.33	97.53	26.60	12.67	98.00	22.77	7.77	2.60	5.77	2.23	3.53	42.61
25	Nidhi	93.00	102.70	22.67	12.33	123.33	18.63	9.60	2.27	6.83	1.90	4.20	47.68
26	NLR34449	95.67	83.27	19.00	13.33	121.67	14.07	7.90	2.30	5.77	1.90	3.76	37.77
27	Pankaj	100.67	75.93	19.40	14.00	127.33	21.53	8.20	2.87	6.17	2.40	3.88	22.35
28	Pothana	100.33	123.60	28.50	11.33	125.33	16.23	7.80	2.50	5.80	2.00	3.71	38.12
29	PR106	103.67	102.53	24.00	14.00	128.33	17.83	8.80	2.40	6.37	2.13	4.06	56.19
30	Pusa Basmati-1	105.33	98.87	27.50	15.67	132.00	22.63	9.63	2.47	6.90	2.20	4.21	51.47
31	PUSA1121	99.00	121.13	23.80	14.33	126.67	27.77	9.67	2.83	7.40	1.90	3.75	61.83
32	Rajeswari	100.67	116.90	19.00	15.00	125.67	22.73	8.23	2.43	6.37	2.17	3.85	47.82
33	Rasi	93.67	102.13	24.67	17.67	123.67	13.23	7.27	2.17	5.53	1.90	3.76	98.50
34	RP Bio -226	106.00	99.43	23.27	13.67	131.33	13.47	7.60	2.23	5.57	1.80	3.80	41.01
35	Sasyasree	94.33	102.97	22.60	14.67	123.00	22.37	8.93	2.70	6.33	2.20	3.87	45.51
36	Shobini	99.67	102.63	27.47	16.00	124.33	15.70	8.80	2.20	6.27	1.80	4.28	45.32
37	Siddhi	115.33	101.10	23.00	14.33	144.67	13.07	7.33	2.13	5.50	1.93	3.69	52.54
38	Sona Mahsuri	116.00	102.80	23.80	12.00	145.67	18.43	7.70	2.63	5.73	2.20	3.51	42.52

Cont…

Table 4.5. Cont…

S. No.	Variety	Time of heading (50% of plants with panicles) (days)	Stem length (excluding panicle; excluding floating rice) (cm)	Panicle		Time of maturity (days)	Weight of 1000 fully developed grains (g)	Grain		Decorticated grain			Grain Yield plant^{-1} (g)
				Length of main axis (cm)	Number per plant			Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length/ breadth ratio	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
39	Sonasali	98.00	122.37	28.03	12.67	123.67	23.77	9.13	2.60	6.83	2.10	4.02	74.68
40	Swarna sub1	117.00	101.53	23.50	13.67	147.00	18.33	7.80	2.60	5.87	2.27	3.58	45.46
41	Tellahamsa	82.00	101.70	22.60	12.67	118.67	23.13	9.43	2.63	6.37	2.17	3.77	34.40
42	Triguna	96.67	101.70	27.60	12.33	124.33	28.03	8.93	2.90	6.80	2.50	3.73	48.81
43	Udayagiri	63.67	82.20	23.30	12.00	97.67	23.10	7.57	2.97	5.80	2.67	3.31	29.01
44	Vajram	94.67	99.93	22.90	14.00	124.33	22.20	9.00	2.70	7.03	2.20	4.20	44.15
45	Vandana	66.33	124.47	23.23	12.33	97.00	22.40	8.37	2.40	6.40	2.23	3.88	42.72
46	Varalu	66.33	83.37	23.03	8.67	98.33	17.33	9.17	2.43	6.30	2.10	3.85	25.44
47	Varadhan	95.67	102.80	23.37	9.33	124.67	26.53	8.40	3.17	6.17	2.43	3.57	44.36
48	Vasumati	114.00	97.87	23.50	16.67	145.67	17.87	7.37	2.63	5.80	2.23	3.54	58.34
49	Vikramarya	108.33	103.07	22.97	12.33	137.33	28.20	7.57	2.33	5.90	1.90	3.73	66.97
50	Warangal Samba	117.33	118.23	22.97	12.33	149.33	16.27	8.10	2.37	5.80	2.00	3.87	40.72
51	WGL32100	102.33	99.33	23.80	8.33	128.67	17.00	7.97	2.33	5.77	1.90	3.67	40.26
52	Saket-4	87.00	85.43	22.73	12.00	118.00	21.47	8.40	2.63	6.37	2.10	3.67	25.10
	General mean	**99.34**	**104.59**	**23.93**	**11.87**	**127.88**	**20.89**	**8.35**	**2.57**	**6.18**	**2.13**	**3.82**	**45.65**
	C.V%	2.62	6.00	5.00	10.33	1.84	4.05	2.84	3.05	2.27	2.43	3.47	16.37
	C.D. 5%	4.22	10.16	1.94	1.99	3.82	1.37	0.38	0.13	0.23	0.08	0.21	12.11
	C.D. 1%	5.58	13.45	2.57	2.63	5.05	1.81	0.51	0.17	0.30	0.11	0.28	16.02
	Range lowest	63.67	75.93	18.73	6.67	97.00	13.00	7.27	2.13	5.30	1.80	3.31	22.35
	Range highest	118.33	124.73	28.60	17.67	150.67	29.77	9.67	3.17	7.40	2.67	4.37	98.50

4.2.2.11 Length/breadth ratio

This trait varied from 3.31 to 4.37 with a general mean of 3.82. The genotype IR64 (4.37) recorded maximum length/breadth ratio and the genotype Udayagiri (3.31) recorded minimum length/breadth ratio.

4.2.2.12 Grain yield plant^{-1}

The grain yield plant^{-1} ranged from 22.35 g (Pankaj) to 98.50 g (Rasi) with a general mean of 45.65 g.

4.2.3 Genetic Variability, Heritability and Genetic Advance

The genotypic and phenotypic coefficients of variation, heritability (broad sense) and genetic advance as per cent of mean were estimated for 52 rice genotypes. Results are furnished in Table 4.6. for PCV, GCV, heritability (broad sense) and genetic advance as per cent of mean respectively.

4.2.3.1 Variability

Phenotypic Coefficient of Variation (PCV) is the resultant of combined action of genotype and environment over a genotype. Genotypic Coefficient of Variation (GCV), a component of total variation reflects the heritable portion. In the present study, the GCV values ranged from 6.09 to 32.97 per cent whereas, PCV values ranged from 7.01 to 36.81 per cent. As the PCV estimates were slightly higher than the corresponding GCV for the characters studied, it indicates that the characters were less influenced by the environment. Therefore, phenotypic selection would be effective for the improvement of these traits.

4.2.3.1.1 Low GCV and PCV

The GCV and PCV values were low for the traits *viz*., grain length (7.76 and 8.26%), decorticated grain length (8.16 and 8.47%) and length/ breadth ratio (6.09 and 7.01%) indicating that the variability of these characters among the genotypes was meagre. Similar findings were reported by Islam *et al.* (2015) and Senapati and Kumar (2015) and Bhinda *et al.* (2017) for grain length, Babu *et al.* (2012), Pratap *et al.* (2012), Dhurai *et al.* (2014) and Aravind *et al.* (2019) for decorticated grain length and Subbaiah *et al.* (2011) and Dhurai *et al.* (2014) for length/breadth ratio.

4.2.3.1.2 Low GCV and moderate PCV

The traits *viz.*, panicle length of main axis, time of maturity and grain width showed low GCV (9.73, 9.83 and 9.95%) and moderate PCV (10.94, 10.00 and 10.40%) estimates respectively. These results were in accordance with Chuchert *et al.* (2018), Ranjith *et al.* (2018) and Aravind *et al.* (2019) for panicle length of main axis, Abebe *et al.* (2017) for time of maturity and Bhinda *et al.* (2017) for grain width.

4.2.3.1.3 Moderate GCV and PCV

Moderate GCV and PCV values were observed for the traits *viz.*, time of heading (13.29 and 13.54%), stem length (11.36 and 12.85%) and decorticated grain width (10.00 and 10.29%). These observations were in agreement with the earlier reports of Veni *et al.* (2013), Gyawali *et al.* (2018), Ranjith *et al.* (2018) for time of heading, Sao *et al.* (2019) for stem length and Umarani *et al.* (2017b) for decorticated grain width.

4.2.3.1.4 High GCV and PCV

High GCV and PCV values were observed for the traits *viz.*, panicle number per plant (21.95 and 24.26%), weight of 1000 fully developed grains (20.30 and 20.70%) and grain yield plant^{-1} (32.97 and 36.81%) indicating that large amount of variation is present among the genotypes. Similar results were obtained by Chuchert *et al.* (2018) and Aravind *et al.* (2019) for panicle number per plant, Gangashetty *et al.* (2013) and Veni *et al.* (2013) for weight of 1000 fully developed grains, and Gangashetty *et al.* (2013), Veni *et al.* (2013), Dhurai *et al.* (2014), Gyawali *et al.* (2018) for grain yield plant^{-1}.

4.2.3.2 Heritability (h^2_{bs})

Heritability is a good measure of the transmission of characters from parents to their offspring. It is the ratio of genotypic variance to the phenotypic variance or total variance and is generally expressed in per cent. Genotypic coefficient of variation along with heritability should be considered in plant breeding programs as they would provide a better picture of the amount of genetic advance to be expected by the phenotypic selection (Burton and Devane, 1953).

In the present study all the characters *viz.*, time of heading (96.25%), stem length (78.19%), panicle length of main axis (79.07%), panicle number per plant (81.86%), time of maturity (96.6 %), weight of 1000 fully developed grains (96.18%), grain length (88.20%), grain width (91.40%), decorticated grain length (92.81%), decorticated grain width (94.91%), length/breadth ratio (75.49%) and grain yield plant^{-1} (80.21 %) showed high heritability (broad sense) indicating that these characters were least influenced by environmental effect and selection may be effective.

4.2.3.3 Genetic advance as per cent of mean

The genetic advance as per cent of mean was high for the characters *viz.*, time of heading (26.86%), stem length (20.69%), panicle number per plant (40.92%), weight of 1000 fully developed grains (41.02%), decorticated grain width (20.02%) and grain yield plant^{-1} (60.82%) implying that these characters were governed by additive gene action and selection for these characters will be effective. On the other hand, moderate genetic advance as per cent of mean was noted for panicle length of main axis (17.82%), time of maturity (19.90%), grain length (15.01%), grain width (19.59%), decorticated grain length (16.19%) and length/breadth ratio (10.90%) indicating that these traits were under the control of non - additive gene action and selection may be ineffective.

High heritability alone provides no indication of the amount of genetic progress that would result from selection of the better individuals. Heritability coupled with genetic advance are more reliable and useful genetic parameters in predicting the genetic gain under selection than heritability estimates alone (Kundu *et al.,* 2008).

High heritability coupled with high genetic advance as per cent of mean was reported for time of heading, stem length, panicle number per plant, weight of 1000 fully developed grains, decorticated grain width and grain yield plant^{-1} indicating that these traits were mostly under the control of additive gene action and direct selection of these traits would be effective for crop improvement. Similar results were reported by Gyawali *et al*. (2018) and Ranjith *et al*. (2018) for time of heading, Sao *et al*. (2019) for stem length and decorticated grain width, Chuchert *et al*. (2018) and Sao *et al*. (2019) for panicle number per plant, Dhurai *et al*. (2014), Devi *et al*. (2016), Chuchert *et al*. (2018) and Aravind *et al*. (2019) for weight of 1000 fully developed grains, Shanthi and Singh (2001), Gangashetty *et al*. (2013), Dhurai *et al*. (2014) and Rahangdale *et al*. (2019) for grain yield plant^{-1}.

High heritability coupled with moderate genetic advance as per cent of mean was observed for the traits *viz*., panicle length of main axis, time of maturity, grain length, grain width, decorticated grain length and length/ breadth ratio indicating the presence of both additive and non-additive genetic effects and genetic improvement for these characters can be exploited through heterosis breeding. Similar reports were published by Shanthi and Singh (2001), Abebe *et al*. (2017), Singh and Verma (2018) and Ranjith *et al*. (2018) for panicle length of main axis, Dhurai *et al*. (2015) and Islam *et al*. (2015) for time of maturity, Islam *et al*. (2015) and Bindha *et al*. (2017) for grain length and grain width, Babu *et al*. (2012), Aditya and Bhartiya (2013), Singh and Verma (2018) for decorticated grain length, Aditya and Bhartiya (2013) and Dhurai *et al*. (2015) for length/breadth ratio.

