

# GENETIC CHARACTERIZATION OF UPLAND RICE LAND RACES OF ODISHA

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*BY*

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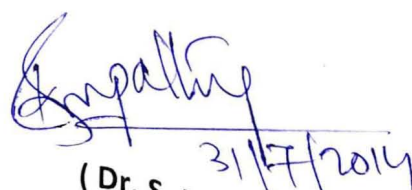
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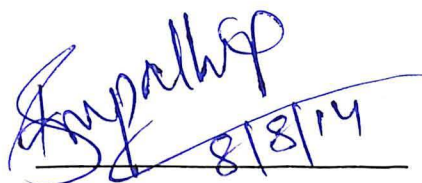
  
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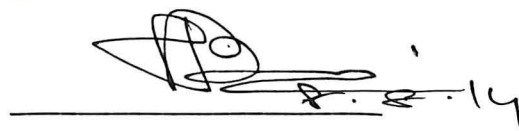
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### **ABSTRACT**

Orissa is the genetic paradise of rice and Jeypore tract (Koraput) of Odisha is considered as the home for a rich diversity of landraces. Rice contains tens of thousands of genes and finding a successful way to hunt for desirable genes is a great task. The existing varieties bred so far have low yield potential and are mostly vulnerable to drought stress and diseases. The paradox is that in order to enable to develop truly revolutionary new cultivars of tomorrow, plant breeders will need to have access to the wealth of genes which exist now, only in exotic and/or local genetic backgrounds. India and Odisha in particular have a vast upland stretches of marginal lands which mostly remain fallow even during rainy season. Therefore, the proposed pursuit entails to characterize and formulate a core germplasm from ninety six upland land races of rice including standard ruling varieties; based on DUS characteristics and agro-economic traits including drought tolerance for their suitability in upland condition. All genotypes were assessed for 54 out of 62 standard DUS parameters recommended by IRRI. Besides, the test genotypes were evaluated for 23 agro-economic traits including three drought tolerance parameters (LRS, DRS and LDS), two physiological traits (LAI and Chlorophyll Index), and tolerance to diseases and nutritional stresses (tolerance zinc and BLB) following Augmented Design. Bhogi - a local land race matures within 78 days. Besides, some of the early maturing land races are Setka 1, Dangarchudi, Podasankara, Dangaradhan, Dasaharadhan, Paradhan, Nandigiri, Dhalashree-B, Saria-B and Zhu 11-26. Among the test entries, Saria B, kalakeri, Browngora, N22 and CR Dhan 40 recorded high degree of tolerance to drought stress. Kinari and setka 1 had shown significantly higher value for both chlorophyll index and leaf area index under drought stress condition. Kutiarasi, Pora, Kusuma, Kenduphula, Ambajhuka, Kunor, Padarabank, Jhitipiti, Sanarasi and Dasaharadhan recorded extreme tolerance to zinc at seedling stage. But, only two local land races, e.g., Nagina 22(N22) and Salampikit was shown to have

high field tolerance to BLB. Bastul, Setka-2, Dhubasaria, Malkadna, Bhogi, and Jhulipuagi had shown plant stature between 90-95cm. suitable for upland situation. Kutiarasi, Badi, Hiran, and CR 143-2-2 revealed high tillering ability, while Padarabank and Pustak excelled in panicle weight and grains per panicle. Lalubadikaberi, Kahnei, Jhulipuagi and Dasaharadhan were characterized as slender grain and kernel genotypes. Grain fertility status is an important criterion for drought stress situation. Dhanisaria, Khursudi, Dular, Kandasuri, kanding, , Asumakunda, Nandigiri, Pustak and CR 143-2-2 were identified to maintain high grain fertility even more than 90% in drought stress upland condition. Asumakunda and Khursudi performed well with significantly high seed yield (>36q/ha) than the best check variety Vandana(34.8q/ha). Number of effective bearing tillers (EBT/m<sup>2</sup>), panicle weight, grains per panicle, grain length, kernel length and kernel breadth had shown significantly high positive correlation with seed yield. However, path analysis revealed importance of EBT, panicle weight, grain length and grain breadth. A regression equation  $Y(\text{Seed yield}) = -12.365 + 0.420X_1 + 0.051X_2 + 2.978**X_3 + 0.014X_4 + 0.046X_5 + 1.773X_6 - 2.550*X_7 - 0.438X_8 + 2.736**X_9 + 0.067X_{10}$  with R<sup>2</sup> value 0.76 was formulated for assessment of productivity of test genotypes. The regression coefficient of panicle weight (X3), grain breadth(X7) and kernel breadth(X9) was significant suggesting importance of these traits for selection of desirable upland genotypes for higher productivity. Analysis of genetic divergence revealed wide array of diversity among the land races. Principal component -1 contributed 79.05% of the total variation while, PC-2 and PC-3 had shown negligible contribution (3.26-11.05%). Among the agro-economic characters, number of ear bearing tillers contributed maximum to the genetic divergence. Baspatri was found most divergent based on DUS characteristics while, Setka-1 and Rangahazari revealed high genetic distance from rest of the varieties based on agro-economic traits. Considering both DUS and agro-economic traits; Baspatri, N22, Damaraphuli and Lalubadikaberi may be sorted out as highly divergent genotypes. Further, it revealed that Baspatri with either Kalakeri, Kantadumer, Dhobasaria, N22 or Dular had shown very high genetic divergence. Inter-varietal crossing between Baspatri with above genotypes may result useful transgressive segregants for desirable selection of plant types suitable for upland situation. A set of 26 drought tolerant genotypes identified in this pursuit and three popular upland genotypes (Khandagiri, Mandakini, Sneha) were characterized using total seed storage protein fingerprinting. Nineteen polypeptide bands were identified including four monomorphic bands with 78.94% polymorphism. The test genotypes were distributed over nine clusters. N22, khursudi, Kinari, Khandagiri and Zhu 11-26 were identified to be highly divergent in seed protein expression. Besides, a few genotype-specific polypeptide markers identified in this pursuit may be useful for varietal identification.

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## CHAPTER-I

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# *Introduction*

# INTRODUCTION

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Rice (*Oryza sativa* L) is one of the most important food crop of the developing world. About more than 40% the world's population, especially the people in many developing countries, are living on this important crop (Bellon *et al.*, 1998.). It provides up to two thirds of calories for more than two billion people in Asia. Nearly 100 million people now depend on upland rice as their daily staple food. Almost two-thirds of the upland rice area is in Asia. Bangladesh, Cambodia, China, India, Indonesia, Myanmar, Thailand, and Vietnam are important producers. Many upland farmers plant local rices that do not respond well to improved management practices but these are well adapted to their environments and produce grains that meet local needs (Dhakal *et al.* 2006).

Thus, understanding valuable genetic diversity and using it to breed new rice varieties will provide the foundation for improving rice production. Genetic improvement of rice in India and many other rice growing countries has already achieved yield platuae for medium land irrigated rice ecosystems, but areas pertaining to rainfed rice ecosystems particularly high land situations are constrained with low productivity owing to erratic rainfall distribution. Grain yields are generally as low as 0.5 to 1.5 tonnes/ha in Asia, about 0.5 metric tonnes/ha in Africa and 1 to 4 metric tonnes/ha in South America (Fageria *et al.*, 1982) The existing genotypes particularly the short duration rice varieties so far developed are rarely adaptable to pre and post-monsoon drought situation. It has been contemplated that climatic change will compel rice breeders to reorient the breeding strategies and develop high yielding short duration rice varieties to combat the recurrent occurrence of drought in coming years.

About 90% of the world's rice is produced and consumed in Asia. Rice provides 20% of the world's dietary energy supply in different region of the world. 100g of rice produce 330 kcal energy, 79 g carbohydrates, 8.0g proteins, 0.69 g fates and is a good source of thiamine, riboflavin and niacin (FAO, 2009). The proteins of rice are mainly

compose of glutelin while prolamin is in lower quantity, that's why rice kernels are consider to have high quality proteins (Mullins, 1999).

Rice (*Oryza sativa* 2n=24 AA) belong to the family "Poaceae. The genus *oryza* comprises 25 species distributed throughout the world including tropical and subtropical region of world. Out of 25 common species of *oryza*, two species i.e., *Oryza sativa* and *Oryza glaberrima* are cultivated widely. *Oryza sativa* is grown world wide while *Oryza glaberrima* is only confined to West Africa (Grist, 1986). A lot of genetic diversity was lost during Asian rice domestication that led to the *indica*(adapted to the tropics) and japonica subspecies(adapted to temperate regions) (Kochko, 1987). However, rice relative species belonging to the *Oryza* AA genome complex contain a high amount of diversity. The indigenous African rice, *O. glaberrima*, has acquired adaptive or protective mechanisms against many of the major biotic and abiotic stresses during its evolution. It has good weed-competitiveness due to early vigor and excellent ground cover and tolerance to drought, soil acidity and other stresses. The reproductive barriers with *O. sativa* make the utilization of *O. glaberrima* in rice improvement difficult (Ndjioudjop *et al.* 2010). It is essential to determine genetic diversity and identify a subset of alleles from the wild or exotic germplasm and produce elite breeding lines that would contain only specific "wild QTLs" or "genes". A number of useful traits such as cytoplasmic male sterility, resistance plant hopper, have been introgressed from wild-species into cultivated rice (Brar and Khush 1997). Considering the large number of wild species germplasm available, it is difficult to choose which accession should be used in order to maximize the chances of finding new and useful genes. Seed protein profiles, DNA profiles and genome-specific markers can be generated to determine genetic diversity and alien introgression into elite breeding lines. Morphological, isozyme and molecular makers have been used to determine genetic diversity and phylogenetic relationships in *Oryza* ( Dally and Second 1990; Wang *et al.* 1992; Aggarwal *et al* 1999).

Orissa is the genetic paradise of rice and Jeypore tract (Koraput) of Odisha is considered as the home for a rich diversity of landraces (Ramiah, 1953). More than 1,750 traditional cultivars of rice existed during 1955-60. Sharma *et al.* (2000) concluded that this area could be a center of origin of the aus (early maturing upland varieties) ecotypes. The aus rices of the Jeypore tract have many special features such as early maturity, short

height, thin culm, few tillers, small panicles with awns, and often (though not always) black /brown husk, red/ light red kernel. Traditional rice varieties were forgotten in the past decades; but they are the “heart and soul of rice”. They require little fertilizer and no chemical inputs. Traditional rice varieties are more nutrient-rich, tastier and friendlier to the soil. They allow farmers to protect their soil and ecosystem and have control of the seeds that their forefathers have reared for centuries. The paradox is that "If few of these rice types share a favourable trait, like drought tolerance, high yield, or even desirable cooking quality characteristics, they are likely to also share the same DNA variation responsible for that trait." Rice contains tens of thousands of genes, so finding a successful way to hunt is a great task. IRRI maintains the International Rice Gene Bank containing over 109,000 types of rice, yet relatively few have been used in breeding programs. "If breeders know more about the genetic makeup of rice, they can use it more effectively. As we face more erratic changes in climate, we will increasingly rely on using the untapped diversity of rice to develop new and improved rice varieties." The comprehensive SNP information is enabling the exploration of rice diversity not only for understanding how genes function in a growing and developing plant, but also for improving important rice traits related to disease resistance, drought tolerance, increased productivity, and human health benefits. Compared to Asian rices(*O. sativa*); the African rices (*O. glaberrima*) are well-adapted to the marginal lands and responsive to even low inputs. Such vars. are preferred by many African farmers and are grown in 25-40% of the upland area. New, putatively highly weed-competitive and drought-tolerant plant types based on interspecific crosses of Asian and African rice would pave the way for increase of rice productivity in upland situation (Craufurd *et al.* 2000).