Table 4.6. Mean, Range, Coefficients of variation, Heritability (broad sense) and Genetic advance as per cent of mean for grain yield and its component traits in 52 rice varieties

S. No.	Character	Mean	Range		Coefficient of variation (%)		Heritability (%) (broad sense)	Genetic Advance	Genetic advance as per cent of mean (5% level)
			Minimum	Maximum	Genotypic	Phenotypic			
1	Time of heading (50% of plants with panicles) (days)	99.34	63.67	118.33	13.29	13.54	96.25	26.68	26.86
2	Stem length (excluding panicle; excluding floating rice) (cm)	104.59	75.93	124.73	11.36	12.85	78.19	21.64	20.69
3	Panicle length of main axis (cm)	23.93	18.73	28.60	9.73	10.94	79.07	4.26	17.82
4	Panicle number per plant	11.87	6.67	17.67	21.95	24.26	81.86	4.85	40.92
5	Time of maturity (days)	127.88	97.00	150.67	9.83	10.00	96.61	25.45	19.90
6	Weight of 1000 fully developed grains (g)	20.89	13.00	29.77	20.30	20.70	96.18	8.57	41.02
7	Grain length (mm)	8.35	7.27	9.67	7.76	8.26	88.20	1.25	15.01
8	Grain width (mm)	2.57	2.13	3.17	9.95	10.40	91.40	0.50	19.59
9	Decorticated grain length (mm)	6.18	5.30	7.40	8.16	8.47	92.81	1.00	16.19
10	Decorticated grain width (mm)	2.13	1.80	2.67	10.00	10.29	94.41	0.43	20.02
11	Length/breadth ratio	3.82	3.31	4.37	6.09	7.01	75.49	0.42	10.90
12	Grain yield plant^{-1} (g)	45.65	22.35	98.50	32.97	36.81	80.21	27.76	60.82

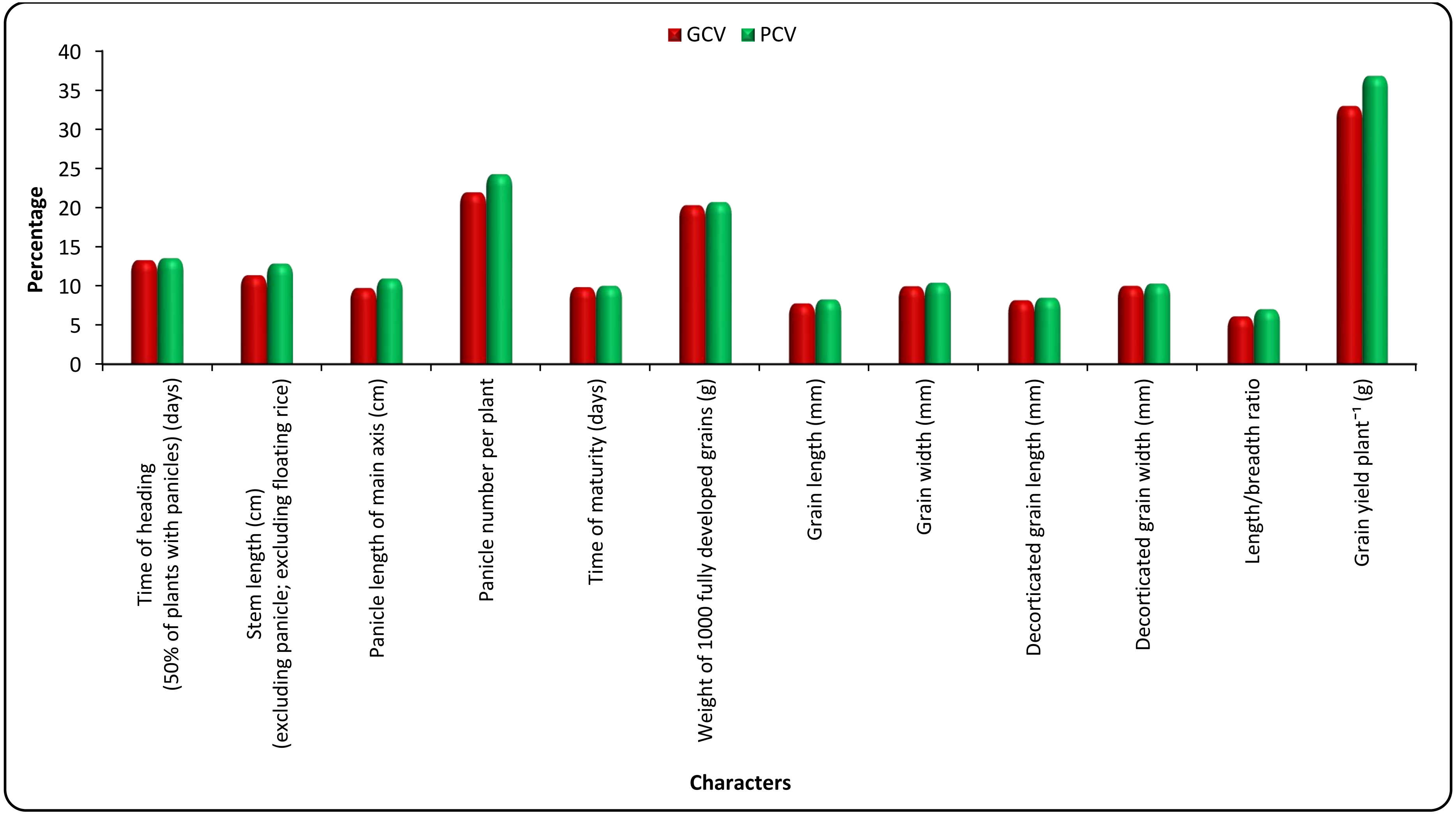


Fig. 4.4. Genotypic and phenotypic coefficients of variation of various traits

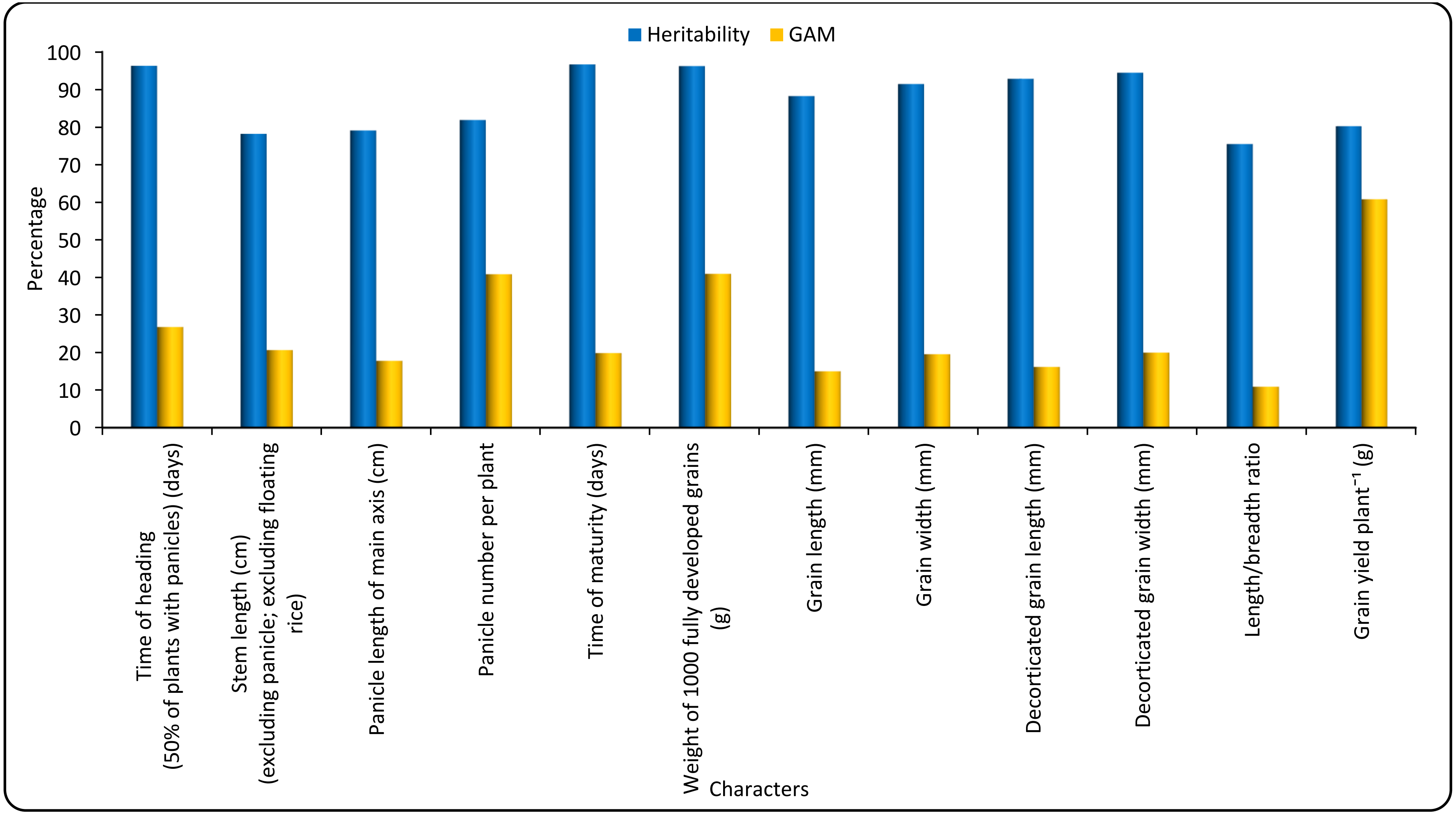


Fig. 4.5. Heritability and Genetic Advance as per cent of Mean (GAM) for various traits in rice genotypes

4.3 IDENTIFICATION OF RICE VARIETIES USING MOLECULAR MARKERS

4.3.1 Molecular Marker Analysis

In the present study, 25 gene-specific markers were used to screen 52 rice genotypes. These gene-specific markers were linked to genes controlling the traits such as early heading date, heading date, short and thick culm, submergence tolerance, panicle length, erect panicle, hybrid sterility, grain length and width, grain size, grain weight, grain length-width ratio, grain number, kernel length, awn length, grain yield, blast and gall midge resistance (Table 3.3.). Of the 25 primers used, 18 were polymorphic (72%) and seven were monomorphic (28%). The 18 polymorphic markers were distributed on nine chromosomes (1, 2, 3, 5, 6, 8, 9, 10 and 11) covering uniformly and nearly the entire rice genome, which satisfied the standards proposed by the UPOV (http://www.upov.int) with respect to genome distribution of molecular markers for DNA profiling of cultivars. Hence, these markers could be of great value in cultivar identification.

The number of alleles produced by these markers ranged from two to three with an average of 2.05 alleles per locus. The Polymorphism Information Content (PIC) values of these gene-specific markers ranged from 0.037 (PAY1SP6 and sd1-h) to 0.369 (RGS-1) with an average of 0.212. The PIC and number of alleles of the polymorphic markers used in the study are mentioned in the Table 4.7. The number of alleles and PIC values of these gene-specific markers were much less than the generally used SSR markers from previous studies due to the selection imposed by the breeders for only particular traits which naturally favours only few alleles. For instance, Choudhary *et al.* (2013) reported an average of 3.6 alleles per locus with PIC of 0.87 using 52 SSR markers in 100 rice genotypes. However, in line with the present study, Ngangkham *et al.* (2018) reported a total of 21 alleles with an average of 2.1 allele per locus and the PIC ranging from 0.13 to 0.58 with an average value of 0.31 using 10 gene-specific markers regulating grain size in 89 rice genotypes.