The biophysical constraints that limit upland rice yield in highlands are numerous. The most important biotic constraint is weeds, followed by blast. Major knowledge gaps remain on weed competitiveness under drought conditions, which is frequently associated with weed problems. Leaf area index (LAI), specific leaf area(SLA) and tillering ability are the major determinants of early vegetative vigour which confer to compete with weed growth. Among the abiotic stresses, the major constraints are depleted soils in major elements, soil chemical disorders related to acidity (low pH between 4 and 7) and drought caused by erratic rainfall. The acidity present in the subsoil of many upland areas

prevents plant roots from reaching the moisture and nutrients therein, thus reducing crop yield. On the other hand, the complex nature of drought tolerance, genotype × environment interaction, and the difficulty of effective drought tolerance screening complicate the development of drought tolerant varieties. Several varieties that have been widely grown in rain fed environments in India for many years are indeed lack of drought tolerance leading to low productivity. The cultivation of upland rice is a non-flooded alternative to lowland rice culture that can reduce the demand for irrigation water by 50–70%. Rice varieties adapted specifically to upland conditions are typically characterized by a deep root system, tall stature, thicker stem and fewer tillers. These characteristics are believed to confer adaptability to the upland conditions. It is reported that the long and thick root system of upland rice contributes greatly to its drought resistance. Additional studies have shown that the ratio of root weight to shoot weight, and root penetration ability is also correlated with drought resistance. Identification of useful markers linked to drought resistant trait(s) would help to develop drought tolerant rice varieties using marker assisted selection.

India and Odisha in particular have a vast upland stretches of marginal lands which mostly remain fallow even during rainy season. These areas are often inhabited by socially and politically disadvantaged ethnic minorities. For these populations, food security remains a daily battle. These areas may be brought under cultivation to feed the rural poor. Therefore, the proposed pursuit entails to formulate a core germplasm based on DUS characteristics and characterize them for drought tolerance. The drought tolerant donors may be used for future breeding and development of high yielding drought tolerant upland rice varieties.

It is in vogue to evaluate varieties for DUS (Distinctness, Uniformity, and Stability) characteristics based on morphological expressions (in the field) which are largely influenced by environmental conditions (Goodrich et al., 1985). The morphological markers were not quite enough to expose the genetic diversity between the morphological overlap cultivars and the morphological identical accessions. Therefore, there is a need for a new tool. The advent of electrophoretic analytical tool provides indirect methods for genome probing by exposing structural variations in enzymes and proteins (Cook 1984; Gilliland 1989). Electrophoretic markers appear to be due to neutral

genes which do not affect expression of any loci of the genome. Therefore, these are independent of cultivar morphology and physiology, and offer significant advantages over morphological methods of variety and/or species identification in that they are rapid, relatively cheap, eliminate the need to grow plants to maturity and largely unaffected by the growth environment. The biochemical methods have some disadvantages e.g. that they are profoundly influenced by tissue specificity and developmental stage. This disadvantages can be overcome by using the electrophoretic markers of a conservative protein e.g. seed storage proteins.

Seed storage protein profiles have been used to study evolutionary relation of several crop plants (Ravi et al., 2003). Besides, the polypeptide banding pattern of seed storage proteins can reveal a considerable degree of polymorphism, some homology across taxa and a simple genetic control subject to minimum environmental influence. This is also used to study genetic control of protein expression, linkage of polypeptide bands inter se, stability of genotype-specific polypeptide banding pattern, centre of genetic diversity and dissemination pathways by several workers. Recently, the number of available DNA markers in plants has increased dramatically with the use of molecular biology techniques. With these techniques, it is now possible to identify variation among the individuals which otherwise may not be differentiated based on visible or protein phenotype. Moreover, these techniques need enough capital outlay which is not available to most of the laboratories in the developing countries.

The polypeptide banding pattern based on SDS-PAGE of seed proteins does not require amplification as in case of random amplified polymorphic DNA (RAPD) or radio-isotope labelling as in case of restriction fragment length polymorphism(RFLP). In spite of the fact that, the degree of intra-specific variation exhibited by polypeptide banding pattern in SDS-PAGE is lower as compared to RAPD and RFLP; this simpler and cheaper technique could be a viable alternative to study genetic variation and genetic amelioration in crop plants.

The intelligent exploitation of rice varieties for crop improvement programme requires a detailed knowledge of genetic and historical relationships among varieties and an understanding of the partitioning of genetic diversity among them. This would help

identify a set of germplasm lines that have maximum diversity in their genetic background. In the present study, therefore, an attempt has been made to use drought tolerance parameters and agro-economic traits under upland condition for phenotyping; and the potential of SDS-PAGE of total seed storage proteins in genotype profiling of a set of 96 early maturing rice varieties including 83 land races of Odisha. The specific objectives of the present research pursuit were outlined to:

- ❖ characterize germplasm lines using standard DUS characteristics.
- ❖ select suitable plant types with biotic and abiotic stress tolerance.
- ❖ formulate a suitable selection strategy for identification of promising rice genotypes suitable for upland condition.
- ❖ standardize the protocol for SDS-PAGE of total seed storage proteins in short duration rice genotypes.
- ❖ study polypeptide banding pattern in a set of short duration land races of rice
- ❖ select high yielding plant types with improved agro-economic traits.
- ❖ identify highly divergent example local land races of rice for drought tolerant traits.
- ❖ explore genotype-specific protein type or profile and specific polypeptide marker in particular for reliable identification of test genotypes.

CHAPTER-II

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*Review of Literature*

# REVIEW OF LITERATURES

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Among the rice cultivating Asian countries, India is a major producer of many rice varieties. Rice crop can potentially thrive and grow successfully in a wide range of agro-climatic conditions. However, for the last few decades, drastic changes in climatic conditions particularly extremes of ecological adversities including extremes of water availability and temperature. affected the cultivation and quality of rice varieties (Naz *et al.*,2006).

Repeated use of selected rice breeding lines in various breeding programs not only limits the genetic base but also develop susceptibility to various abiotic and biotic stresses. In these circumstances, genetic variability of existing rice germplasm should be maintained for several economical traits by conserving landrace genotypes and broadening the gene pool of rice for future breeding programs (Rabbani *et al.*, 2008). It can be possible only by the availability of genetic variations (local and exotic germplasm) (Javid *et al.*,2004) and their assessment for varietal improvement (Sadia *et al.*, 2009). Genotyping of different species is also necessary for characterization of different accession of crop germplasm, testing varietal purity and registration of newly developed cultivars (Chowdhury *et al.*, 2002).

## MORPHOLOGICAL CHARACTERIZATION

### **Genetic variability:**

The amount of genetic variability present in a set of test materials is a prerequisite for effective selection. It is, therefore, essential to assess and quantify genetic variability for recovery of superior genotypes.

Genetic variability on agro-economic traits of rice under different ecosystem have been extensively documented and reviewed by several workers. However, in the present pursuit, an attempt has been taken to review recent reports on genetic variability of grain yield and yield related traits with special reference to upland rice.

Gomathinayagam *et al* (1990) studied the genetic variability in 40 upland rice genotypes. The coefficient of variation was high for number of effective tillers per plant, grain yield per plant and weight of roots. High heritability estimate and genetic advance were observed for the characters which may be considered reliable for selection under rain fed condition.

Sharma *et al.* (1990) measured awning, pigmentation, length: breadth (L/B) ratio and 1000-grain weight in 43 traditional rice varieties. Grain length ranged from 5.7 to 9.9 mm, breadth from 1.8-3.0 mm and L/B ratio from 2.4 to 4.3. A range of 8.4 to 25.5g was recorded for 1000-grain weight.

Singh *et al.* (2000) studied genetic variability and character association in comprising 42 rice genotypes in Boro season. Significant genotypic variation was observed for most of the characters. The yield attributing characters had high heritability coupled with high genetic advance.

Rao (2000) observed highest genetic variation in grain yield followed by percentage of filled spikelets in very early rice genotypes under upland condition. High heritability estimates coupled with high genetic advance was also observed for 1000-grain weight suggesting the dominant role of additive gene effects in expression of the trait.

Yadav (2000) observed appreciable amount of genetic coefficient of variation, heritability and genetic advance for total grains /panicle, grain yield /plant, total grains/plant in 25 genotypes of rice, which indicates the additive gene effect in inheritance of these traits.

Das *et al.* (2001) studied 29 boro rice genotypes and reported that the estimates of PCV is more than GCV for the characters like panicle number, panicle length, plant height, number of tilled grains/panicle, grain yield and harvest index suggesting the influence of environmental factors for the expression of these traits. High heritability values along with high genetic advance for characters like plant height, panicle number, harvest index, total grain yield /plant indicate additive gene action for these characters.

Roy *et al.*(2001) observed high phenotypic and genotypic variance for grain yield, followed by number of filled grains per panicle. Heritability ranged from 50%( grain yield per hill ) to 90% (grain breadth). Genetic advance as percentage of mean was highest for number of filled grains per panicle(70.34) followed by grain yield (68.72). Number of filled grains per panicle and 1000-grain weight, grain length and breadth exhibited less environmental effect and high heritability coupled with moderate to high genetic advance.

Yadav *et al.*(2001) evaluated genetic variability for yield and its component characters for 124 rain fed landraces of rice during kharif 1999 in Chhattisgarh. The results obtained in respect of different metric traits, viz days to maturity, plant height, number of tillers per plant, panicle length, number of grains per panicle, 100-seed weight and grain yield per plant revealed the presence of significant variability for all these character studied.

Zuo Qingfan *et al.*(2001)in their study of genetic control of quality traits of rice grain reported that the endosperm nuclear genes mainly influenced amylose content, gelatinization temperature and grain length with an additive effect and mainly influenced gel consistency and protein content with dominant gene action .The effect of genotype x environmental interactions on the quality traits of rice appeared mainly as maternal effect x environment and cytoplasm x environment interactions.

Tair *et al.*(2002) reported highly significant mean sum of squares for almost all economic traits suggesting the presence of genetic difference among the rice genotypes. Phenotypic and genotypic coefficients of variation(PCV and GCV) were of comparable magnitude except grain yield per plant and grains per panicle , where the environmental coefficients of variation (ECV) might have contributed more to the PCV than GCV. Shirame and Muley (2003) reported maximum coefficient of variation in grain yield /hill, dry matter /plant and number of sterile spikelets/panicle.

Chand *et al.* (2004) reported the estimates of GCV and PCV was high in case of the characters viz., grain yield, panicle height and number of filled grains/plant.

Similarly, Sinha *et al.*(2004) reported high value of PCV and GCV for the characters like grain yield , days to heading , panicle number and grain weight .

Shukla *et al.* (2004) reported high value of PCV and GCV for the characters like grain yield ,panicle number , number of filled grains, grain weight ,harvest index and low values of GCV and PCV for days to heading and plant height. While, Raju *et al.*(2004)reported high value of PCV and GCV for the characters like grain yield, and panicle number, moderate values for plant height and low values for panicle length. Das *et al.*(2005) observed high value of PCV and GCV for the characters like grain yield, panicle length, panicle number, plant height and low value in case of days to flowering. Sinha *et al.*(2007) reported high value of PCV and GCV for grain yield ,panicle number and biomass yield and low values for days to flowering ,plant height and panicle length. Sarkar *et al.* (2007) observed high values of PCV and GCV in case of 100 - grain weight.

Kundu *et al.*(2008) studied 35 genotypes of tall indica aman rice for nine panicle characters under two environments to find out the extent of genetic variability present in the population. Genotypic and phenotypic coefficient of variation was high for unfilled florets/ panicle, grain yield/ plant, panicle number/plant, grains/panicle, panicle weight and 1000- grain weight.

Laxuman and Salimath *et al.* (2010) studied backcross inbred line (BIL) population developed from an inter-specific cross between *Oryza sativa* cv. *Indica* and NERICA-L-20 (New Rice for Africa) and assessed genetic variability for yield and its component traits. It is observed that phenotypic coefficients of variance and genotypic coefficients of variance for most of the traits were either high or moderate except for productivity traits like days to heading, days to 50% flowering, panicle length and plant height at maturity.

Immanuel *et al.* (2011) reported high heritability coupled with high genetic advance and high GCV for number of tillers per plant followed by number of productive tillers per plant, plant height and grain yield per plant.

Prajapati *et al.* (2011) showed high estimates of heritability coupled with high genetic advance for harvest index followed by number of spikelets per panicle, number of panicle per hill, and number of tiller per hill.

### Character association:

The extent of genetic improvement depends on selection of desirable plants basing on mutual relationship between various plant characters including seed yield. But, it is often constrained with unfavorable linkages resulting in genetic slippage and limited genetic advance. The nature and extent of association inter se among yield and its component characters including quality parameters have been extensively documented. A brief review of recent works is summarized below.