Table 4.7. Details of number of alleles and Polymorphism Information Content (PIC) of gene-specific markers

S. No.	Primer	No. of alleles	PIC
1	Dep1S9	3	0.106
2	Ehd1 -2	2	0.356
3	GLW7	2	0.183
4	OsSnb-2	2	0.132
5	Hd1 InDel-2	2	0.132
6	Hd3a	2	0.254
7	LRR DEL	2	0.305
8	PAY1SP6	2	0.037
9	RGS 1	2	0.369
10	RM 202	2	0.356
11	RM 208	2	0.311
12	RM228	2	0.350
13	RM 7289	2	0.070
14	RMw-513	2	0.316
15	S5-1	2	0.183
16	sd1-h	2	0.037
17	SPIKE-indel3	2	0.183
18	Sub1	2	0.132
	Total	**37**	**0.212(Av.)**

PIC : Polymorphism Information Content
Av. : Average

4.3.2 Development of Varietal-specific DNA Fingerprints

The polymorphic gene-specific markers were used to develop varietal-specific DNA fingerprints for all the 52 rice varieties. The alleles of the polymorphic markers were assigned with codes *i.e.,* A, B and C based on their allele sizes in ascending order (Plate 4 and 5). The pattern of allele codes of these polymorphic markers were used to depict the DNA fingerprints. The differences in the pattern with respect to even a single allele code could distinguish one variety from the other. For instance, the grains of popular fine grain varieties such as BPT5204 with excellent cooking quality and NLR34449, a fine grain but short of cooking quality traits of BPT5204 are difficult to distinguish visually. Due to the market price difference between BPT5204 and NL34449, NLR34449 is being used extensively to adulterate the BPT5204 by the unscrupulous millers to get some benefit out of it. Therefore, now it is easy to distinguish these two varieties using the DNA fingerprints developed in the present study. Furthermore, using the varietal-specific DNA fingerprints, it is possible to distinguish premium cultivars such as Pusa Basmati-1 (ABBAABBABBBBABBBBB - allele code) and PUSA1121 (ABBADAABBBAABDBBBB - allele code). In addition, the variety developed through marker-assisted selection, RP Bio-226 (Improved Samba mahsuri) (ABBAABBBABAABBBBBB - allele code) can be distinguished from the original variety, BPT5204 (Samba mahsuri) (ABBAABDBABBABABBBB-allele code). Employing these codes of the polymorphic markers, 50 of 52 rice varieties could be unambiguously distinguished. The diagrammatic representation of varietal-specific fingerprints is given in the Figure 4.6. Only two varieties *i.e.,* BPT2295 and Jaya could not be distinguished as they were possessing similar allele codes (DNA fingerprints) possibly due to a limited number of polymorphic markers. Earlier, Vemireddy *et al*. (2015) developed molecular profiles for 90 Indian varieties using only eight hypervariable SSR markers.

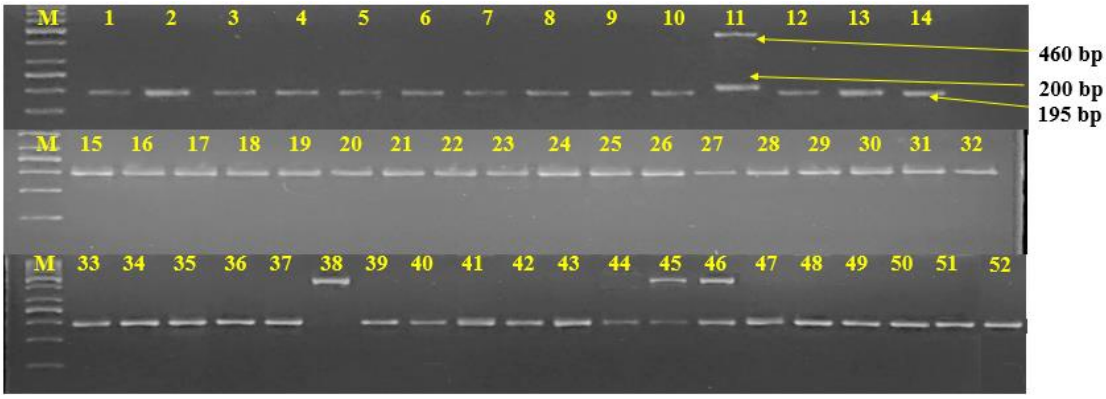


Plate 4. **Representative of PCR amplified fragments of Dep1S9.**
Lane: M =50 bp DNA ladder; 1-52 represents the rice genotypes mentioned in the Table (3.1). The size of the DNA fragments is indicated on the right side of the figure.

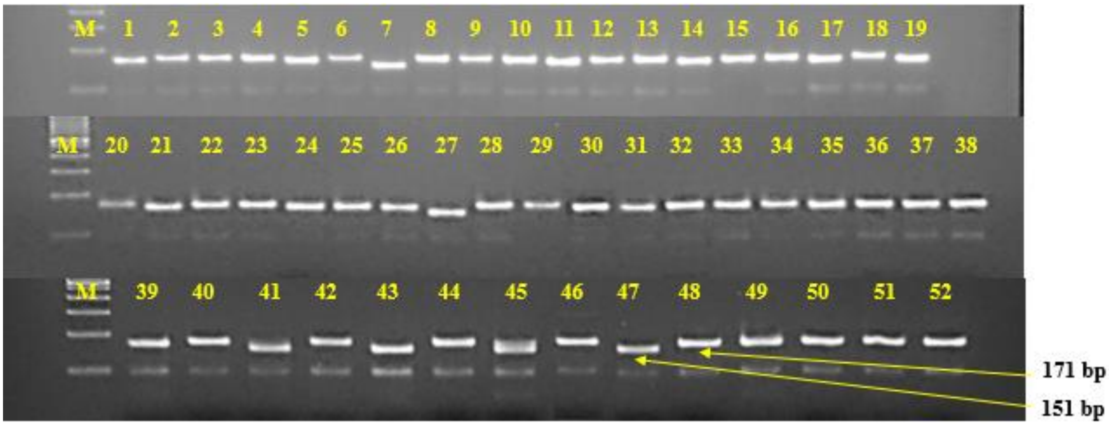
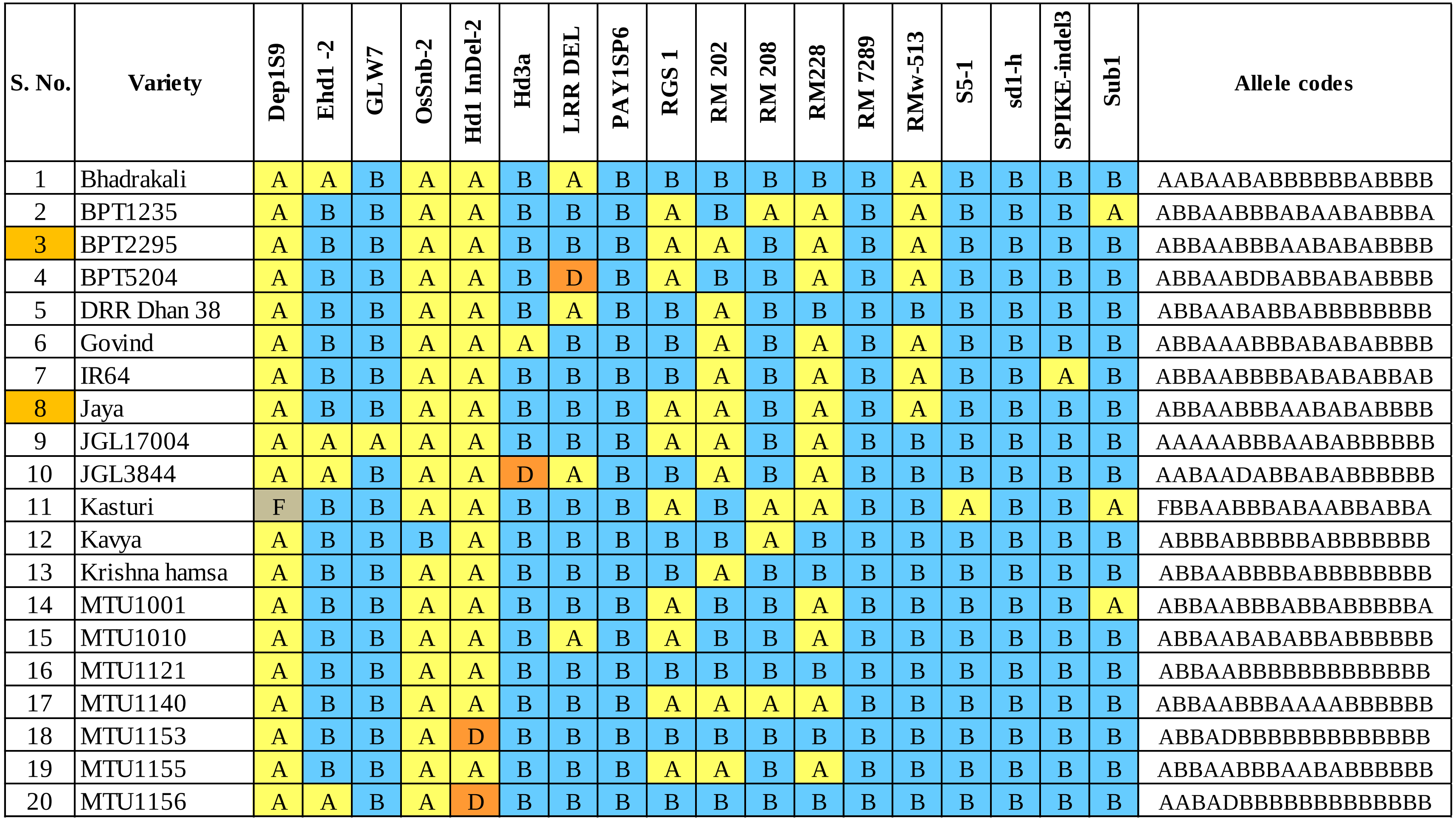


Plate 5. **Representative of PCR amplified fragments of SPIKE-indel3.**
Lane: M =100 bp DNA ladder; 1-52 represents the rice genotypes mentioned in the Table (3.1). The size of the DNA fragments is indicated on the right side of the figure.