<b>Characters reported to have significant positive association with grain yield in scented rice</b>	<b>References</b>
No. of grains per panicle and 100-grain weight at the genotypic level	Nath and Talukdar (1997)
Plant height, productive tillers per plant, panicle weight, total spikelets per panicle and spikelet fertility	Basavaraja <i>et al.</i> (1997)
Panicles per plant, panicle length and grains per panicle in F <sub>2</sub> generation of eight crosses	Gupta <i>et al.</i> (1997)
Panicle number per unit area, grain numbers per panicle and fertility percentage	Goswami <i>et al.</i> (2000)
Plant height, panicle number per plant, panicle length, total number of spikelets per panicle and total no. of grains per panicle at both genotypic and phenotypic levels.	Nayak <i>et al.</i> (2001)
Number of effective tillers per plant, biological yield and harvest index at genotypic and phenotypic levels in 16 cultivars and 72 F <sub>1</sub> hybrids.	Mishra and Verma (2002)
Effective tillers per plant, spikelet density and biological yield per plant.	Chaudhury and Motiramani(2003)
Days to flowering	Khedikar <i>et al.</i> (2004)
Plant height and spikelet fertility	Das <i>et al.</i> (2000)
Productive tillers per plant (but negative association of grain number is having yield)	Chuhan <i>et al.</i> (2003)

Productive tillers per plant, grain no. per panicle, harvest index and biological yield.	Satish <i>et al.</i> (2003)
Productive tillers per plant	De <i>et al.</i> (2005)
Grain weight (test weight)	Sadhukhan & Chattopadhyay(2000) Singh <i>et al.</i> (2002)
Flag leaf area	Sharma and Haloi (2001)
Chlorophyll content	Ghosh <i>et al.</i> (2003)
Number of effective tillers per plant, number of grains per panicle (but negative association with sterility percentage) at both genotypic and phenotypic levels.	Talukdar and Talukdar (1998)

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## **B) Inter-relationship in different yield component characters:**

Among yield component characters- days to 50% flowering, number of panicles per plant, number of primary branches per panicle and number of filled grains per panicle were positively and significantly inter-correlated among themselves (Mahto *et al.* 2003), Cai *et al.*(1989) reported positive significant correlation of total dry matter with panicle number and number of spikelets per panicle. However, harvest index revealed positive significant correlation with number of fertile grains per panicle (Satish *et al.*2003) and 100-grain weight (Sarawagi *et al.* 1997). Fertile grains per panicle registered positive significant association with panicle length (Gupta *et al.*1999), number of productive tillers per plant( Satish *et al.*2003) and total spikelets per panicle but significant negative correlation with seed sterility(Prasad *et al.*1998). Gupta *et al.*(1999) observed significant positive correlation of panicle weight with number of panicles per plant, panicle length and grains per panicle in F<sub>2</sub> generation of eight crosses of basmati rice.

Goswami *et al.*(2000) observed positive association between panicle number per unit area, grain numbers per panicle and filled grain percent with grain yield per plant. Nayak *et al.* (2001) observed positive association of grain yield/ plant with the characters as plant height, panicle number per plant, panicle length, total

number of spikelets per panicle and total number of grains per panicle at both genotypic and phenotypic level. Chaudhury and Motiramani (2003) reported positive correlation between grain yield per plant and characters like effective tillers per plant, spikelet density and biological yield per plant.

Sinha *et al.* (2004) reported positive relation between productive tillers/ plant and grain yield. Swain and Reddy *et al.* (2005) found days to 50% flowering to have positive association with grain yield/ plant. Babu *et al.* (2006) observed positive correlation between plant height and grain yield/ plant.

Bastia *et al.* (2007) observed positive association of days to 50 % flowering, plant height, number of productive tillers/ plant and panicle length with grain yield/ plant. Reddy *et al.* (2008) studied correlation and component analysis for 12 characters in 20 genotypes and found that a +ve (positive) and significant correlation was found for the majority of the characters as panicle length, number of spikelets per panicle, flag leaf area, biological yield and harvest index, highly significant and positive correlation was observed between number of tillers & number of panicles per plant.

### **Path analysis:**

The concept of path analysis was originally developed by Wright in 1921, but the technique was first used for plant selection by Dewey and Lu (1959). Wigon and Mather stated that the linkage of polygenes of different traits could be the most plausible cause of genetic correlation and revealed the importance of causal factors in producing the effect or end product. Path coefficient analysis involves computation of simple standardized partial regression coefficients which split the correlation coefficient of an independent variable with the depended variable into the measures of direct and indirect effects. It reflects the direct and indirect contribution of independent variables on the depended variables and therefore, it is crucial for selection of genotypes. A brief review of recent reports on path analysis of component traits on grain yield in upland rice is summarized below.

**Direct effects of different characters on grain yield:**

<b>Characters</b>	<b>Direction of effects</b>	<b>Reference(s)</b>
Days to 50% flowering	Positive	Khedikar <i>et al</i> (2003)
Productive tillers per plant	Positive	Nayak <i>et al</i> (2001) Singh <i>et al</i> (2002) Chaudhury and Motiramani(2003)
Productive tillers per plant	Positive	Khedikar <i>et al</i> (2003) Basavaraja <i>et al.</i> (1997) Satish <i>et al.</i> (2003) Behera (2007)
Panicle length	Positive	Singh <i>et al.</i> (2002) Mishra and Verma (2002) Khedikar <i>et al</i> (2003)
Grain number per panicle	Positive	Nayak <i>et al</i> (2001) Singh <i>et al</i> (2002) Satish <i>et al.</i> (2003) Jena (2002)
Harvest index	Positive	Mishra and Verma (2002)
Flag leaf area	Positive	Mishra and Verma (2002)
100-grain weight	Positive	Jena(2002) Singh <i>et al.</i> (2002) Khedikar <i>et al</i> (2003) Satish <i>et al.</i> (2003)
Panicle weight	positive	Gupta <i>et al.</i> (1999)
Dry matter production, leaf area index, relative growth rate, crop growth rate	Positive	Das <i>et al.</i> (2000)
Linear elongation ratio, breadth wise expansion ratio, kernel L/B ratio	Positive	Vivekananda and Giridharan(1998)
Leaf area index, harvest index, net assimilation rate, fertile grain number/panicle.	Positive	Kar (2008)
HRR%, elongation ratio, water uptake , volume expansion ratio	Positive	Jena (2002)

### **Genetic divergence:**

Each genotype differs from the other depending upon the genetic architecture producing contrasting characters. The genotypes which are more distantly related are likely to produce transgressive segregants in the recombination breeding owing to vast scope of shuffling of genes through recombination. Therefore, study of genetic divergence is a pre-requisite in a set of breeding material or germplasm lines to select desirable parents for hybridization.

Multivarietal analysis as a potent tool for assessment of diversity was first postulated by Mahalanobis (1928). Murty and Arunachalam (1966) and Vanaja *et al.*(2003) did not find parallelism between genetic diversity and geographical distribution and stated that genetic drift and selection in different environments could cause greater diversity than geographic distance. Mahalanobis' generalized distance ( $D^2$  - statistics) has been effectively used in assessing genetic divergence in rice by number of workers. Similarly, Bansal *et al.*(1990) reported genetic distance of fifty breeding lines and ten cultivars of diverse parentage using data from eleven quantitative traits and made twelve clusters. They also found that clustering pattern was influenced by the pedigree of the breeding lines.

Sinha *et al.*(1991) assigned six clusters to thirty traditional varieties on the basis of Mahalanobis  $D^2$  - statistics calculated for 10 growth and yield related traits and found cluster-1 combined with 66.6% of all genotypes while cluster IV, V and VI were monogenotypic and varieties from North-Eastern region showed the greatest diversity represented in all cluster except VI. However, De *et al.* (1992) grouped twenty eight early maturing genotypes grown under direct seeded and transplanted conditions during Rabi into five and six clusters under direct sowing and transplanting conditions respectively, after evaluating the genotypes for twelve quantitative characters.

Reddy and Mahana (1992) derived cluster means and intra-cluster distances, using  $D^2$ -statistics from data on seven yield related characters in twenty-five diverse genotypes of early duration. The genotypes were distributed among eight clusters,

differentiated mainly by days to 50% flowering. Roy and Panwar (1995) reported that genetic diversity was not related to geographic diversity. Out of eleven characters studied, yield per plant, panicles per plant and spikelets per panicle were responsible for genetic divergence.

Vivekanandan and Subramanian(1993) assessed genetic divergence in twenty - eight genotypes of rain fed rice using Mahalanobis D<sup>2</sup>-statistics. On the basis of yield and eight yield related character of fifty rice genotypes, Bhardwaj *et al.* (2001) concluded that length: breadth ratio contributed maximum towards genetic divergence followed 100-grain weight and grain yield per plant accounting to 80% of total divergence.

Rather *et al.*(2001) grouped fifty-six genotypes in to eight clusters. The clusters-I comprised of thirty-eight genotypes, whereas the clusters IV, V, VII and VIII were monogenotypic. The genotypes from different regions were grouped in the same cluster as revealed by the cluster I, II and VI; whereas in cluster III all entries were from the same region (Hungary).On the other hand, the study also revealed the existence of genetic diversity within the same region, as the cultivars of the same region could be grouped in to different clusters. The 100-grain weight and length/breadth ratio of grain was important components of divergence.

Roy *et al.*(2002) revealed the nature and magnitude of genetic diversity in 50 high yielding varieties and traditional germplasm of rice. The genotypes were grouped into 10 clusters. The pattern of distribution of genotypes within various clusters was random and independent of geographical origin of adaptation. Days to 50% flowering, grain length, kernel breadth and grain yield/ plant were found to be major component characters contributing towards genetic diversity. Joshi *et al.*(2008) studied the genetic divergence among rice varieties in Punjab and found days to 50% flowering and plant height contributing to 61% of total divergence.

Mini and Mohanan (2009) studied the genetic diversity of 39 cultivars in Kerala and found that cultivars are very rich in genetic diversity and many of them showed their competence in terms of many agronomic characters. Similarly,

Manonmani and Khan (2003) estimated the nature and magnitude of genetic divergence in fourteen indica rice genotypes. The genotypes were grouped into 5 clusters using the Mahalanobis D<sub>2</sub> analysis. The cluster-I considered of 10 genotypes and the clusters II, III, IV and V had one genotype each. Number of filled grains per panicle, days to 50% flowering and plant height showed maximum contribution to the genetic divergence. The cluster III exhibited relatively high mean values for days to 50% flowering and grain yield.

## **BIOCHEMICAL CHARACTERIZATION**

Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, seed storage protein analysis represents a valid alternative and/or improved approach to varietal identification (Mennella *et al.*, 1999). It is a useful tool for studying genetic diversity via sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Sadia *et al.*, 2009) in a short period of time (Netra and Prasad, 2007). Seed storage protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts *et al.*, 1986; Sadia *et al.*, 2009) compared to other group of proteins.

Systematic studies have been carried out, notably by T. B. Osborne (1859–1929) who can be regarded as the father of plant protein chemistry. Osborne developed a classification of plant proteins based on their solubility in a series of solvents, for example, albumins in water, globulins in dilute saline water. Although ‘Osborne fractionation’ is still widely used, it is more usual today to classify seed proteins into three groups: storage proteins, structural and metabolic proteins, and protective proteins. Seed storage proteins fall into three different Osborne fractions and occur in three different tissues of the grain.

Rice (*Oryza sativa*) seed storage proteins (SSPs) are synthesized and deposited in storage organelles in the endosperm during seed maturation as a nitrogen source for germinating seedlings and serve as a nutrient source for humans and livestock. Plant endosperm storage proteins are coded by a group of enormous gene families, and a subfamily genes code a kind of protein. These proteins are deposited in protein bodies and maintain structural stability during seed formation. Thus, storage proteins as direct,

stable products of genes can reflect DNA diversity of plants. The kind of diversity can be easily detected by various electrophoresis.

Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as characterization of germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005), varietal identification (Song *et al.* 1996 and Zhang *et al.* 1998), hybrid seed purity test(Wei-dong *et al.* 2006) screening of mutants for seed storage proteins (Kumamaru *et al.* 1988 and Qu *et al.* 2001), germplasm resource analysis (Sun *et al.*2000 and Yu *et al.* 2003), genetic diversity analysis(Gan *et al.* 1999 and Yang *et al.*2005), determination of phylogenetic relationship between different species and generation of pertinent information to complement evaluation (Sammour, 1991; Isemura *et al.*, 2001; Ghafoor *et al.*, 2002). There have been a substantial number of studies that have used SDS-PAGE to profile seed storage proteins in rice (Saruyama and Shinbasi, 1993; Montalvan *et al.*, 1998; Thanh and Hirata, 2002; Netra and Prasad, 2007). Hence, it becomes highly imperative to assess genetic diversity within and between rice landraces for varietal improvement, evaluation and modification, using better methods of germplasm evaluation and characterization strategies. It is also required to investigate the present gene pool for selection of diverse parent cultivar and to broaden the germplasm base of rice breeding programs in the future for sustainable management of the genetic resources.

Cereal grains contain relatively little protein compared to legume seeds, with an average of about 7–12%dry wt. Nevertheless, they provide over 200 mt of protein for the nutrition of humans and livestock, which is about three times the amount derived from the more protein-rich (20–40%) legume seeds. In addition to their nutritional importance, cereal seed proteins also influence the utilization of the grain in food processing. This is particularly important in wheat, which is largely consumed by humans after processing into bread and other foods. It is not surprising, therefore, that cereal seed proteins have been a major topic of research for many years, with the aim of understanding their structures, control of synthesis and role in grain utilization.

#### **Cereal seed storage proteins:**

The scientific study of cereal grain proteins extends back for over 250 years, with the isolation of wheat gluten first being described in 1745 (Beccari, 1745). Since then more systematic studies have been carried out, notably by TB Osborne (1859–1929) who

can be regarded as the father of plant protein chemistry. Osborne developed a classification of plant proteins based on their solubility in a series of solvents, for example, albumins in water, globulins in dilute saline. Although 'Osborne fractionation' is still widely used, it is more usual today to classify seed proteins into three groups: storage proteins, structural and metabolic proteins, and protective proteins. Seed storage proteins fall into three different Osborne fractions and occur in three different tissues of the grain.

### **Storage globulins**

The embryo and outer aleurone layer of the endosperm contain globulin storage proteins, and those from maize embryos have been characterized in some detail (Kriz, 1989, 1999; Kriz and Schwartz, 1986; Kriz and Wallace, 1991; Wallace and Kriz, 1991). These proteins are readily soluble in dilute salt solution and have sedimentation similarity with, and may be homologous to, the 7S vicilins of legumes and other dicotyledonous plants; they also have similar structures and properties (Kriz, 1999). Related proteins have been found in embryos and/or aleurone layers of wheat, barley and oats (Burgess and Shewry, 1986; Yupsanis *et al.*, 1990; Heck *et al.*, 1993). 7S globulins from rice embryos have also been characterized (Horikoshi and Morita, 1975), but their relationships to other plant 7S globulins have not been established. The 7S globulins are stored in protein bodies and appear to function solely as storage proteins. However, they do not appear to be absolutely required for normal seed function, at least in maize, where a null mutant behaves normally in terms of development and germination (Kriz and Wallace, 1991). Furthermore, although the aleurone and embryo are rich in proteins compared with the starchy endosperm, the globulins in these tissues have limited impact on the end use properties of the grain. In small grained cereals, such as wheat, the aleurone and embryo account only for about 10% of the grain dry weight and are usually removed by milling (wheat), polishing (rice), pearling (barley) or decortication (sorghum), before human consumption. By contrast, the embryo of maize accounts for 10–11% of the grain and its high contents of protein and oil are important for livestock nutrition. Storage globulins of 11–12S located in the starchy endosperm are also present in at least some cereal grains. In fact, in oats and rice these proteins form the major endosperm storage protein fraction, accounting for about 70–80% of the total protein. It

is now known that these proteins are related to the widely distributed 'legumin' type globulins which occur in most dicotyledonous species (Casey, 1999). The rice proteins are not readily soluble in dilute salt solutions and hence are classically defined as glutelins, but they clearly belong to the 11–12S globulin family. They comprise subunits of Mr approx. 55 000 that are post-translationally cleaved to give acidic (Mr approx. 33 000 in oats, 28–31 000 in rice) and basic (Mr approx. 23 000 and 20–22 000, respectively) polypeptide chains linked by a single disulphide bond (Shotwell, 1999; Takaiwa *et al.*, 1999). The oat globulin also resembles the legumins in forming a hexameric structure with a sedimentation coefficient of about 12. Proteins related to legumins, called 'triticins', are present in starchy endosperm of wheat, although they account only for about 5% of the total seed protein (Singh *et al.*, 1988). Triticins consist of large (Mr about 40 000) and small (Mr about 22–23 000) polypeptide chains, but the subunits appear to form dimeric structures rather than the typical legumin hexamers (Singh *et al.*, 1988, 1993; Singh and Shepherd, 1985). The high content of globulin storage proteins in oat grain may contribute to the high nutritional value when compared with other cereals, such as barley and wheat, an important factor in view of the widespread use of oats for livestock feed (Lockhard and Hurt, 1986; Cuddeford, 1995).

#### **Prolamin storage proteins:**

With the exceptions of oats and rice, the major endosperm storage proteins of all cereal grains are prolamins. This name was originally based on the observation that they are generally rich in proline and amide nitrogen derived from glutamine, but it is now known that the combined proportions of these amino acids actually vary from about 30–70% of the total among different cereals and protein groups. Similarly, although prolamins were originally defined as soluble in alcohol-water mixtures (e.g. 60–70% (v/v) ethanol, 50–55% (v/v) propan-1-ol or propan-2-ol), some occur in alcohol-insoluble polymers. Nevertheless, all individual prolamin polypeptides are alcohol-soluble in the reduced state. The prolamins vary greatly, from about 10 000 to almost 100 000, in their molecular masses.

It is clear, therefore, that prolamin storage proteins are much more variable in structure than the 7S and 11-12S globulins, and it is possible that the major groups of prolamins in the Triticeae (wheat, barley, rye) and the Panicoideae (maize, sorghum,

millet) have separate evolutionary origins. Nevertheless, most prolamins share two common structural features. The first is the presence of distinct regions, or domains, which adopt different structures to each other and may have different origins. The second is the presence of amino acid sequences consisting of repeated blocks based on one or more short peptide motifs, or enriched in specific amino acid residues, such as methionine. These features are responsible for the high proportions of glutamine, proline and other specific amino acids (e.g. histidine, glycine, methionine, phenylalanine) in some prolamins groups.

### **The prolamins superfamily:**

Discussions of prolamins structure and properties can be confusing for the non-expert because of the complexity of the fractions and their specialized nomenclature. However, the availability of complete amino acid sequences of representatives of all the major prolamins groups has allowed the redefinition of their classification in relation to structural and evolutionary relationships (Shewry and Tatham, 1990). This new system of classification assigns all of the prolamins of the Triticeae (wheat, barley and rye) to three broad groups: sulphur-rich (S-rich), sulphur-poor (S-poor) and high molecular weight (HMW) prolamins, with several subgroups within the S-rich group. These groups do not correspond directly to the polymeric and monomeric fractions in wheat (glutenins and gliadins, respectively) recognized by cereal chemists, as both monomeric and polymeric forms of S-rich and S-poor prolamins occur. The typical S-rich, S-poor and HMW prolamins of wheat contain extensive repeated sequences based on proline-rich and glutamine-rich motifs with the repeat motifs of the S-rich and S-poor groups being clearly related. Similarly, sequence similarity is clearly present between the non-repetitive domains of the S-rich and HMW prolamins, particularly in positions of conserved cysteine residues and amino acid residues adjacent to these. On the basis of such comparisons, it can be concluded that the S-rich, S-poor and HMW prolamins have a common evolutionary origin. Wider comparisons show further evolutionary and structural relationships to several groups of zein proteins, the prolamins of oats and rice, 2S albumin storage proteins of dicotyledonous seeds, cereal seed inhibitors of  $\alpha$ -amylase and trypsin and a range of low cysteine-rich plant proteins including lipid transfer proteins and cereal grain puroindolines. These proteins are, therefore, together defined as

the Cereal Prolamin Superfamily of plant proteins (Kreis *et al.*, 1985). In wheat, the prolamins form the major components of the gluten protein fraction which is largely responsible for their ability to process wheat to form bread, pasta and many other food products.

### **The prolamins of maize**

The prolamins of maize (called zeins) and of other panicoid cereals (e.g. sorghum and many millets) are comprised of one major group of proteins (a-zeins) and several minor groups (b, c, d-zeins) (Coleman and Larkins, 1999; Leite *et al.*, 1999) (Fig. 2). Amino acid sequence comparisons demonstrate that the b,c and d-zeins are all members of the prolamin superfamily, but only the c-zeins contain repeated amino acid sequences (either two or eight tandem repeats of Pro-Pro-Pro-Val-His- Leu). The b- zeins and d-zeins are both rich in methionine with these residues being clustered in a region close to the C-terminus in the former. By contrast, the a-zeins do not appear to be related to any other prolamins except the a-type prolamins of other panicoid cereals. They consist of two major subclasses called the 19K and 22K zeins based on their Mr determined by SDS-PAGE although they have true molecular masses of 23–24 000 and 26 500–27 000, respectively. Both subclasses contain degenerate repeats of about 20 amino acid residues, with nine such blocks present in the Z19 and ten in the Z22 zeins. The a-zeins contain only one or two cysteine residues per molecule and are present in the grain as monomers or oligomers, while the b-, c- and d-zeins are all richer in cysteine and form polymers.

### **Synthesis and deposition of cereal seed storage proteins:**

Cereal seed storage proteins are produced by the secretory pathway and deposited in discrete protein bodies. However, the origins of the protein bodies and the mechanisms that determine the pathway of storage protein trafficking and deposition are still incompletely understood. The 7S and 11S storage globulins, which are present in the embryo and the aleurone layer; and in the starchy endosperm, respectively, of some cereals are believed to follow the same route as the homologous proteins in dicotyledonous seeds. Thus, they are synthesized on rough endoplasmic reticulum (ER) membranes, transported co-translationally into the lumen and then pass via the Golgi apparatus into a specific population of protein storage vacuoles, which differ from the lytic vacuoles that are also present in developing seeds. The precise details of this process

have been reviewed by Kermode and Bewley, 1999. The precise mechanisms are incompletely understood but physical aggregation within the Golgi appears to be important leading to the formation of electron dense aggregates which form the contents of dense vesicles. Globulin storage proteins do not contain cleavable pro-domains which confer vacuolar targeting (Kermode and Bewley, 1999).

The mechanisms of prolamin transport and deposition are less well understood than those of globulins, but two pathways may occur. In maize, other panicoid cereals (sorghum, millets) and rice the prolamins appear to accumulate directly within the lumen of the ER, leading to the formation of discrete protein bodies surrounded by a membrane of ER origin (Coleman and Larkins, 1999; Muench *et al.*, 1999). In rice, this leads to the presence of two populations of protein bodies, PB-I which is of ER origin and contains prolamins and PB-II which is vacuolar in origin and contains globulins/glutelins (Yamagata and Tanaka, 1986; Krishnan *et al.*, 1986). Okita and co-workers also provided evidence that prolamins and globulins/glutelins are synthesized in separate subdomains of the ER, with mRNAs for prolamins being targeted to the rough ER associated with the developing prolamin-containing protein bodies and globulin/glutelin mRNAs to more typical cysternal ER membranes (Li *et al.*, 1993a; Choi *et al.*, 2000). More recent work indicates that prolamin mRNA localization results from binding to a specific site on the tubulin and actin cytoskeleton (Muench *et al.*, 1998).

Oats resemble rice in having a high proportion of globulin-type storage proteins in starchy endosperm cells, but in this case the globulins and prolamins are co-located in the same protein bodies, with the prolamins present as lighter-staining inclusions (Lending *et al.*, 1989). This results from the fusion of two populations of protein bodies, of ER origin (containing prolamins) and of vacuolar origin (containing globulins).