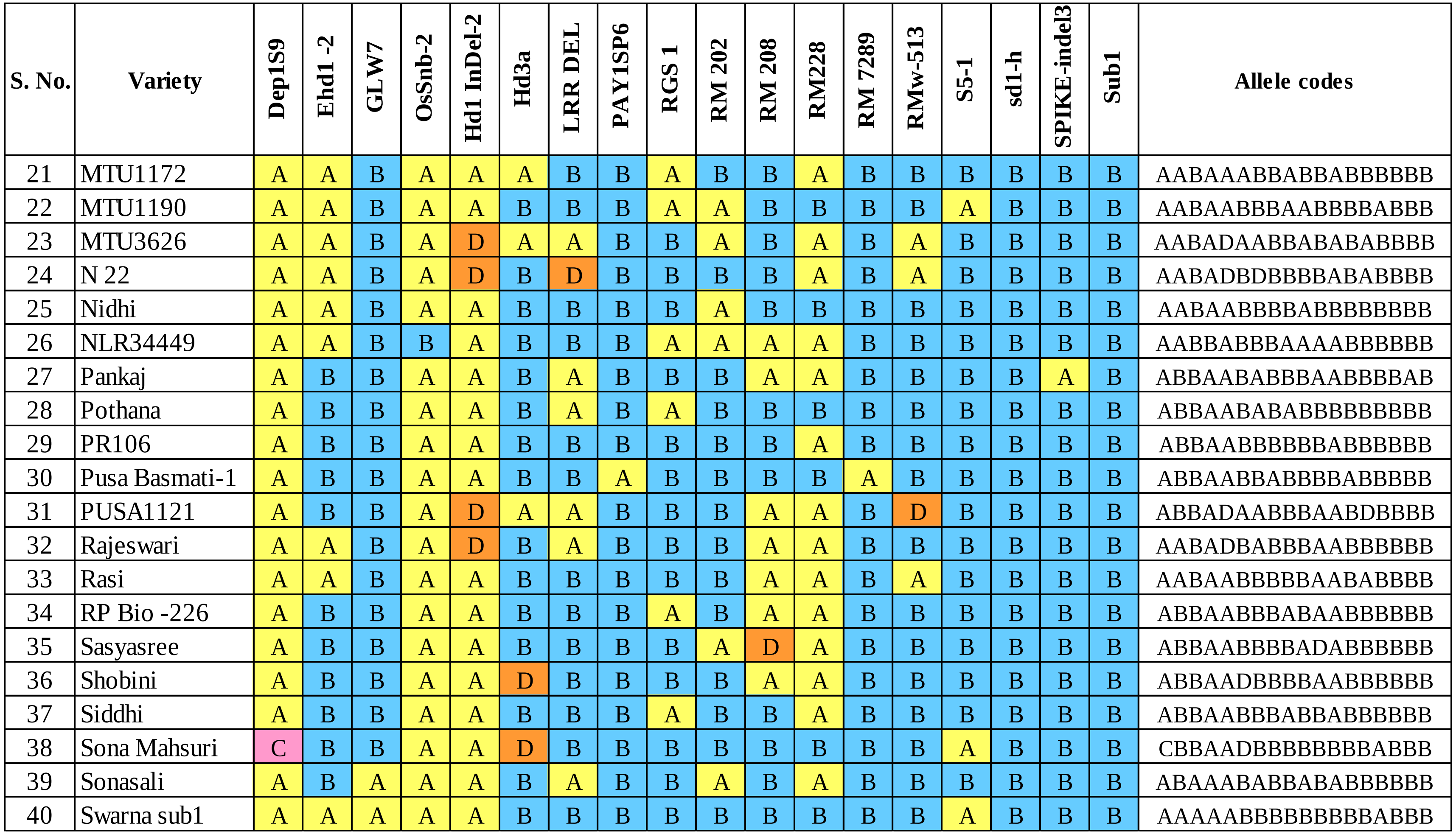
S. No.	Variety	Dep1S9	Ehd1 -2	GLW7	OsSnb-2	Hd1 InDel-2	Hd3a	LRR DEL	PAY1SP6	RGS 1	RM 202	RM 208	RM228	RM 7289	RMw-513	S5-1	sd1-h	SPIKE-indel3	Sub1	Allele codes
1	Bhadrakali	A	A	B	A	A	B	A	B	B	B	B	B	B	A	B	B	B	B	AABAABABBBBBBABBBB
2	BPT1235	A	B	B	A	A	B	B	B	A	B	A	A	B	A	B	B	B	A	ABBAABBBABAABABBBA
3	BPT2295	A	B	B	A	A	B	B	B	A	A	B	A	B	A	B	B	B	B	ABBAABBBAABABABBBB
4	BPT5204	A	B	B	A	A	B	D	B	A	B	B	A	B	A	B	B	B	B	ABBAABDBABBABABBBB
5	DRR Dhan 38	A	B	B	A	A	B	A	B	B	A	B	B	B	B	B	B	B	B	ABBAABABBABBBBBBBB
6	Govind	A	B	B	A	A	A	B	B	B	A	B	A	B	A	B	B	B	B	ABBAAABBBABABABBBB
7	IR64	A	B	B	A	A	B	B	B	B	A	B	A	B	A	B	B	A	B	ABBAABBBBABABABBAB
8	Jaya	A	B	B	A	A	B	B	B	A	A	B	A	B	A	B	B	B	B	ABBAABBBAABABABBBB
9	JGL17004	A	A	A	A	A	B	B	B	A	A	B	A	B	B	B	B	B	B	AAAAABBBAABABBBBBB
10	JGL3844	A	A	B	A	A	D	A	B	B	A	B	A	B	B	B	B	B	B	AABAADABBABABBBBBB
11	Kasturi	F	B	B	A	A	B	B	B	A	B	A	A	B	B	A	B	B	A	FBBAABBBABAABBABBA
12	Kavya	A	B	B	B	A	B	B	B	B	B	A	B	B	B	B	B	B	B	ABBBABBBBBABBBBBBB
13	Krishna hamsa	A	B	B	A	A	B	B	B	B	A	B	B	B	B	B	B	B	B	ABBAABBBBABBBBBBBB
14	MTU1001	A	B	B	A	A	B	B	B	A	B	B	A	B	B	B	B	B	A	ABBAABBBABBABBBBBA
15	MTU1010	A	B	B	A	A	B	A	B	A	B	B	A	B	B	B	B	B	B	ABBAABABABBABBBBBB
16	MTU1121	A	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	ABBAABBBBBBBBBBBBB
17	MTU1140	A	B	B	A	A	B	B	B	A	A	A	A	B	B	B	B	B	B	ABBAABBBAAAABBBBBB
18	MTU1153	A	B	B	A	D	B	B	B	B	B	B	B	B	B	B	B	B	B	ABBADBBBBBBBBBBBBB
19	MTU1155	A	B	B	A	A	B	B	B	A	A	B	A	B	B	B	B	B	B	ABBAABBBAABABBBBBB
20	MTU1156	A	A	B	A	D	B	B	B	B	B	B	B	B	B	B	B	B	B	AABADBBBBBBBBBBBBB

Fig. 4.6. Diagrammatic representation of DNA fingerprints for 52 rice varieties using gene-specific markers

The letters, A, B and C are the alleles of specific marker recorded by the genotypes. While, the rice genotypes containing both alleles like, AB, AC and BC are heterozygotes represented with the letters D, E and F respectively. The genotypes highlighted (BPT2295 and Jaya) were possessing similar allele codes.

Cont…

Fig. 4.6. Cont…



S. No.	Variety	Dep1S9	Ehd1 -2	GLW7	OsSnb-2	Hd1 InDel-2	Hd3a	LRR DEL	PAY1SP6	RGS 1	RM 202	RM 208	RM228	RM 7289	RMw-513	S5-1	sd1-h	SPIKE-indel3	Sub1	Allele codes
21	MTU1172	A	A	B	A	A	A	B	B	A	B	B	A	B	B	B	B	B	B	AABAAABBABBABBBBBB
22	MTU1190	A	A	B	A	A	B	B	B	A	A	B	B	B	B	A	B	B	B	AABAABBBAABBBBABBB
23	MTU3626	A	A	B	A	D	A	A	B	B	A	B	A	B	A	B	B	B	B	AABADAABBABABABBBB
24	N 22	A	A	B	A	D	B	D	B	B	B	B	A	B	A	B	B	B	B	AABADBDBBBBABABBBB
25	Nidhi	A	A	B	A	A	B	B	B	B	A	B	B	B	B	B	B	B	B	AABAABBBBABBBBBBBB
26	NLR34449	A	A	B	B	A	B	B	B	A	A	A	A	B	B	B	B	B	B	AABBABBBAAAABBBBBB
27	Pankaj	A	B	B	A	A	B	A	B	B	B	A	A	B	B	B	B	A	B	ABBAABABBBAABBBBAB
28	Pothana	A	B	B	A	A	B	A	B	A	B	B	B	B	B	B	B	B	B	ABBAABABABBBBBBBBB
29	PR106	A	B	B	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	ABBAABBBBBBABBBBBB
30	Pusa Basmati-1	A	B	B	A	A	B	B	A	B	B	B	B	A	B	B	B	B	B	ABBAABBABBBBABBBBB
31	PUSA1121	A	B	B	A	D	A	A	B	B	B	A	A	B	D	B	B	B	B	ABBADAABBBAABDBBBB
32	Rajeswari	A	A	B	A	D	B	A	B	B	B	A	A	B	B	B	B	B	B	AABADBABBBAABBBBBB
33	Rasi	A	A	B	A	A	B	B	B	B	B	A	A	B	A	B	B	B	B	AABAABBBBBAABABBBB
34	RP Bio -226	A	B	B	A	A	B	B	B	A	B	A	A	B	B	B	B	B	B	ABBAABBBABAABBBBBB
35	Sasyasree	A	B	B	A	A	B	B	B	B	A	D	A	B	B	B	B	B	B	ABBAABBBBADABBBBBB
36	Shobini	A	B	B	A	A	D	B	B	B	B	A	A	B	B	B	B	B	B	ABBAADBBBBAABBBBBB
37	Siddhi	A	B	B	A	A	B	B	B	A	B	B	A	B	B	B	B	B	B	ABBAABBBABBABBBBBB
38	Sona Mahsuri	C	B	B	A	A	D	B	B	B	B	B	B	B	B	A	B	B	B	CBBAADBBBBBBBBABBB
39	Sonasali	A	B	A	A	A	B	A	B	B	A	B	A	B	B	B	B	B	B	ABAAABABBABABBBBBB
40	Swarna sub1	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	B	B	B	AAAAABBBBBBBBBABBB

The letters, A, B and C are the alleles of specific marker recorded by the genotypes. While, the rice genotypes containing both alleles like, AB, AC and BC are heterozygotes represented with the letters D, E and F respectively. The genotypes highlighted (BPT2295 and Jaya) were possessing similar allele codes.

Cont…

Fig. 4.6. Cont…

S. No.	Variety	Dep1S9	Ehd1 -2	GLW7	OsSnb-2	Hd1 InDel-2	Hd3a	LRR DEL	PAY1SP6	RGS 1	RM 202	RM 208	RM228	RM 7289	RMw-513	S5-1	sd1-h	SPIKE-indel3	Sub1	Allele codes
41	Tellahamsa	A	A	A	A	A	B	B	B	B	A	B	B	B	B	B	B	A	B	AAAAABBBBABBBBBBAB
42	Triguna	A	B	A	A	A	B	B	B	A	B	B	A	B	A	B	B	B	B	ABAAABBBABBABABBBB
43	Udayagiri	A	A	A	B	A	D	B	B	A	B	B	B	B	A	B	B	A	B	AAABADBBABBBBABBAB
44	Vajram	A	A	B	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	AABAAABBBBBBBBBBBA
45	Vandana	E	B	B	B	A	A	A	B	A	A	A	A	B	B	B	A	A	B	EBBBAAABAAAABBBAAB
46	Varalu	E	B	B	A	A	A	B	B	A	A	B	B	B	D	B	B	B	B	EBBAAABBAABBBDBBBB
47	Varadhan	A	A	B	A	B	B	B	B	B	B	B	A	B	A	A	B	A	B	AABABBBBBBBABAABAB
48	Vasumati	A	B	B	A	A	B	B	B	A	B	B	B	B	B	A	B	B	B	ABBAABBBABBBBBABBB
49	Vikramarya	A	B	B	A	A	D	B	B	B	B	B	A	B	B	B	B	B	B	ABBAADBBBBBABBBBBB
50	Warangal Samba	A	A	B	A	A	B	B	B	A	B	A	A	B	B	B	B	B	B	AABAABBBABAABBBBBB
51	WGL32100	A	A	B	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	AABAABBBBBBABBBBBB
52	Saket-4	A	B	B	A	A	B	A	B	B	B	B	A	A	B	B	B	B	B	ABBAABABBBBAABBBBB

The letters, A, B and C are the alleles of specific marker recorded by the genotypes. While, the rice genotypes containing both alleles like, AB, AC and BC are heterozygotes represented with the letters D, E and F respectively. The genotypes highlighted (BPT2295 and Jaya) were possessing similar allele codes.