Whereas the prolamins of maize, rice and probably also oats appear to accumulate directly within the ER with little or no evidence for transport to the vacuole. There is now overwhelming evidence that both routes of protein transport and protein body formation operate in wheat, barley and probably also rye. Evidence for this was reviewed by Galili, 1997 which includes immunogold labelling of prolamins within Golgi complexes (Shewry, 1999), the observation of small protein bodies within or associated with the ER, the association of ER marker enzymes with protein bodies prepared by sub-

cellular fractionation and the expression of wild-type and mutant proteins in heterologous systems. Some prolamins, particularly gliadins, are transported via the Golgi to the protein storage vacuole whereas others, particularly glutenins, are retained within the ER. Galili and co-workers also suggested that the ER-derived protein bodies are subsequently absorbed by protein storage vacuoles in a process similar to autophagy. The protein bodies in developing wheat grains also contain dark-staining globulin storage protein triticin (Bechtel *et al.*, 1991), which is presumably transported via the Golgi to the vacuolar protein bodies.

The precise mechanism of protein body fusion in wheat remains to be resolved. However, the net result is the presence in mature, dry endosperm cells of a continuous matrix of proteins that surrounds starch granules and engulfs the remains of other cell structures. This matrix is the basis for the formation of the gluten network when wheat flour is mixed with water to form dough.

The mechanisms which determine whether prolamins are retained within the ER or transported via the Golgi to the vacuole are not known, and it is not possible to recognize either classical ER retention signals (i.e. the C-terminal tetrapeptides KDEL or HDEL) or vacuolar targeting sequences in these proteins. Expression of wildtype and mutant forms of c-zein and c-gliadin in heterologous systems has demonstrated that the proline-rich repetitive sequences are required for ER retention (Torrent *et al.*, 1994; Geli *et al.*, 1994; Altschuler *et al.*, 1993; Altschuler and Galili, 1994), and it is possible that these regions form protein : protein interactions leading to the formation of insoluble accretions which directly in the ER rather than being transported to the Golgi and vacuole (Coleman and Larkins 1999, Shewry 1999).

Okita and co-workers have proposed the existence of a specific mechanism leading to the retention of prolamins in the ER of rice. This involves an interaction with the molecular chaperone BiP (binding protein) which may bind the nascent polypeptide and retain it in the ER until assembly into a protein body (Li *et al.*, 1993b; Muench *et al.*, 1999). This mechanism has not so far been reported for other cereals.

#### **Organization of prolamins with protein bodies:**

The clear separation of prolamins and globulin components in the biphasic protein bodies of oats and wheat may result from the initial deposition of these components in

separate populations of protein bodies (as in rice), which subsequently fuse. However, it could also result from phase separation of the prolamin and globulin proteins due to their different structures and properties.

Evidence for the spatial separation of different types of prolamins within protein bodies is less clear cut as the different prolamins tend to show similar staining properties on preparation for electron microscopy. However, Lending and Larkins presented elegant studies showing differences in the distribution of zeins within protein bodies related to their position in the developing endosperm (Lending and Larkins, 1989). Thus, the protein bodies in the youngest cells in the sub-aleurone region contain mainly b- and c-zeins which are distributed throughout the protein body. Passing into the endosperm the protein bodies increased in size, with the appearance of centrally-located 'locules' containing a-zeins. Finally, these locules fused to form a continuous central region of a-zeins, with the b- and c-zeins being located peripherally in the mature protein bodies of the central endosperm cells.

The analysis of protein body development in maize was facilitated by the availability of highly specific antibodies for a, b and c-zeins, and similar studies have not so far been reported for other cereals. However, recent work carried out with different gliadin fractions indicates that separation could occur, resulting in the formation of microphases. Purified a-gliadin and v-gliadin fractions were dissolved in 70% (v/v) ethanol, mixed in ratios of 1 : 3, 1 : 1 and 3 : 1, applied to a mica surface and the solvent allowed to evaporate. Analysis of the surface properties of the dried films with atomic force microscopy showed two phases in proportions approximately related to the ratios of the components (McMaster *et al.*, 1999). This suggests that gliadins and glutenins could partition into separate microphases in protein bodies, which would not be observed by conventional electron microscopy.

Expression in heterologous systems also indicated that a mixture of zein classes may be required for the formation of normal protein bodies. Thus, co-expression of c-zein was required for the accumulation of a-zein in transgenic tobacco (Coleman *et al.*, 1996) while b- and d-zeins formed abnormal protein bodies when expressed alone in tobacco, but apparently normal protein bodies when expressed together (Bagga *et al.*, 1995, 1997). It is clear from these studies that there is still much to learn about the

mechanisms of protein body formation in cereals and the roles and organization of the different protein groups.

#### **Spatial distribution of prolamins within the starchy endosperm:**

Because the aleurone cells continue to divide periclinally in the developing cereal endosperm the youngest cells are present in the sub-aleurone layer and the oldest cells in the central part of the endosperm. In both small grain cereals and panicoid species the sub-aleurone cells contain few starch granules, which tend to be smaller than those in the central endosperm cells. Consequently, these cells contain high proportions of proteins, although the total protein content per cell varies little across the whole wheat endosperm (Evers, 1970). In maize, the protein bodies in the sub-aleurone and outer parts of the endosperm are enriched in c- and b-zeins and low in a-zeins, the latter being more uniformly distributed across the endosperm (Geetha *et al.*, 1991). This distribution is consistent with the ontogeny of maize protein bodies discussed above.

Differences in protein distribution throughout the endosperm also occur in barley (and probably also in wheat), although the developmental basis for this is unclear. The sub-aleurone cells of both species are rich in proteins, but immunocytochemical and pearling studies of barley show that they contain mainly S-rich and S-poor prolamins (principally B and C hordeins), with the HMW prolamins (D hordein) only occurring in significant amounts below the sub-aleurone (Shewry *et al.*, 1996; Tecsi *et al.*, 2000). This may have significance for the utilization of wheat and barley, as D hordeins are major components of the gel protein fraction that may limit the modification of barley during malting (Smith and Lister, 1983), while the homologous HMW subunits of wheat glutenin are major components of the elastomeric polymers that underpin breadmaking and other food uses (Shewry *et al.*, 1995).

#### **Regulation of prolamins gene expression:**

Prolamin genes are subject to tissue-specific and developmental regulation, being expressed exclusively in the starchy endosperm during mid- and late-development, and nutritional regulation, responding sensitively to the availability of nitrogen and sulphur in the grain (Duffus and Cochrane, 1992; Giese and Hopp, 1984). This control of gene expression is exerted primarily at the transcriptional level (Bartels and Thompson, 1986; Sørensen *et al.*, 1989).

Little, if anything, is known about the mechanisms by which the expression of prolamin genes responds to sulphur. However, a motif involved in the response of S-poor and S-rich prolamin genes of wheat, barley and rye to nitrogen has been identified. This motif (the N motif or nitrogen element) (Hammond-Kosack *et al.*, 1993; Muller and Knudsen, 1993) is present within a highly conserved sequence called the prolamin box. The prolamin box (sometimes called the endosperm element) was the first prolamin gene regulatory sequence to be reported and was identified through a comparison of the promoters of several gliadin and hordein genes (Forde *et al.*, 1985). This revealed the presence of the conserved sequence, which is approximately 30 bp long, around 300 bp upstream of the transcription start site (it was first called the  $\_300$  element). The consensus sequence for the element is: 59-TGACATGTAA AGTGAATAAG ATGAGTCATG. The N motif is at the 39 end of the box and has the consensus sequence G(AuG)TGAGTCAT in S-rich prolamin genes. It is present in the reverse orientation within the prolamin box of S-poor prolamin genes (Shewry *et al.*, 1999). It has similarity with the binding site of the GCN4 transcription factor, which is a component of the nitrogen signalling pathway in yeast, and is sometimes called the GCN4-like motif (GLM). The prolamin box contains a second highly conserved motif, sequence TGTAAGT, that has been called the endosperm or E motif (Hammond-Kosack *et al.*, 1993). Promoter regions containing the prolamin box have been shown to be functional by introducing promoter chloramphenicol acetyl transferase (CAT) reporter gene constructs into transgenic tobacco (Colot *et al.*, 1987; Marris *et al.*, 1988). A regulatory role for the prolamin box itself has been established previously (Muller and Knudsen, 1993; Hammond-Kosack *et al.*, 1993). Muller and Knudsen used an homologous, transient expression system, involving particle bombardment of cultured barley endosperms with C hordein promoterub-glucuronidase (GUS) constructs. These experiments confirmed that the E and N motifs are separate elements and showed that the N motif exerts a negative effect on gene expression at low nitrogen levels and interacts with the E motif and other upstream elements to give high expression when nitrogen levels are adequate.

Hammond-Kosack *et al.* used *in vivo* footprinting and gel retardation assays to show that E motifs within the prolamin box and further upstream in the promoter of a

wheat low molecular weight (LMW) subunit gene bound a putative transcription factor, ESBF-1 (Hammond- Kosack *et al.*, 1993). A second putative transcription factor, ESBF-II, bound the N motif prior to maximum expression of the gene. A third putative transcription factor, SPA, has since been shown to recognize the N motif (Albani *et al.*, 1997). Together, the results of these experiments suggest that the N motif is an important component of the nitrogen regulatory mechanism for S-rich and S-poor prolamin genes. Its function requires interaction with the E motif, the two motifs together making up the prolamin box. However, the prolamin box is not present in all prolamin genes. Zein gene promoters, for example, contain a highly conserved 15 bp element that has been suggested to act as a tissue-specific enhancer (Quayle and Feix, 1992). It contains the sequence TGTAAG, which resembles the E motif, but the N motif is absent (Coleman and Larkins, 1999). The N motif is present in c-zein promoters, but it is separated from the E motif and its function, if any, has not been investigated (Coleman and Larkins, 1999).

The prolamin box in its entirety is also not present in HMW prolamin gene promoters (Shewry *et al.*, 1999). Instead, HMW prolamin promoters contain a major regulatory element (identified by Thomas and Flavell, 1990) which is located in a 38 bp sequence with the consensus: 59-GTTTTGCAA GCTCCAATTG CTCCTTGCTT ATCCAGCT. The location of this sequence is highly conserved in all HMW prolamin promoters (Shewry *et al.*, 1999). The element contains the sequence TGCAAAG, which is similar to the E motif sequence TGTAAG that is also present in zein genes, but it does not contain anything resembling the N motif. Sequences corresponding to parts of the N and E motifs are present in HMW prolamin promoters upstream from the major enhancer (Lamacchia *et al.*, 2001). However, deletion of these sequences does not appear to affect activity of the promoter, at least when driving reporter gene expression in transgenic tobacco (Halford *et al.*, 1989; Thomas and Flavell, 1990).

#### **Cereal seed proteins and grain utilization:**

The total protein contents of cereal seeds vary from about 10–15% of the grain dry weight, with about half of the total being storage proteins. Nevertheless, proteins have major impacts on the end use properties of the grain. The prolamins, which form the major storage protein fraction in all the major cereals except oats and rice, are deficient in

the essential amino acids lysine and in threonine and tryptophan (particularly in maize). This results in nutritional deficiencies in these amino acids when the whole grain are fed to monogastric livestock such as pigs and poultry. It is therefore usual to combine cereals with other sources of these amino acids for animal feed, for example, legume seeds (notably soybean), oilseed cake, fish meal or synthetic amino acids. The combination of cereals and legume seeds is particularly favoured, as these two types of seeds are essentially complementary in their compositions of essential amino acids: cereals tend to be rich in sulphur-containing amino acids and low in lysine and legume seeds vice versa. The nutritional quality of cereals is not generally an important consideration for human diets in the developed world, although it is still important in some developing countries. The major consideration is the impact of the grain proteins on functional properties for food processing, since the bulk of all cereals, except rice, are consumed in processed foods. Processing quality is particularly important for wheat where the gluten proteins are the major determinant of end use quality.

#### **Rice Seed Storage Proteins:**

Rice grain consists mainly of starch (90%) and small proportions of proteins (~7%), lipids (~2%) and minerals (~1%)(Aryan 2006). Grain starch is comprised of two components e.g., amylose and amylopectin, which differ in their amounts among various sub-species of rice. It is the amount and structure of these two starch components that play the key role in defining cooking properties of a rice variety. Grain lipids and proteins also influence the pasting properties of cooked rice through interaction with the amylose and amylopectin molecules.

The rice has the lowest content of seed storage proteins among cereals and it ranges from 5-10% of seed weight depending upon varieties. Major storage protein is an acid and/or alkaline soluble protein (glutelin), which accounts for 80% of the total protein. Salt and alcohol soluble proteins (globulin and prolamin respectively) are present in relatively low amounts(5%) in rice endosperm (Cagampang *et al.* 1966).

Rice glutelin has a mol. wt. of  $6 \times 10^5$  according to Tecson *et al.* (1971), but Takeda *et al.*(1970) demonstrated that it has a heterogeneous mol wt. The increase in glutelin in the developing rice grain coincides with the appearance of Protein Bodies' in

the starchy endosperm 7 to 8 DAF (Del Rosario *et al.* 1968, Villareal and Juliano 1978, and glutelin is found exclusively in Protein bodies (Del Rosario *et al.* 1968, Tanaka *et al.* 1980, Wu and Chen 1978). Two types of protein bodies (PBs) develop in the starchy endosperm of rice grains. The PB-I is spherical with a concentric ring structure, whereas the other type (PB-II) is stained homogeneously by osmium tetroxide. PB-I contains prolamin which accumulate within the lumen of the rough endoplasmic reticulum (ER), while PB-II is rich in glutelin and globulin that are deposited into protein storage vacuoles (PSVs) (Krishnan *et al.*, 1986; Li *et al.*, 1993). The glutelin in PB-II is composed of two principal subunits, the 22- to 23- and 37- to 39-kD complexes, and the prolamin in PB-I is composed mainly of 13-kD polypeptide (Yamagata *et al.* 1982). Transmission electron microscopy and fractionation by sucrose density gradient centrifugation of the starchy endosperms at various stages of development showed that PB- II was formed faster than PB- I.

Cereals particularly rice is deficient in lysine but rich in sulphur containing amino acids. A reduction in the levels of 13-kD prolamin resulted in enhancement of the total lysine content by 56% when compared with the wild type. Knock down of 13kD prolamins not only reduced the size of endoplasmic reticulum derived protein bodies (PBs) but also altered the rugged peripheral structure. In contrast, PBs became slightly smaller or unchanged by severe suppression of 10- or 16-kD prolamins, respectively, indicating that individual prolamins have distinct functions in the formation of PBs and seed quality in terms of amino acid composition.

In rice, 60% to 80% of total seed protein is composed of glutelins, encoded by 15 genes in the rice genome, and 20% to 30% of total seed protein are prolamins that are encoded by 34 genes (Kawakatsu *et al.*, 2008; Xu and Messing, 2009). Glutelins can be classified into four groups (GluA, GluB, GluC, and GluD) based on amino acid sequence similarity (Kawakatsu *et al.*, 2008) while, prolamins are classified into three groups (10, 13, and 16 kD) by their molecular mass according to their electromobility on SDS-PAGE gels. The 13-kD prolamins can be further classified into three subgroups (class I, II, and III) by their Cys residue content (Muench *et al.*, 1999). Based on amino acid sequence similarity, 16- and 13-kD prolamins correspond to maize  $\lambda_2$ -zein, and 10-kD prolamins correspond to  $\delta$ -zeins (Xu and Messing, 2009).

Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of the starchy endosperm protein of rice (*Oryza sativa* L. Japonica cv Koshihikari) during seed development confirmed that storage protein begins to accumulate about 5 days after flowering (Yamagata *et al.* 1982). Two polypeptide groups, 22 to 23 and 37 to 39 kD, the components of glutelin, the major storage protein in rice seed, appeared 5 days after flowering. In vivo pulse-chase labeling studies showed the 57kD polypeptide to be a precursor of the 22 to 23 and 37 to 39 kD subunits. The 57kD polypeptide was salt-soluble, but the mature glutelin subunits were almost salt insoluble. In vitro protein synthesis also showed that the mRNAs directly coding the 22 to 23 and 37 to 39kD components were absent in developing seeds and that the 57-kilodalton polypeptide was the major product. Thus, it was concluded that 57kD peptide was synthesized at an earlier stage than the glutelin subunits and such two subunits of rice glutelin subunits are formed through post-translational cleavage of the 57-kD polypeptide. A 26kD polypeptide globulin component also appeared 5 days after flowering. While, smaller polypeptides (10- 16kD) including prolamin components, appeared about 10 days after flowering. In contrast, the levels of the 76 and 57kD polypeptides were fairly constant throughout seed development.

Wei-dong *et al.* (2006) revealed that storage protein polymorphism in rice can not distinguish different japonica varieties. Although they had got 19 types of profile in varieties studied, the profiles of 80% varieties were similar. Jahan *et al.* (2003) reported nine variations of glutelin in Bangladeshi rice varieties. Aung *et al.* (2003) found there were two varietal types for glutelin, five varietal types for prolamin in Myanmar rice varieties. However, glutelin and prolamin of Pakistani rice varieties had six and four variation types respectively (Sarker and Bose 1984).

Generally, there is one band at the site of 57 kDa by SDS-PAGE. But, one out of five hormone mutants of RT series derived from a variety "Ochikara" by Sodium azide in Japan, had two bands at this site (Wei-dong *et al.* 2006). It is therefore speculated that the additional band was the result of chemical mutagenesis. Further, Wei-dong *et al.* (2006) revealed that the RT series mutants e.g., RT60, RT61, RT62 and RT64 had high staining intensity of band 60 kDa indicating high amylose content compared to the parent variety "Ochikara". This phenomenon is worthwhile to study further.

The amount of amylose in the grain determines the texture and appearance of cooked rice. A gene known as the Waxy gene determines the levels of amylose in the grain. In glutinous rice, the Waxy gene is non-functional (mutated), resulting in very low amylose (<2%) resulting very sticky kernels upon cooking. Non-glutinous rices have a fully or partly functional Waxy gene. There are two types of Waxy genes – ‘a’ and ‘b’. Rice with the ‘a’ type Waxy gene in indica rice, has a fully functional Waxy gene and amylose levels can be up to 30%. When cooked, high amylose rices (>24%) are firm in texture and opaque in appearance. The japonica rices contain the ‘b’ type Waxy gene, which has lower amylose levels (16–18%) than the ‘a’ type. When cooked, these rices have softer, translucent cooked grains, which is a unique characteristic of most Australian cultivars. The 60 kDa seed storage protein is the translation product of waxy (Wx) gene (Sano *et al.* 1985, Sano *et al.* 1986 and Shu *et al.* 1999) , which control amylose content. Extent of staining intensity of such 60kDa protein band in different rice varieties can be a reliable parameter to assess amylose content.

#### **Gene specific protein markers:**

Hong *et al.* (2001) found a protein marker  $\alpha$ -4f (approx.35kDa) can be used as genetic marker of restorer gene to help restorer line selection and to distinguish Liuqianxin A, Liuqianxin B seeds from F<sub>1</sub> seeds. Hong *et al.* reported that moving rate / migration rate of glutelin  $\alpha$ -4f band (35kDa) during SDS-PAGE in restorer lines was fast than that in Liuqianxin A. Some researchers (Chen *et al.* 1998 and Liu *et al.* 2003) reported that sterile lines in sorghum were related with deficient and unstable heat shock protein of 70 kDa (HSP70) in seeds.

Staining intensity of some of the polypeptide band as in case of erstwhile mentioned 60kD band can be considered important protein marker for assessing extend of amylose content in a set of rice genotypes.

CHAPTER-III

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*Materials and Methods*

## MATERIALS AND METHODS

The experimental materials used in the present investigation consisted of ninety six upland genotypes including standard ruling varieties and popular locally adapted land race(s). The details of these genotypes are given in Table 1.

**Table 1. List of upland test genotypes.**

Sl. No.	Genotype	Source (Village/Block, District of Odisha)	Sl. No.	Genotype	Source (Vill./Block, Dist. of Odisha/.Inst.)
1.	<b>Koliha</b>	Dharuamunda, Nuapada	49.	<b>Pora</b>	Khalibhata/Jaipatna, Kalahandi
2.	<b>Kantadumer</b>	Saliha, Nuapada	50.	<b>Karanga</b>	Ghosa, Sambalpur
3.	<b>Bastul</b>	Pagarpani, Nuapada	51.	<b>Kenduphula</b>	Jhurapali/Bamara, Sambalpur
4.	<b>Biramani-1</b>	Thikpali/Komona,	52.	<b>Malkadua</b>	Badmal/Maneswar, Sambalpur
5.	<b>Setka-1</b>	Kantapali/Komana, Nuapada	53.	<b>Ambajhuka</b>	Jharmunda/Rairakhol, Sambalpur
6.	<b>Kharkoili</b>	Gahira padar/Komana, Nuapada	54.	<b>Kunor</b>	Barididinga/Jujumura, Sambalpur
7.	<b>Kuliha</b>	Dharamsagar/Komana, Nuapada	55.	<b>Haladigundi</b>	Langabahal/Jujumura, Sambalpur
8.	<b>Chinger-1</b>	Banjibahal/Boden,	56.	<b>Padarabank</b>	Paluakhalia/Sanakhemundi, Ganjam
9.	<b>Kusuma-1</b>	Makarbirhi/Boden, Nuapada	57.	<b>Mahulakunchi</b>	Paluakhalia/Sanakhemundi, Ganjam
10.	<b>Hiran-1</b>	Dhungiamunda/Sinapali, Nuapada	58.	<b>Somo</b>	Hariharnagar/Aska, Ganjam
11.	<b>Ninibudhi</b>	Hinguput/Lamaput, Kalahandi	59.	<b>Jhitipiti</b>	Adipur/Sukruli, Mayurbhanj
12.	<b>Badi</b>	Sasahandi/Bariguma, Koraput	60.	<b>Raas</b>	Jhadadumuria/Karanjia, Mayurbhanj
13.	<b>Rangahazari</b>	Jamunahandi/Kotpad, Koraput	61.	<b>Sanarasi</b>	Pansi/Raruam, Mayurbhanj
14.	<b>Lalubadikaberi</b>	Mahuli/ Boipariguda, Koraput	62.	<b>Dasaharadhan</b>	Deesaripada/Bissam Cuttack, Raigada
15.	<b>Dangarchudi</b>	Dhamanahandi, Koraput	63.	<b>Bhatasakli</b>	Bankamba/Kasipur, Raigada
16.	<b>Danisaria</b>	Siletпали/Padampur, Baragarh	64.	<b>Paradhan</b>	Singari/Singhpur, Raigada