Allele size code key:

Allele code	Dep1S9	Ehd1 -2	GLW7	OsSnb-2	Hd1 InDel-2	Hd3a	LRR DEL	PAY1SP6	RGS 1	RM202	RM208	RM228	RM7289	RMw-513	S5-1	sd1-h	SPIKE-indel3	Sub1
A	195	650	137	994	247	100	350	200	179	297	282	200	190	171	321	600	151	250
B	200	680	140	1020	403	140	600	206	193	300	300	240	195	188	457	800	171	277
C	460																	

4.3.3 Development of varietal-specific fingerprints using both DUS descriptors and gene-specific markers

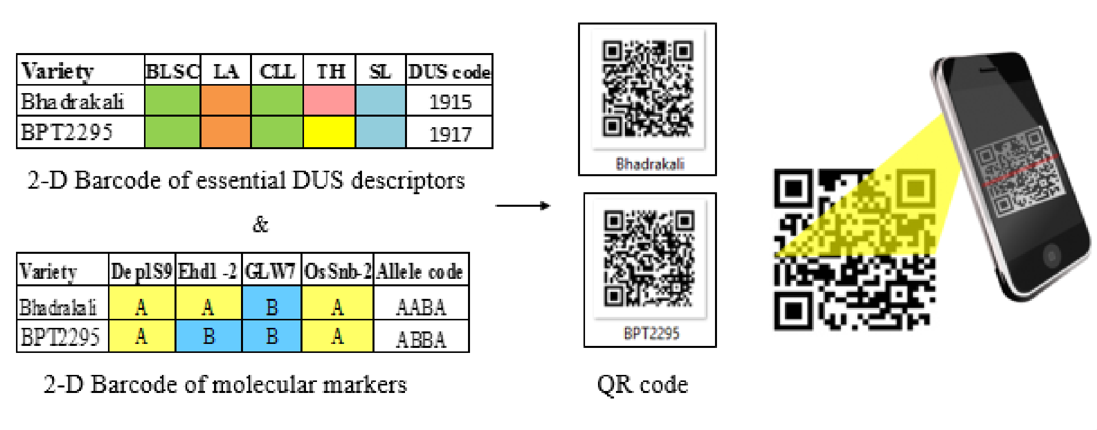
In the present study, we developed varietal-specific fingerprints for 52 rice varieties using 10 DUS descriptors and 18 gene-specific markers, which were able to distinguish 26 and 50 varieties respectively when we analysed them separately. However, using the combination of DUS traits and gene-specific markers, it is possible to distinguish all the 52 rice varieties including BPT2295 and Jaya, which could not be distinguished using gene-specific markers alone. Hence, to our knowledge, this is the first report to identify the rice varieties using both DUS traits and gene-specific markers. However, as of now more than 1200 rice varieties have been released in India and several new varieties releasing every year makes it difficult to identify each variety accurately using either DUS traits or gene-specific markers or in combination also. This situation warrants to identify more gene-markers to develop molecular profiles of all the released varieties and upcoming varieties in near future. This DUS descriptor and gene-specific markers information of all the rice varieties can be stored in a database.

The conventional method of storing the breeding information in field books often leads to manual errors during data entry and risk of missing datasheet or book etc. An alternate approach to minimize the errors, for easy maintenance and retrieval, data sharing among breeders and other end users is essential. Hence, there is an urgent need to develop the rice database comprising of all the released varieties in India along with the germplasm. Furthermore, these databases can be used to feed, retrieve, analyze and share data like already developed databases such as MusaBase (https://musabase.org), MGIS (https://www.crop-diversity.org/mgis/), ProMusa (http://www.promusa.org/) and MusaNet (http://www.musanet.org/). It is worth noting that, ICAR-NRCB (National Research Centre for Banana) developed a Banana Breeding Tracker Database (BBTbase) to monitor the progress of the banana breeding program. Similar kind of Breeding tracker has been developed for potato (Kozub *et al.*, 2000), cotton (Yu *et al.*, 2014) and apple (Evans *et al.*, 2013) and are being used in their respective breeding programs.

In all these databases, the huge information generated during the breeding programmes will be converted into QR (Quick Response) codes using a QR code generator. The information stored in the databases during the breeding programs can be viewed and retrieved in the form of QR codes and can be printed using handheld devices. The printed QR codes can be used as labels for tagging the breeding material both in field and laboratory. Any user can access the information on QR code by scanning the QR codes using the hand-held data capture devices such as barcode or QR code scanner or by using smartphones, tablet or laptop with a camera, using freely available software.

In the present investigation, descriptor notes of the 10 essential DUS traits and the allele codes of the gene-specific markers in combination were converted to Quick Response (QR) codes (Figure 4.7.) using online tool (available at www.barcode-generator.org). These QR codes developed in combination of DUS traits and gene-specific markers are capable of distinguishing BPT2295 and Jaya (Figure 4.8.) which were not distinguished using the DNA fingerprinting codes of gene-specific markers alone. The QR (Quick Response) codes generated for all the 52 rice genotypes using combined DUS and allele codes are represented in the Figure 4.9.

There are several applications of this QR code system in plant breeding. This system is rapid and efficient for unambiguous identification of rice varieties, with minimum laboratory facilities consisting of a PCR, gel electrophoresis and gel documentation. In future, by including all the released varieties in India, we can develop a rice database with QR codes for their easy access and exploitation by different laboratories in the country. Recently, a web-based model of crop variety identification based on 2-D barcode system, *VISTa* (Variety Identification System of *Triticum aestivum*) was developed by Singh *et al*. (2019) using SNP array in *Triticum aestivum* which is based



Variety	BLSC	LA	CLL	TH	SL	DUS code
Bhadrakali	🟩	🟧	🟩	🟥	🟦	1915
BPT2295	🟩	🟧	🟩	🟨	🟦	1917

2-D Barcode of essential DUS descriptors

&

Variety	DeplS9	Ehdl -2	GLW7	OsSnb-2	Allele code
Bhadrakali	A	A	B	A	AABA
BPT2295	A	B	B	A	ABBA

2-D Barcode of molecular markers

Fig. 4.7. Conversion of 2-D barcodes of DUS descriptors and molecular markers into QR codes

BPT2295

Name: BPT2295
Combined DUS and allele code:
19173517151ABBAABBBAABABABBBB

Jaya

Name: Jaya
Combined DUS and allele code:
19153715575ABBAABBBAABABABBBB

Fig. 4.8. QR codes generated for BPT2295 and Jaya using combined DUS and allele codes

Bhadrakali	BPT1235	BPT2295	BPT5204	DRR Dhan 38	Govind	IR64
Jaya	JGL3844	JGL17004	Kasturi	Kavya	Krishna hamsa	MTU1001
MTU1010	MTU1121	MTU1140	MTU1153	MTU1155	MTU1156	MTU1172
MTU1190	MTU3626	N22	Nidhi	NLR34449	Pankaj	Pothana

Fig. 4.9. QR codes generated for all the 52 rice genotypes using combined DUS and allele codes Cont…

Fig. 4.9. Cont…

PR106
Pusa Basmati-1
PUSA1121
Rajeswari
Rasi
RP Bio -226
Saket-4
Sasyasree
Shobini
Siddhi
Sona Mahsuri
Sonasali
Swarna sub 1
Tellahamsa
Triguna
Uydyagiri
Vajram
Vandana
Varadhan
Varalu
Vasumati
Vikramarya
Warangal Samba
WGL32100

on 54 SNPs and 36 DUS features that can differentiate more than 350 varieties. Including more additional gene-specific markers from key agronomic traits, the QR code system can be extended to identify even upcoming varieties in the future. In addition, this code system can be utilized to represent the recovery of recurrent parent genome content by replacing GGT (Graphical GenoTypes) software output in marker-assisted backcross breeding approach, as this system comparatively provides more information utilizing less space. It also helps in rapid identification of germplasm or breeding material management and establishes rapid, stable and accurate results across laboratories and detection platforms over time, which enables direct comparison of the data across various sources. Furthermore, in combination with capillary electrophoresis and High Resolution Melting (HRM) capable real-time (RT) PCR, this QR code system can be extended to quantify the adulteration in rice seed samples that enables to supply pure samples to the farmers, if Government adopts this system for varietal identification. While the advances in plant genomics is apparent, the complementary developments in plant phenotyping is warranted. To this end, recently, the first user-friendly field-based phenotyping app, Field Book has been developed and successfully deployed for management of breeding programs around the world. Similarly, PhenomApp is another application for collecting plant phenotypes using tablets and smartphones. It provides offline capabilities to view existing information on plots and record new observations – traits and images, in the field, which are then synchronized back into the main computers in the laboratory.

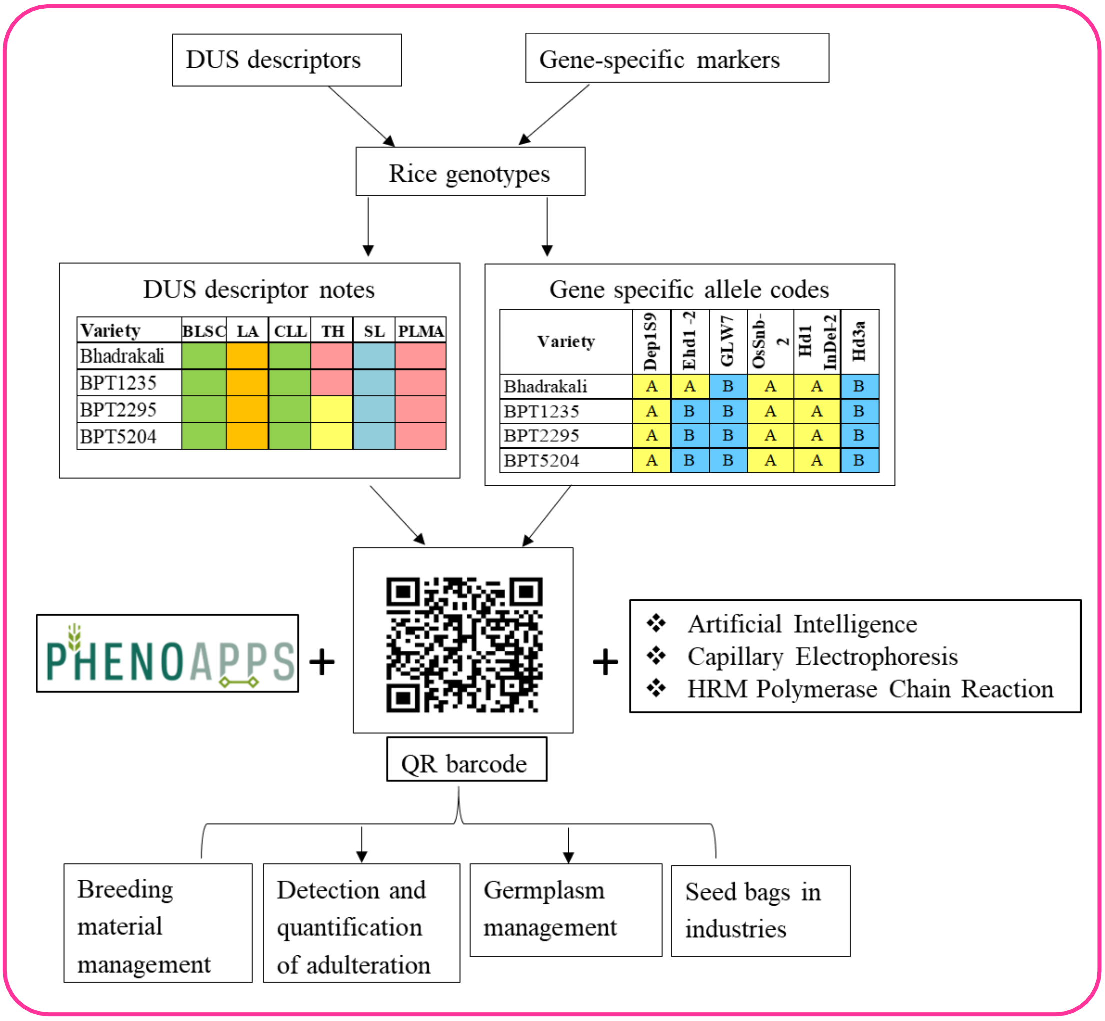
4.3.3 Model for Development of QR Code System in Rice

Unambiguous differentiation of rice cultivars is a crucial aspect for protection of Plant Breeder’s Rights and trade. Here, based on the findings of the present investigation, a model for development of QR code system has been proposed in the Figure 4.10. By screening all released rice varieties or presently cultivated varieties with large number of gene-specific markers linked to various agronomically important traits, a crop fingerprinting database can be developed with all their QR codes. This QR code system coupled with PhenoApps such as Field Book can be applied for cultivar identification, germplasm, breeding material management and trade. However, Government has to establish a dedicated Institute for executing these tasks for providing pure seeds to the farmers good quality.