17.	<b>Sunamukhi</b>	Bhatigaon/Bijepur, Bargarh	65.	<b>Kandasuri</b>	Parikhiti/Ramana- guda, Raigada
18.	<b>Baspatri</b>	Antapali/Bhatli,Bargarh	66.	<b>Kanding</b>	Binida/Ramanaguda, Raigada
19.	<b>Karni</b>	Jamutbahal/Gaisilet, Bargarh	67.	<b>Asumakunda</b>	Dahani/Gudari, Raigada
20.	<b>Harisankar</b>	Bhutibahal/Gaisilet, Bargarh	68.	<b>Nandigiri</b>	Baramania/kujanga, Jagatsinghpur
21.	<b>Dengabari</b>	Kotpad, Koraput	69.	<b>Dhobasaria</b>	Rampur/Agalpur, Balangir
22.	<b>Kahnei</b>	Dhungiaput/Mathili, Malkangiri	70.	<b>Dhalashree –B</b>	Mirdhapali, Balangiri
23.	<b>Pugukal</b>	Kichipali/Korukunda, Malkangiri	71.	<b>Saria-B</b>	Khaliapali, Balangir
24.	<b>Butasori</b>	Jharpalli, Malkangiri	72.	<b>Hiran-2</b>	Kundapathar, Balangir
25.	<b>Pandeydhan</b>	Jharpalli, Malkangiri	73.	<b>Mahularani</b>	Kundapathar, Balangir
26.	<b>khursudi</b>	Kumbharguda/Kalimela, Malkangiri	74.	<b>Kapaanthi</b>	Baidipali, Balangir
27.	<b>Kinari</b>	Maharajpali/Kalimela, Malkangiri	75.	<b>Pustak</b>	Ampali, Balangir
28.	<b>Jirkubanji</b>	Nalagunthi/Kalimela, Malkangiri	76.	<b>IR 87707-445- B-B<sup>b</sup></b>	IRRI, Philippines
29.	<b>Dular</b>	Somanathpur/K.Gumma, Malkangiri	77.	<b>CR 143-2-2<sup>a</sup></b>	CRRI, Cuttack
30.	<b>Kusuma-2</b>	Dhamanguda/Rampur, Kalahandi	78.	<b>CR Dhan 40<sup>a</sup></b>	CRRI, Cuttack
31.	<b>Podasankara</b>	Baipariguda,Sonepur	79.	<b>Kalakeri<sup>b</sup></b>	CRRI, Cuttack
32.	<b>Merlo</b>	Gunpur/Rampur, kalahandi	80.	<b>Safri -17<sup>b</sup></b>	CRRI, Cuttack
33.	<b>Pankapota</b>	Mundighati/Rampur, Kalahandi	81.	<b>IR 20<sup>a</sup></b>	CRRI, Cuttack
34.	<b>Rasakadali</b>	Bholedapadar/Rampur, Kalahandi	82.	<b>Anjali<sup>a</sup></b>	CRRI, Cuttack
35.	<b>Setka-2</b>	Mahulpatna/Rampur, Kalahandi	83.	<b>Browngora<sup>b</sup></b>	CRRI, Cuttack
36.	<b>Damaraphuli</b>	Kandulguda/Junagarh, Kalahandi	84.	<b>Salampikit<sup>b</sup></b>	CRRI, Cuttack
37.	<b>Chinger-2</b>	Ghotia/Golamunda, Kalahandi	85.	<b>Sadabahar<sup>b</sup></b>	CRRI, Cuttack
38.	<b>Biramani-2</b>	Kanderai/Karlamunda, Kalahandi	86.	<b>Sahbhagi dhan<sup>b</sup></b>	CRRI, Cuttack
39.	<b>Dangaradhan</b>	Podaumer/Lanjigarh, Kalahandi	87.	<b>Saria</b>	Para/Chhendipada, Angul
40.	<b>Kalakusuma</b>	Balsinga/Narla, kalahandi	88.	<b>Annada<sup>a</sup></b>	CRRI, Cuttack

41.	Sarian	Nunpur/Rampur, Kalahandi	89.	Sneha <sup>a</sup>	EB-I Section, OUAT, BBSR
42.	Dal	Niali/Langigarh, Kalahandi	90.	Zhu 11-26 <sup>b</sup>	CRRI, Cuttack
43.	Jhulipuagi	Bankel/Narla, Kalahandi	91.	Heera <sup>a</sup>	CRRI, Cuttack
44.	Litipiti	Nichemaska/Rampur, Kalahandi	92.	Vanaprava <sup>a</sup>	EB-I Section, OUAT, BBSR
45.	Bhogi	Gunpur/Rampur, Kalahandi	93.	N 22 <sup>b</sup>	CRRI, Cuttack
46.	Barei	Kukudipastipada/ Bhawanipatna, Kalahandi	94.	Vandana <sup>b</sup>	CRRI, Cuttack
47.	Kutiarasi	Dangaripadar /Bhawanipatna, Kalahandi	95.	Khandagiri <sup>a</sup>	EB-I Section, OUAT, BBSR
48.	Kusuma-2	Tungala/Kesinga, Kalahandi	96.	Mandakini <sup>a</sup>	EB-I Section, OUAT, BBSR

<sup>a</sup>Standard high yielding varieties, <sup>b</sup> Popular drought tolerant donors, Rest of the genotypes are upland rice land races.

#### Field Plot Technique:

A field experiment comprising above 96 germplasm lines were laid out in augmented design with four blocks and 23 varieties plus four promising standard check varieties( N22, Vandana, Khandagiri and Mandakini) in each block to assess their comparative performance for agro-economic traits including seed yield and assessment for tolerance to drought during Kharif 2013 at EB II Section, Department of Plant Breeding and Genetics, OUAT, Bhubaneswar. Each genotype was sown in three rows of 3m length with a spacing of 20cm between rows and 10cm between plants. The experiment was conducted with fertilizer dose of 60:30:30 of N: P<sub>2</sub>O<sub>5</sub> : K<sub>2</sub>O kg/ha. Half of nitrogenous, half of potassic fertilizers and whole of phosphatic fertilizers were applied in lines as basal at the time of sowing. The rest amount of nitrogen and potassic fertilizers were applied after 25 days of sowing as top dressing. Weeding was done before top dressing of fertilizers. Normal plant protection measures were taken to check development of diseases and population of insect pests. . Other management practices were followed as per recommended package of practices. Similar field experiment comprising the same set of test genotypes was necessitated for proper assessment of drought tolerance (0-9 scale) during Summer 2014.

## **Observations:**

Each genotype was assessed for DUS characteristics (Table 2) at different stages of crop growth. Besides, observations on agro-economic traits were recorded on 5 randomly selected plants from middle row of each plot for 20 biometric traits except days to maturity and grain yield which were recorded on plot basis. For 100-grain weight, observation was taken from random sample of seeds of each plot respectively.

**Days to 50% flowering:** This was recorded as the period from date of sowing to 50% flowering assessed on plot basis.

**Days to maturity:** Period from date of sowing to date of physiological maturity.

**Leaf rolling:** It was scored on 0-9 scale at panicle initiation stage.

**Drought Recovery** : It was scored on 0-9 scale based on percentage of plants recovered day after irrigation following drought stress. **1:** 90-100%, **2:** 80-89%, **3:** 70 - 79%, **4:** 60-69%, **5:** 50-59%, **6:** 40-49%, **7:** 30-39%, **8:** 20-29%, **9:** 0-19%

**Leaf drying** : It was scored on 0-9 scale at 10 days after P.I. Stage based on percentage of leaf area dried from tip. **0:** No dried, **1:** < 5%, **2:** 5-10%, **3:** 10-20%, **4:** 20-30%, **5:** 30-40%, **6:** 40-50%, **7:** 50-60%, **8:** 60-90%, **9:** >90%.

**Leaf area (cm<sup>2</sup>):** Length X breadth measured in centimeter and the product is multiplied an adjustment factor(0.75).  $LA = 0.75 \times (LL \times LB)$

**Chlorophyll Index:** It was measured using the SPAD meter at P.I stage.

**Zinc tolerance:** It was scored on 0-9 scale at early seedling stage (10-12 days after sowing. **0-**Resistant, **1-** highly tolerant, **3-** Tolerant, **5-**Moderately tolerant, **7-**Suseptible, **9-**Highly susceptible

**BLB tolerance:** It was scored on 0-9 scale based on the extent of infestation.

**Plant height :** Height from the base of the plant to the tip of the top most panicle at maturity and expressed in cm.

**No. of effective bearing tillers/m<sup>2</sup>** : No of effective ear bearing tillers at maturity counted in a square meter.

**Panicle length** :Length of panicle from the ciliate base to the tip of the top most panicle of the main culm measured in the centimeter at the time of recording plant height.

**Panicle weight (g)**: Average of the weight of five panicles sampled from each genotype.

**Number of grains per panicle** :Average number of fertile grains per panicle counted from 5 top most panicles(each of 5 randomly selected plants).

**1000-grain weight**:Weight of 1000 filled grains from random sample of seeds of each plot

**Grain and kernel dimension**: Average of the length and breadth of 10 grains and 10 kernels in millimeter, were measured using Dial thickness micrometer and L/B ratio was calculated for grains and kernels of each genotype.

**Grain Fertility percentage** : The ratio of fertile grains to the total number of the spikelets per panicle expressed in percentage.

**Seed yield per ha**: Weight of cleanly threshed dry seeds (in kilogram) per m<sup>2</sup> was estimated from plot yield and multiplied with 10000.

**Table 2. DUS characteristics in rice as per DUS guidelines of DRR, Hyderabad**

Sl. No	DUS Characters	Morphological descriptor with code in 1-9 scale
1	Coleoptile: colour	Colourless/green/purple(1,2,3)
2	Basal leaf: sheath colour	Green/light purple/purple(1,2, 3)
3	Leaf: intensity of green colour	Light/medium/Dark(2,5,7)
4	Leaf: anthocyanin colouration	Absent/Present(1,9)
5	Leaf : distribution of anthocyanin colouration	On tip only, on margin only, Blotches, uniform(1, 2, 3, 4)
6	Leaf sheath: anthocyanin colouration	Absent/Present(1,9)
7	Leaf sheath : intensity of anthocyanin colouration	Very weak/Weak/Med./strong/very strong(1, 3, 5, 7, 9)
8	Leaf: pubescence of blade surface	Absent/weak/Med./strong/very strong(1, 3, 5, 7, 9)
9	Leaf : auricles	Absent/Present(1, 9)
10	Leaf: anthocyanin colouration of auricles	colourless/light purple/ purple(1, 2, 3)
11	Leaf: collar	Absent/Present(1, 9)
12	Leaf: anthocyanin colouration of collar	Absent/Present(1, 9)
13	Leaf: ligule	Absent/Present(1, 9)
14	Leaf: shape of ligule	Truncate/Acute/Split(1, 2, 3)
15	Leaf: colour of ligule	White/light Purple/Purple(1, 2, 3)

16	Leaf: length of blade	Short/Med. /Long(3, 5, 7)
17	Leaf: width of blade	Broad/ Med./Narrow(3, 5, 7)
18	Culm: attitude (for floating rice only)	Non-procumbent/ Procumbent(1, 9)
19	Culm: attitude (Late Stage)	Erect/Intermediate/open/spreading(1, 3, 5, 7)
20	Time of heading (50% of plants with panicles)	VE/E/M/L/VL(1, 3, 5, 7, 9)
21	Flag leaf: attitude of blade (early observation)	Erect/Semierect/horizontal/drooping(1, 3, 5, 7)
22	Spikelet: density of pubescence of lemma	Absent/weak/med./strong/very strong((1, 3, 5, 7, 9)
23	Male sterility	Absent/Present(1,9)
24	Lemma: anthocyanin colouration of keel	Absent/Weak/Medium/Strong/ Very strong
25	Lemma: anthocyanin colouration of area below apex	Absent/Weak/Medium/Strong/ Very strong
26	Lemma: anthocyanin colouration of apex	Absent/Weak/Medium/Strong/ Very strong
27	Spikelet: colour of stigma	White/light green/yellow/light purple/purple(1,2,3,4,5)
28	Stem: thickness	Thin/Med./ Thick (3, 5, 7)
29	Stem: length (excluding panicle; excluding floating rice)	Very short/short/Med./long/V.long(1,3,5,7,9)
30	Stem: anthocyanin colouration of nodes	Absent/Present(1,9)
31	Stem : intensity of anthocyanin colouration of nodes	weak/Med./Strong(3,5,7)
32	Stem: anthocyanin colouration of internodes	Absent/Present(1,9)
33	Panicle: length of main axis	Very short/short/med./long/ very long(1,3,5,7,9)
34	Flag leaf: attitude of blade (late observation)	Erect/Semierect/horizontal/deflexed(1,3,5,7)
35	Panicle: curvature of main axis	Straight/semistraigh/deflexed/drooping(1,3,5,7)
36	Panicle: number per plant	Few(<11), Med.(11-20)/Many(>20per Plant (3,5,7).
37	Spikelet : colour of tip of lemma	White/yellowish,Brown/Red/purple/ Black(1,2,3,4,5,6)
38	Lemma and Palea: colour	Straw(1)/Gold and Gold furrows on straw background(2)/brown spots on straw(3)/ brown furrows on straw(4)/brown(5)/Reddish to light purple(6)/Purple spots/furrows on straw(7)/purple(8)/Black(9)
39	Panicle : awns	Absent/Present(1,9)
40	Panicle: colour of awns (late observation)	Yellowish white(1)/Yellowish brown(2)/Brown(3)/reddish brown(4)/Light red(5)/Red(6)/light purple(7)/Purple(8)/Black(9)
41	Panicle: length of longest awn	Very short/short/med./long/very