4.4 GENETIC DIVERGENCE

4.4.1 Genetic Diversity Analysis Based on Gene-Specific Markers Data

The genetic relationship among the 52 rice varieties is presented in the form of a dendrogram constructed by UPGMA (Unweighted Pair Group Method with Arithematic mean) using Jaccard’s similarity coefficient (Appendix-I). The dendrogram is given in the Figure 4.11. The cluster analysis of the 18 gene-specific markers revealed similarity coefficients ranging from 0.19 to 1.00. The dendrogram grouped the 52 genotypes into seven clusters at the similarity coefficient of 60%. Cluster I comprised 7 genotypes (Bhadrakali, N22, JGL3844, MTU3626, Pankaj, PUSA1121 and Rajeswari), Cluster II contained the highest number of genotypes (37), Cluster III comprised 4 genotypes (JGL17004, Sonasali, Swarna sub1and Tellahamsa) and the genotypes *viz.*, Varadhan (Cluster IV), Kasturi (Cluster V), Udayagiri (Cluster VI) and Vandana (Cluster VII) formed into 4 distinct monogenic clusters indicating that they are genetically divergent from the remaining genotypes. UPGMA Cluster analysis of 52 rice varieties based on Jaccard’s similarity coefficient is represented in the Table 4.8. BPT2295 and Jaya fell into the same cluster with a genetic similarity of 100% possibly due to a limited number of polymorphic markers used in the study. The efficacy of



Variety	**BLSC**	**LA**	**CLL**	**TH**	**SL**	**PLMA**
Bhadrakali	🟩	🟧	🟩	🟥	🟦	🟥
BPT1235	🟩	🟧	🟩	🟥	🟦	🟥
BPT2295	🟩	🟧	🟩	🟨	🟦	🟥
BPT5204	🟩	🟧	🟩	🟨	🟦	🟥

Variety	**Dep1S9**	**Ehd1 -2**	**GLW7**	**OsSnb-2**	**Hd1 InDel-2**	**Hd3a**
Bhadrakali	A	A	B	A	A	B
BPT1235	A	B	B	A	A	B
BPT2295	A	B	B	A	A	B
BPT5204	A	B	B	A	A	B

Fig. 4.10. Proposed model for development of QR code system and its applications in rice breeding and trade

Table 4.8. Clustering of genotypes based on diversity analysis using molecular data

Cluster No.	No. of genotypes	Name of genotypes
Cluster I	7	Bhadrakali, N22, JGL3844, MTU3626, Pankaj, PUSA1121, Rajeswari.
Cluster II	37	BPT1235, MTU1140, RP Bio-226, Warangal samba, NLR34449, BPT2295, Jaya, MTU1155, BPT5204, Triguna, MTU1001, Siddhi, MTU1010, Pothana, DRR Dhan 38, Krishna hamsa, Nidhi, MTU1121, MTU1153, MTU1156, PR106, Vikramarya, Shobini, Sasyasree, Rasi, WGL32100, Kavya, Pusa Basmati-1, Saket-4, Govind, IR64, Varalu, MTU1172, Vajram, MTU1190, Vasumati, Sona mahsuri.
Cluster III	4	JGL17004, Sonasali, Swarna sub-1, Tellahamsa.
Cluster IV	1	Varadhan
Cluster V	1	Kasturi
Cluster VI	1	Udayagiri
Cluster VII	1	Vandana

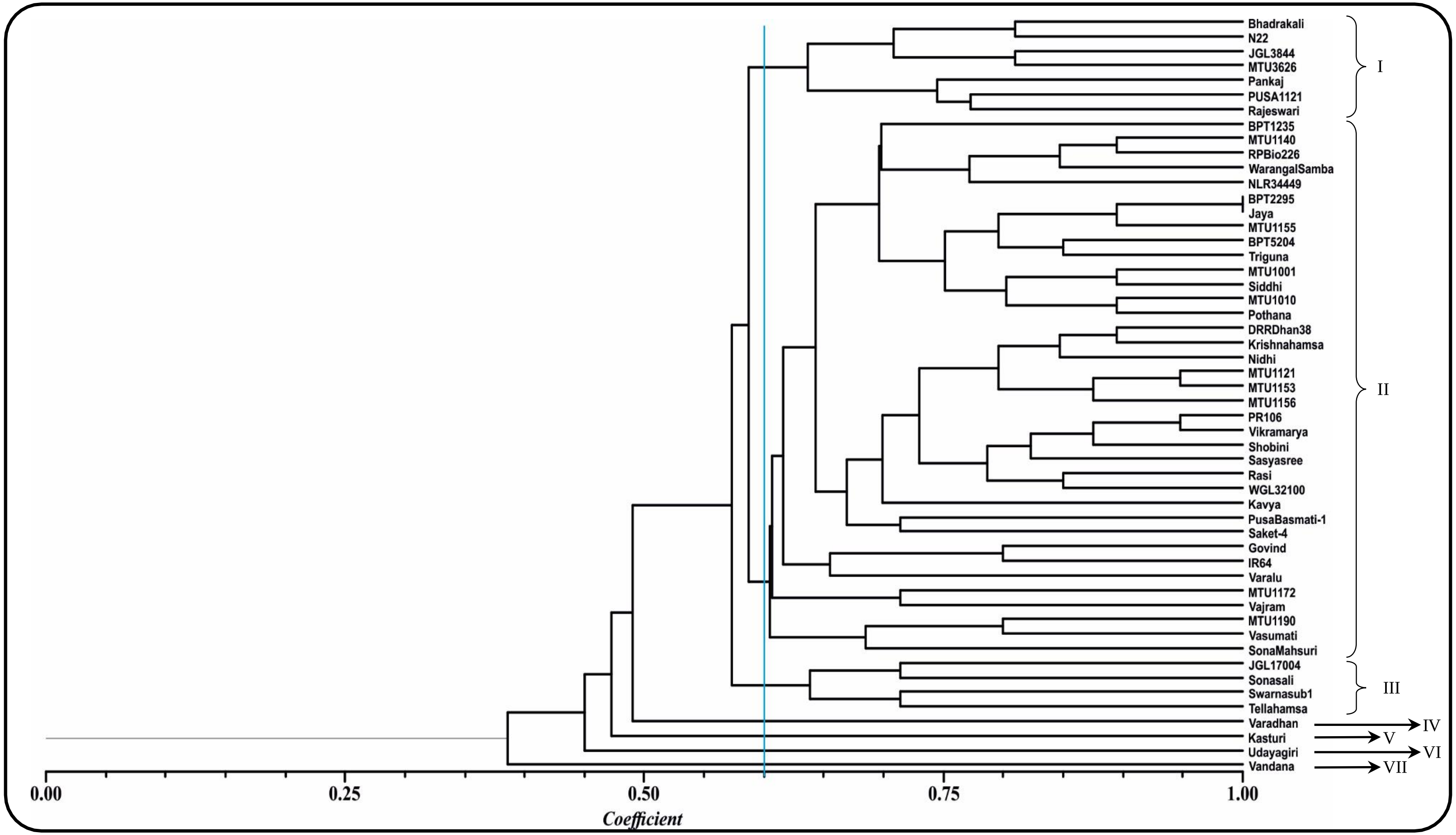


Fig. 4.11. UPGMA cluster analysis of 52 rice varieties based on Jaccard's similarity coefficient

SSR markers in determining the degree of relatedness was reported by Islam *et al.* (2017) and Reddy and Rani (2018) and the number of clusters obtained in the present study were less than the clusters obtained by Islam *et al.* (2017) and more than the clusters obtained by Reddy and Rani (2018) in their genetic diversity studies.

Varieties *viz.*, MTU1153 and MTU1121, PR106 and Vikramarya recorded the highest genetic similarity (95%) among all the genotypes studied and all these 4 genotypes were grouped into the same cluster (Cluster II) even if these genotypes did not have common parentage. Vandana and Swarna sub-1 recorded the lowest genetic similarity (19%) among all the genotypes studied and they fell into two different clusters. BPT5204 and RP Bio-226 sharing common parentage were grouped into the same cluster (Cluster II) with a genetic similarity of 76%. Saket-4 and Sasyasree being common in their parentage were grouped into the same cluster (Cluster II) with a genetic similarity of 68%. The results of clustering of the genotypes according to their pedigree were in agreement with the previous reports of Choudhary *et al.* (2013), Becerra *et al.* (2015) and Vigneshwari *et al.* (2017).

4.4.2 Genetic Diversity Analysis Based on Morphological Characteristics

Hierarchical clustering of the 52 genotypes based on morphological data using nearest neighbour methods based on squared Euclidean distance (Appendix-II) classified the genotypes into four major clusters. The dendrogram obtained is represented in the Figure 4.12. The first cluster comprised of 18 short slender, 19 long slender and all the 10 long slender (for Basmati type) genotypes and second cluster consisted of two short slender (Udayagiri and N22) and one long slender (Varalu) genotypes. However third and fourth cluster consisted of only one genotype *i.e.*, Vandana (long slender) and Rasi (short slender) respectively. The first cluster was further sub-

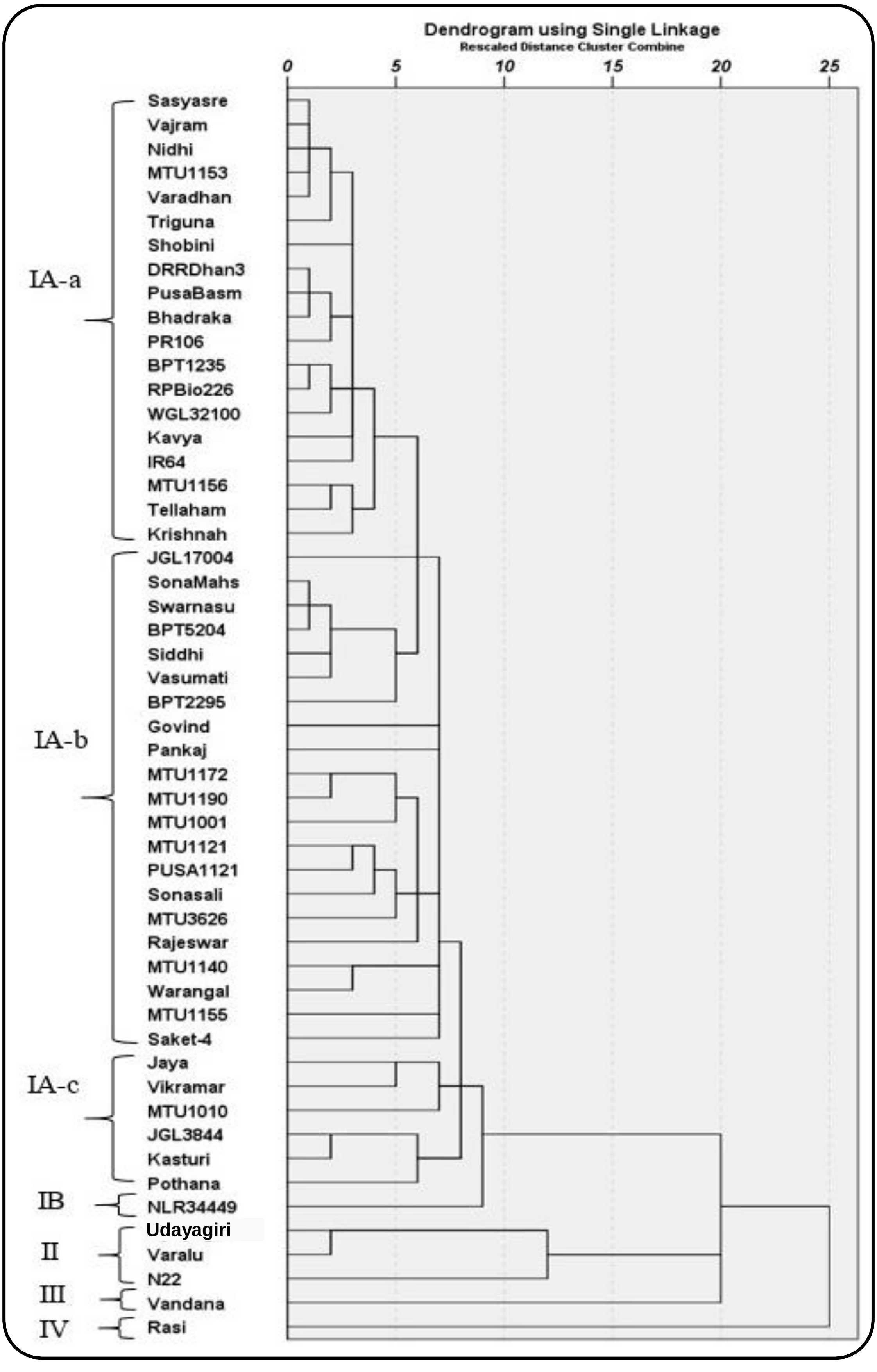


Fig. 4.12. Hierarchial clustering using morphological characters showing relationships between all the varieties

grouped into IA and IB. The number of genotypes under IA and IB are 46 and one respectively. IA of was further sub-clustered into three groups (IA-a, IA-b and IA-c). IA-a consisted of 19 genotypes, IA-b consisted of 21 genotypes and IA-c consisted of only 6 genotypes. The third and fourth clusters were monogenic consisting of only one genotype per each cluster which showed significant difference from other two groups. The genotypes, Vandana and Rasi appeared to be more diverse and may be useful as a source for variable characters for rice improvement. Similarly Vigneshwari *et al.* (2017) obtained two clusters based on their grain type in rice.

Chapter ~ V

Summary & Conclusions

Chapter – V

SUMMARY AND CONCLUSIONS

In the present investigation entitled, "Identification of rice (*Oryza sativa* L.) varieties using DUS descriptors and gene-specific markers", an attempt was made to identify 52 rice varieties using 22 DUS descriptors and 25 gene-specific markers and also to study the magnitude of diversity present among the varieties using both DUS descriptors and gene-specific markers. The salient features of the investigation have been given hereunder.

Morphological characterization of 52 rice varieties using 22 DUS descriptors revealed existence of ample polymorphism for these characters. Out of 22 morphologically assessed DUS descriptors, nine were dimorphic, nine were polymorphic and four were monomorphic. Of these 18 traits (both dimorphic and polymorphic), 10 were essential traits as per PPV and FRA. The descriptor notes of these 10 essential traits were used for creating DUS 2-D barcode for identification of rice varieties. The 2-D barcode developed using these 10 DUS descriptors was able to distinguish only 26 varieties and the remaining 26 genotypes were unable to be distinguished.

Analysis of variance for 12 characters *viz.*, time of heading (days), stem length (cm), panicle length of main axis (cm), panicle number per plant, time of maturity (days), weight of 1000 fully developed grains (g), grain length (mm), grain width (mm), decorticated grain length (mm), decorticated grain width (mm), length/breadth ratio and grain yield plant^{-1} (g) revealed significant differences among the genotypes for all the characters indicating the presence of greater variability among the genotypes.

High PCV and GCV were recorded for the traits *viz.*, panicle number per plant, weight of 1000 fully developed grains and grain yield plant^{-1} indicating that large amount of variation was present among the genotypes.

Whereas, low PCV and GCV were recorded for the traits *viz*., grain length, decorticated grain length and length/breadth ratio indicating that the variability for these characters among the genotypes was meagre. The variability studies indicated that the estimates of PCV were slightly higher than the corresponding GCV estimates for all the characters indicating that the characters were less influenced by the environment. Therefore, selection on the basis of phenotype can be effective for the improvement of these traits.

High heritability coupled with high genetic advance as per cent of mean was recorded for time of heading, stem length, panicle number per plant, weight of 1000 fully developed grains, decorticated grain width and grain yield plant^{-1} indicating that these traits were mostly under the control of additive gene action and direct selection of these traits would be effective for crop improvement. While high heritability coupled with moderate genetic advance as per cent of mean was observed for the traits *viz*., panicle length of main axis, time of maturity, grain length, grain width, decorticated grain length and length/breadth ratio indicating the presence of both additive and non-additive gene action and genetic improvement of these characters can be exploited through heterosis breeding.

Out of the 25 gene-specific markers screened in 52 rice genotypes, 18 were polymorphic. The number of alleles of the polymorphic markers ranged from two to three with a mean of 2.05 alleles per locus. The PIC values of these gene-specific markers ranged from 0.037 to 0.369 with an average of 0.212. The alleles of the polymorphic markers were assigned with codes *i.e.,* A, B and C based on their allele sizes in ascending order. The patterns of allele codes of these polymorphic markers were used to depict the DNA fingerprints. Employing these codes of the polymorphic markers, 50 rice varieties could be unambiguously distinguished. BPT2295 and Jaya were the two varieties possessing similar DNA fingerprints (allele codes) and could not be distinguished using these allele codes.

In the present study, we have developed varietal-specific fingerprints of 52 rice varieties using 10 essential DUS descriptors and 18 gene-specific markers. It was possible to distinguish 26 and 50 varieties respectively, when we analysed them separately. However, in combination of DUS traits and gene-specific markers, it was possible to distinguish all the 52 rice varieties used in the present study. This DUS and allele codes together were used to develop QR codes using an online tool (available at www.barcode-generator.org) for the rapid identification of these varieties. The QR codes generated using the data collected from the present study made it possible to distinguish similar looking varieties like BPT5204 and NLR34449.

The molecular genetic diversity among the 52 rice varieties is reported in the form a UPGMA dendrogram based on Jaccard's similarity coefficient. The dendrogram grouped the 52 genotypes into seven clusters at 60% of similarity coefficient. Cluster I comprised of 7 genotypes, Cluster II contained the highest number of genotypes (37), Cluster III comprised of 4 genotypes and the genotypes *viz*., Varadhan (Cluster IV), Kasturi (Cluster V), Udayagiri (Cluster VI) and Vandana (Cluster VII) formed into 4 distinct monogenic clusters indicating that they are genetically divergent from the remaining genotypes.

Hierarchical clustering of the 52 genotypes based on morphological data using nearest neighbour methods based on squared Euclidean distance classified the genotypes into four major clusters. The first cluster comprised of 18 short slender, 19 long slender and all the 10 long slender (for Basmati type) genotypes and second cluster consisted of two short slender (Udayagiri and N22) and one long slender (Varalu) genotypes. However, the third and fourth clusters were monogenic consisting of only one genotype *i.e.,* Vandana (long slender) and Rasi (short slender), respectively. The genotypes Vandana and Rasi appears to be more diverse and may be useful as a source for variable characters for rice improvement.

In conclusion, it was demonstrated in the present study that the combination of DUS traits and molecular markers, it was possible to develop DNA fingerprints which were further converted into QR codes that can be applied for unambiguous identification of all rice varieties. The QR codes developed in the present study can be applied not only for identification of rice varieties but also for breeding material management, germplasm management and for tagging in seed industries. In addition, the QR code system coupled with mobile-based phenotype applications such as Field Book can be used in regular breeding programmes for quick data entry and breeding material management. The molecular marker-based DNA fingerprints along with the capillary electrophoresis can be applied for detection and quantification of adulteration in premium quality rice samples.

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LITERATURE CITED

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Appendices

APPENDICES

Appendix I. Jaccard’s genetic similarity coefficients for 52 rice genotypes based on gene-specific markers

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.00																									
2	0.50	1.00																								
3	0.57	0.71	1.00																							
4	0.68	0.76	0.85	1.00																						
5	0.71	0.44	0.64	0.61	1.00																					
6	0.57	0.57	0.80	0.68	0.64	1.00																				
7	0.57	0.57	0.80	0.68	0.64	0.80	1.00																			
8	0.57	0.71	1.00	0.85	0.64	0.80	0.80	1.00																		
9	0.50	0.50	0.71	0.61	0.57	0.57	0.57	0.71	1.00																	
10	0.68	0.42	0.61	0.58	0.76	0.68	0.61	0.61	0.68	1.00																
11	0.32	0.68	0.48	0.52	0.37	0.37	0.37	0.48	0.42	0.36	1.00															
12	0.57	0.57	0.50	0.54	0.64	0.50	0.50	0.50	0.44	0.48	0.48	1.00														
13	0.64	0.50	0.71	0.61	0.89	0.71	0.71	0.71	0.64	0.68	0.42	0.71	1.00													
14	0.50	0.80	0.71	0.76	0.57	0.57	0.57	0.71	0.64	0.54	0.68	0.57	0.64	1.00												
15	0.64	0.64	0.71	0.85	0.71	0.57	0.57	0.71	0.64	0.68	0.54	0.57	0.64	0.80	1.00											
16	0.71	0.57	0.64	0.68	0.80	0.64	0.64	0.64	0.57	0.61	0.48	0.80	0.89	0.71	0.71	1.00										
17	0.44	0.71	0.80	0.68	0.64	0.64	0.64	0.80	0.71	0.61	0.61	0.64	0.71	0.71	0.71	0.64	1.00									
18	0.68	0.54	0.61	0.65	0.76	0.61	0.61	0.61	0.54	0.58	0.46	0.76	0.85	0.68	0.68	0.95	0.61	1.00								
19	0.50	0.64	0.89	0.76	0.71	0.71	0.71	0.89	0.80	0.68	0.54	0.57	0.80	0.80	0.80	0.71	0.89	0.68	1.00							
20	0.76	0.48	0.54	0.58	0.68	0.54	0.54	0.54	0.61	0.65	0.41	0.68	0.76	0.61	0.61	0.85	0.54	0.90	0.61	1.00						
21	0.57	0.57	0.64	0.68	0.50	0.64	0.50	0.64	0.71	0.68	0.48	0.50	0.57	0.71	0.71	0.64	0.64	0.61	0.71	0.68	1.00					
22	0.57	0.44	0.64	0.54	0.64	0.50	0.50	0.64	0.71	0.61	0.48	0.50	0.71	0.57	0.57	0.64	0.64	0.61	0.71	0.68	0.64	1.00				
23	0.68	0.42	0.61	0.58	0.61	0.76	0.61	0.61	0.54	0.81	0.27	0.37	0.54	0.42	0.54	0.48	0.48	0.52	0.54	0.58	0.61	0.48	1.00			
24	0.81	0.58	0.65	0.77	0.58	0.65	0.65	0.65	0.58	0.70	0.39	0.52	0.58	0.58	0.65	0.65	0.52	0.70	0.58	0.77	0.65	0.52	0.77	1.00		
25	0.71	0.44	0.64	0.54	0.80	0.64	0.64	0.64	0.71	0.76	0.37	0.64	0.89	0.57	0.57	0.80	0.64	0.76	0.71	0.85	0.64	0.80	0.61	0.65	1.00	
26	0.44	0.57	0.64	0.54	0.50	0.50	0.50	0.64	0.71	0.61	0.48	0.64	0.57	0.57	0.57	0.50	0.80	0.48	0.71	0.54	0.64	0.64	0.48	0.52	0.64	1.00

The numbers, 1-52 represents the rice genotypes mentioned in the Table (3.1)

Cont…

Appendix I. Cont...

Genotypes	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
27	1.00																									
28	0.64	1.00																								
29	0.71	0.71	1.00																							
30	0.50	0.64	0.71	1.00																						
31	0.73	0.58	0.65	0.46	1.00																					
32	0.76	0.61	0.68	0.48	0.77	1.00																				
33	0.68	0.54	0.76	0.54	0.70	0.81	1.00																			
34	0.71	0.71	0.80	0.57	0.65	0.68	0.76	1.00																		
35	0.68	0.61	0.85	0.61	0.63	0.65	0.73	0.76	1.00																	
36	0.76	0.61	0.85	0.61	0.77	0.73	0.81	0.85	0.81	1.00																
37	0.64	0.80	0.89	0.64	0.58	0.61	0.68	0.89	0.76	0.76	1.00															
38	0.48	0.61	0.68	0.61	0.50	0.46	0.52	0.54	0.58	0.65	0.61	1.00														
39	0.64	0.64	0.71	0.50	0.58	0.61	0.54	0.57	0.76	0.61	0.64	0.48	1.00													
40	0.44	0.57	0.64	0.57	0.41	0.54	0.61	0.50	0.54	0.54	0.57	0.68	0.57	1.00												
41	0.50	0.50	0.57	0.50	0.36	0.48	0.54	0.44	0.61	0.48	0.50	0.48	0.64	0.71	1.00											
42	0.50	0.64	0.71	0.50	0.52	0.48	0.61	0.71	0.61	0.61	0.80	0.48	0.64	0.57	0.50	1.00										
43	0.37	0.48	0.42	0.37	0.34	0.36	0.46	0.42	0.36	0.41	0.48	0.41	0.37	0.54	0.61	0.61	1.00									
44	0.44	0.57	0.64	0.57	0.52	0.54	0.61	0.50	0.54	0.61	0.57	0.61	0.44	0.64	0.57	0.44	0.48	1.00								
45	0.54	0.42	0.37	0.23	0.50	0.41	0.36	0.48	0.46	0.46	0.42	0.31	0.42	0.19	0.32	0.32	0.36	0.28	1.00							
46	0.41	0.65	0.58	0.52	0.54	0.39	0.50	0.58	0.63	0.56	0.65	0.63	0.52	0.46	0.52	0.58	0.50	0.58	0.50	1.00						
47	0.50	0.38	0.57	0.38	0.46	0.54	0.61	0.44	0.48	0.48	0.50	0.48	0.38	0.57	0.50	0.50	0.48	0.44	0.23	0.36	1.00					
48	0.50	0.80	0.71	0.64	0.46	0.48	0.54	0.71	0.61	0.61	0.80	0.76	0.50	0.71	0.50	0.64	0.48	0.57	0.32	0.65	0.50	1.00				
49	0.68	0.68	0.95	0.68	0.70	0.65	0.73	0.76	0.81	0.90	0.85	0.73	0.68	0.61	0.54	0.68	0.46	0.68	0.41	0.63	0.54	0.68	1.00			
50	0.64	0.64	0.71	0.50	0.58	0.76	0.85	0.89	0.68	0.76	0.80	0.48	0.50	0.57	0.50	0.64	0.48	0.57	0.42	0.52	0.50	0.64	0.68	1.00		
51	0.64	0.64	0.89	0.64	0.58	0.76	0.85	0.71	0.76	0.76	0.80	0.61	0.64	0.71	0.64	0.64	0.48	0.71	0.32	0.52	0.64	0.64	0.85	0.80	1.00	
52	0.71	0.71	0.80	0.71	0.65	0.68	0.61	0.64	0.68	0.68	0.71	0.54	0.71	0.50	0.44	0.57	0.32	0.50	0.37	0.46	0.44	0.57	0.76	0.57	0.71	1.00

The numbers, 1-52 represents the rice genotypes mentioned in the Table (3.1)

Appendix II. The similarity matrix among 52 rice genotypes using Euclidean distance

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.000																									
2	0.993	1.000																								
3	0.987	0.995	1.000																							
4	0.996	0.998	0.996	1.000																						
5	0.998	0.997	0.990	0.996	1.000																					
6	0.983	0.989	0.997	0.988	0.986	1.000																				
7	0.990	0.996	0.997	0.994	0.994	0.997	1.000																			
8	0.991	0.971	0.959	0.977	0.985	0.952	0.963	1.000																		
9	0.981	0.991	0.985	0.984	0.988	0.984	0.994	0.952	1.000																	
10	0.970	0.981	0.969	0.969	0.980	0.969	0.984	0.943	0.996	1.000																
11	0.969	0.980	0.969	0.968	0.979	0.970	0.983	0.940	0.996	0.999	1.000															
12	0.995	0.998	0.996	0.997	0.998	0.994	0.999	0.974	0.991	0.981	0.980	1.000														
13	0.993	0.992	0.984	0.989	0.997	0.981	0.991	0.978	0.993	0.988	0.987	0.994	1.000													
14	0.995	0.991	0.978	0.988	0.996	0.973	0.987	0.987	0.986	0.984	0.982	0.991	0.995	1.000												
15	0.993	0.976	0.962	0.979	0.989	0.956	0.970	0.997	0.966	0.961	0.959	0.979	0.987	0.994	1.000											
16	0.990	0.978	0.961	0.977	0.989	0.954	0.972	0.991	0.973	0.973	0.970	0.980	0.989	0.997	0.997	1.000										
17	0.987	0.996	0.990	0.989	0.993	0.988	0.997	0.961	0.998	0.994	0.993	0.996	0.994	0.991	0.972	0.979	1.000									
18	0.998	0.996	0.989	0.994	0.999	0.986	0.995	0.983	0.990	0.984	0.983	0.998	0.997	0.998	0.989	0.990	0.995	1.000								
19	0.984	0.993	0.988	0.986	0.991	0.988	0.996	0.956	0.998	0.995	0.994	0.994	0.994	0.988	0.968	0.975	0.999	0.993	1.000							
20	0.987	0.989	0.981	0.983	0.992	0.982	0.992	0.967	0.997	0.994	0.994	0.992	0.998	0.993	0.980	0.985	0.996	0.995	0.997	1.000						
21	0.998	0.994	0.983	0.992	0.999	0.978	0.990	0.988	0.987	0.981	0.980	0.994	0.997	0.999	0.993	0.995	0.992	0.999	0.989	0.993	1.000					
22	0.995	0.994	0.980	0.991	0.997	0.974	0.988	0.984	0.988	0.984	0.982	0.993	0.996	0.999	0.991	0.995	0.992	0.997	0.990	0.992	0.999	1.000				
23	0.986	0.968	0.949	0.968	0.982	0.943	0.961	0.993	0.961	0.960	0.957	0.970	0.982	0.992	0.998	0.998	0.967	0.983	0.962	0.976	0.989	0.988	1.000			
24	0.975	0.968	0.949	0.961	0.980	0.949	0.966	0.971	0.978	0.982	0.982	0.970	0.990	0.988	0.985	0.989	0.977	0.982	0.978	0.989	0.986	0.986	0.986	1.000		
25	0.996	0.993	0.982	0.992	0.998	0.977	0.989	0.985	0.990	0.984	0.982	0.993	0.998	0.999	0.993	0.994	0.993	0.998	0.990	0.994	0.999	0.999	0.989	0.988	1.000	
26	0.995	0.998	0.996	1.000	0.996	0.988	0.994	0.975	0.984	0.969	0.969	0.996	0.989	0.988	0.977	0.975	0.989	0.993	0.986	0.983	0.992	0.990	0.967	0.961	0.991	1.000

The numbers, 1-52 represents the rice genotypes mentioned in the Table (3.1)

Cont…

Appendix II. Cont...

Genotypes	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
27	1.000																									
28	0.956	1.000																								
29	0.968	0.984	1.000																							
30	0.979	0.983	0.998	1.000																						
31	0.942	0.987	0.992	0.987	1.000																					
32	0.961	0.995	0.993	0.991	0.996	1.000																				
33	0.873	0.918	0.961	0.949	0.961	0.941	1.000																			
34	0.985	0.987	0.995	0.997	0.980	0.990	0.932	1.000																		
35	0.972	0.992	0.997	0.997	0.994	0.998	0.944	0.995	1.000																	
36	0.974	0.992	0.997	0.997	0.989	0.995	0.943	0.998	0.998	1.000																
37	0.982	0.977	0.996	0.997	0.978	0.985	0.946	0.998	0.992	0.995	1.000															
38	0.991	0.981	0.993	0.997	0.975	0.986	0.925	0.999	0.993	0.995	0.998	1.000														
39	0.917	0.976	0.986	0.977	0.996	0.986	0.978	0.968	0.983	0.980	0.969	0.961	1.000													
40	0.990	0.978	0.993	0.997	0.975	0.985	0.929	0.999	0.992	0.994	0.999	1.000	0.961	1.000												
41	0.969	0.994	0.986	0.988	0.988	0.994	0.919	0.989	0.996	0.992	0.981	0.986	0.972	0.984	1.000											
42	0.971	0.988	0.996	0.997	0.993	0.995	0.946	0.993	0.999	0.996	0.991	0.992	0.984	0.991	0.993	1.000										
43	0.964	0.989	0.980	0.984	0.984	0.989	0.915	0.982	0.992	0.987	0.974	0.980	0.969	0.978	0.998	0.991	1.000									
44	0.977	0.991	0.997	0.998	0.991	0.996	0.941	0.997	1.000	0.998	0.994	0.995	0.980	0.995	0.996	0.999	0.992	1.000								
45	0.874	0.973	0.942	0.932	0.973	0.968	0.904	0.930	0.957	0.948	0.917	0.919	0.968	0.915	0.965	0.951	0.964	0.950	1.000							
46	0.968	0.993	0.981	0.984	0.981	0.989	0.908	0.986	0.992	0.989	0.977	0.984	0.965	0.981	0.998	0.990	0.998	0.992	0.962	1.000						
47	0.974	0.991	0.995	0.996	0.992	0.996	0.938	0.993	0.999	0.996	0.990	0.993	0.981	0.992	0.996	0.999	0.993	0.999	0.954	0.992	1.000					
48	0.979	0.970	0.996	0.997	0.978	0.982	0.953	0.994	0.990	0.992	0.999	0.995	0.970	0.997	0.977	0.990	0.972	0.992	0.911	0.972	0.988	1.000				
49	0.962	0.971	0.996	0.995	0.989	0.986	0.968	0.987	0.991	0.989	0.991	0.986	0.985	0.988	0.978	0.994	0.974	0.992	0.930	0.971	0.991	0.994	1.000			
50	0.984	0.991	0.992	0.994	0.981	0.993	0.922	0.999	0.996	0.997	0.994	0.997	0.966	0.996	0.993	0.993	0.987	0.997	0.940	0.991	0.995	0.989	0.982	1.000		
51	0.983	0.989	0.995	0.997	0.984	0.992	0.931	0.999	0.997	0.998	0.996	0.998	0.971	0.998	0.992	0.996	0.986	0.998	0.937	0.990	0.997	0.993	0.988	0.999	1.000	
52	0.992	0.983	0.982	0.989	0.969	0.983	0.896	0.993	0.990	0.989	0.987	0.995	0.948	0.993	0.992	0.988	0.989	0.992	0.926	0.992	0.991	0.983	0.974	0.995	0.994	1.000

The numbers, 1-52 represents the rice genotypes mentioned in the Table (3.1)

IDENTIFICATION OF RICE (*Oryza sativa* L.) VARIETIES USING DUS DESCRIPTORS AND GENE-SPECIFIC MARKERS

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ABSTRACT

An investigation was undertaken in the wet land farm and laboratory of Department of Genetics and Plant Breeding, S.V. Agricultural College, Tirupati for "Identification of rice (*Oryza sativa* L.) varieties using DUS descriptors and gene-specific markers" by employing 52 released rice varieties using 22 DUS descriptors and 25 gene-specific markers. Of these 22, 18 traits (both dimorphic and polymorphic), only 10 were essential traits. The descriptor notes of these 10 traits were used for generating DUS 2-D barcode for identification of rice varieties. The 2-D barcode developed using these 10 DUS descriptors was able to distinguish 26 varieties. The analysis of variance demonstrated significant differences among 52 genotypes for all the traits studied. High PCV and GCV were recorded for the traits *viz.,* panicle number per plant, weight of 1000 fully developed grains and grain yield plant^{-1} indicating that large amount of variation was present among the genotypes for these characters.

Out of 25 gene-specific markers used for screening, 18 were polymorphic. The PIC values of these gene-specific markers ranged from 0.037 to 0.369 with an average of 0.212. Varietal specific fingerprints were generated using these 18 markers. The order of the alleles of these gene-specific markers were used for generating the fingerprints for all the 52 rice varieties. These fingerprints were able to distinguish 50 genotypes among the 52. However, in combination of both DUS descriptors and gene-specific markers, it was possible to distinguish all the 52 rice varieties. These DUS and allele codes together were used to develop QR (Quick Response) codes using an online tool (available at www.barcode-generator.org) for their rapid identification.

The dendrogram developed by UPGMA using the polymorphism of gene-specific markers grouped all the 52 rice genotypes into seven clusters at 60 per cent similarity coefficient. Cluster I, II and III comprised of seven, 37 and four genotypes respectively. Hierarchical clustering of the 52 genotypes based on morphological data using nearest neighbour methods based on squared Euclidean distance classified the genotypes into four major clusters. Cluster I comprised of 47 genotypes and Cluster II comprised of three genotypes and the remaining two genotypes, Vandana and Rasi formed into two distinct monogenic clusters, appearing to be the more diverse.