		long(1,3,5,7,9)
42	Panicle: distribution of awns	Tip only/upper half only/whole length(1,3,5)
43	Panicle: presence of secondary branching	Absent/Present(1,9)
44	Panicle: secondary branching	Weak /strong/clustered(1,2,3)
45	Panicle: attitude of branches	Erect/Erect-semierect/Semierect/semierect to spreading /spreading(1,3,5,7,9)
46	Panicle: exertion	Partially exerted/mostly exerted/well exerted(3,5,7)
47	Time of maturity	Very early/early/med/late/very late(1,3,5,7,9)
48	Leaf: senescence	Early/Med./late(3,5,7)
49	Sterile lemma colour	Straw/gold/red/purple(1,2,3,4)
50	Grain: weight of 1000 fully developed grains	Very low/Low/Med./high/Very high(1,3,5,7,9)
51	Grain: length	very short/short/med./long/very long(1,3,5,7,9)
52	Grain: width	very narrow/Narrow/Med/Broad/Very broad(1,3,5,7,9)
53	Grain: phenol reaction of lemma	Absent/Present(1,9)
54	Decorticated grain: length	very short/short/med./long/very long(1,3,5,7,9)
55	Decorticated grain: width	Narrow(<2)/med.(2-2.5)/broad(>2.5mm) (3,5,7)
56	Decorticated grain: shape (in lateral view)	Short slender/short bold/medium slender/long bold/long slender/extra long slender(1,2,3,4,5,6)
57	Decorticated grain: colour	White/light brown/variegated brown/dark brown/light red/ red /variegated purple/purple/dark purple (1,2,3,4, 5,6,7,8,9)
58	Endosperm: presence of amylose	Absent/Present(1,9)
59	Endosperm: content of amylose	Very low/Low/Med./high/Very high(1,3,5,7,9)
60	Varieties with endosperm of amylose absent only , Polished grain : expression of white core	Absent/small/med./large/fully chalky(1,3,5,7,9)
61	Gelatinization temperature through alkali spreading value.	Low/ Med./high med./high(1,3,5,7)
62	Decorticated grain: aroma	Absent/Present(1,9)

### Statistical analysis:

### Augmented design :

The replicated sub design (augmented design) is analysed and used to adjust yield of un-replicated varieties for location effects and to estimate common error variance which is used to compare all the varieties statistically as per the standard

procedure adopted in augmented design. Actual values of agronomic data were adjusted as per the standard procedure of augmented design to eliminate block effects from actual data recorded.

- c = number of check varieties : 04
- v = number of test varieties : 92
- b = number of blocks : 04
- n = v/b = number of test varieties per block : 23
- p = c + n = number of plots per block : 27
- N = b (c+n) = total number of plots : 108
- Block effect  $R_j$  for each block
- $R_j = B_j - M$
- $B_j$  = mean of all checks in the  $j^{\text{th}}$  block
- M = Grand mean of all checks
- Adjusted yield = Actual yield – block effect

**ANOVA of Augmented Design for check varieties.**

Source	Df	MSS	F
Block	b-1	MSb	MSb/MSe
Checks	c-1	MSc	MSc/MSe
Error	(b-1)(c-1)	MSe	-

\* Difference between adjusted yields of two varieties in the same block =  $\sqrt{2MSE}$

\* Difference between adjusted yields of two varieties in different blocks =  $\sqrt{2MSE(1+1/c)}$

\* Difference between an adjusted variety yield and check mean =  $\sqrt{\frac{MSE(b+1)(c+1)}{bc}}$

**3.5.1 Estimation of mean, range, standard error and critical differences:**

Mean value of each character was worked out by dividing the total by the corresponding number of observations while the lowest and the highest values for each character was taken as the range.

Standard error of mean =  $\sqrt{2M_e / r}$

Critical difference =  $\sqrt{(2M_e / r)}$  x t value at error degree of freedom at  
5% and 1% level of significance

### 3.5.2 Correlation coefficients:

Correlation coefficients between each pair of characters were estimated as per Panse and Sukhateme (1985) to establish genetic relationship among different characters.

The partial regression coefficients for yield determining traits were worked out with a view to fit the regression equation  $Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6$  according to the method given by Panse and Sukhateme(1985).

### 3.5.4 Multivariate analysis of diversity among 96 genotypes under upland condition

Principal component analysis is a short-cut multivariate analysis where vectors or roots representing different axes of differentiation and the amount of variation accounted by each of such axes, respectively are derived Rao (1952). It is a multivariate statistical method, which seeks to summarize the variation in a multivariate sample with fewer variables than the original set with minimal loss of information. It looks for a few linear combinations of the variables/characters, which give maximal variance among the objects (here genotypes). The linear combinations used in PCA are as follows.

$$\begin{aligned}
 Y_1 &= a_1X_1 + a_2X_2 + \dots\dots\dots + a_nX_n \\
 Y_2 &= a_1X_1 + a_2X_2 + \dots\dots\dots + a_nX_n \\
 &\dots\dots\dots \\
 Y_k &= a_1X_1 + a_2X_2 + \dots\dots\dots + a_nX_n
 \end{aligned}$$

Where,  $Y_1, Y_2, \dots, Y_k$  are  $PC_1, PC_2, \dots, PC_k$  scores,  $a_1, a_2, \dots, a_k$  are the elements of 1<sup>st</sup>, 2<sup>nd</sup>, ..., k<sup>th</sup> eigen vectors of the variance- covariance matrix (V) of the variables/characters and  $x_1, x_2, \dots, x_n$  are means of a genotype for different characters.

A variance-covariance matrix of dimension n will have  $r \leq n$  eigen vectors with their corresponding eigen values ( $\lambda$ 's) and  $\lambda_1 \geq \lambda_2 \dots \geq \lambda_n$ . Some of the tail end  $\lambda$ 's may be equal to zero in which case the corresponding eigen vectors will be null vectors.

The sum of the diagonal elements of  $V$  ( $\text{tr } V$ ) is taken as the total variation in the matrix and this is equal to sum of the  $\lambda$ 's. Thus, the proportion of variation accounted for by any Principal Component can be calculated. The first two principal components PC1 and PC2 scores are used for cluster analysis of objects/genotypes.

Besides, the *inter se* varietal genetic distances between genotypes were estimated following SPSS software programme and dendrograms were constructed based on DUS characteristics, agro-economic traits as well as combined DUS and agro-economic traits separately to assess genotypic relationship among the test genotypes.

### **Extraction of total seed storage proteins for SDS-PAGE**

Five seeds (brown rice) from single plant of each test genotype were ground to fine powder with mortar and pestle. 0.05gm flour of each genotype was used for protein extraction. The extraction buffer contained 0.25M Tris-HCl (pH 6.8), 4% SDS, 10% glycerol, 5%  $\beta$ -mercaptoethanol and 8M urea. The sample buffer and seed powder was mixed thoroughly by a glass rod followed by shaking for four hours using vortex. Each sample was centrifuged at 12,000 rpm for 10min. at 4°C. The supernatant containing the total seed storage protein was collected in fresh 1.5ml micro centrifuge tubes and stored in deep freezer at -20°C until use in electrophoresis.

### **Denaturation of proteins**

2  $\mu$ l of the extracted total seed storage protein was mixed with 14 $\mu$ l cracking buffer (0.125M Tris HCl, pH 6.8, 4% SDS, 20% glycerol; 10% 2-Mercaptoethanol; 0.1% bromophenol blue) and thoroughly mixed by tapping for 2 minutes. The test samples were then denatured at a temperature 100°C in hot water bath for 20 seconds.

### **Electrophoresis**

Total seed protein of each of the genotypes was analysed through one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as per Laemmli (1970) with minor modification using 12.5% polyacrylamide gel in a vertical slab gel apparatus (Tarson Pvt. Ltd.). 0.5  $\mu$ l of marker stain (mix. of 1 mg bromophenol

blue, 100  $\mu$ l glycerin, 900  $\mu$ l water) was loaded to each lane prior to loading of 2 $\mu$ l of each of the denatured sample. 2 $\mu$ l of molecular weight marker (Bangalore Genei, CAT No. 105975) dissolved in erstwhile mentioned cracking buffer was loaded to last lane instead of sample. The molecular weights of the dissociated polypeptides were determined by using molecular weight marker of protein standards which consisted eight standard proteins of known molecular weight i.e., soybean trypsin inhibitor (20.1kd), carbonic anhydrase (29kd), ovalbumin (43kd), bovine serum albumin (66kd) and phosphorylase-b (97.4kd).

Electrophoresis was carried out simultaneously by running two gels at a time taking 10 genotypes in each set of electrophoresis. In order to check the reproducibility of the banding pattern, each set of electrophoresis is repeated twice under similar electrophoretic conditions. The electrophoresis was carried out in a buffer (0.025M Tris, pH 8.3, 0.192M glycine and 0.1 % SDS) at a constant voltage (100 V) and constant current (50 mA) for two and half hours.

### **Staining, Destaining and Photography**

After electrophoresis, the gels were stained with silver staining technique. The gels were placed on trans-illuminator for assessment of banding pattern and photographed with Digital Camera (Canon, 7.1 megapixel).

### **Scoring of polypeptide bands**

The clearly distinguishable polypeptide bands in the electrophoregram were scored as 1.0 (presence) and 0 (absence) from the gel itself. Standard curves were drawn for each gel by plotting the  $R_m$  (relative mobility) values on the X-axis and Log 10 of the known molecular weights of the marker proteins on the Y-axis.  $R_m$ -values were computed as the ratio of distance of polypeptide bands to the distance of tracking dye (Bromophenol blue marker stain) migration in the gel. The molecular weights of the polypeptide bands were obtained in relation to respective  $R_m$ -values from standard curve for each gel. Molecular weights of individual bands at each specific position in different gels were nearly equal except few bands with negligible difference. The molecular weights of the dissociated polypeptides were determined by using molecular weight

marker of protein standards which consisted four standard proteins of known molecular weight i.e., Carbonic anhydrase (29kd), Ovalbumin (43kd), Bovine serum albumin (66kd) and Phosphorylase-b (97.4kd).

### **Cluster analysis and construction of dendrogram**

The morphological data were subjected to SAS statistical software programme (version 9.0) to estimate Root Mean Square(RMS) distance as a measure of Euclidian genetic distance between paired genotypes and clustering of genotypes were done based on Norm RMS tie distance values.

For clustering based on SDS-PAGE analysis, gels were scored for the presence (1) or absence (0) of bands and each band corresponding to specific polypeptide was treated as a unit character. The binary data matrix of 1 / 0 scores were analysed to estimate Jaccard's similarity coefficient (Jaccard 1908) values. The NTSYS software programme was used to estimate the Jaccard's similarity coefficient, a common estimator of genetic relationship which was calculated as follows.

$$\text{Jaccard's similarity Coefficient} = (NAB) / (NAB + NA + NB)$$

Where,  $NAB$  = No. of bands shared by samples

$NA$  and  $NB$  = Amplified fragments in sample A and B

respectively.

The dendrogram was constructed using Unweighted Paired Group method with Arithmetic means (UPGMA)-phenograms (Sokal and Michener, 1958) employing Sequential agglomerative Hierarchic and Non-overlapping Clustering (SAHN).

## CHAPTER-IV

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# *Results & Discussion*

## RESULTS AND DISCUSSION

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With the shrinking of natural resources such as land and water for agriculture and predicted adverse affects of climate change on agriculture, producing food for nine billion people is going to be a challenging task. Hence, “food” has once again come to occupy the centre stage in the international discussions. Productivity depends upon both nature (genotype) of the crop and the way by which it is being nurtured (crop husbandry). Nearly, 50% of the global increase in food production during the past century is attributed to genetic improvement through plant breeding. The last century witnessed an average increase of 32 million tons food production annually. To meet the food demands of 2050, 70% more food will have to be produced at an annual rate of increase of 44 million tons.

It is well known that all biological characteristics have a genetic base. But the involvement of the number and nature of genetic factors is not well known. Invariably, for each character it runs into dozens (if not hundreds) of genes and gene clusters. Nature has created a huge reservoir of genes in some corner of the biological world in plants, animals and microbes. Genes of economic value for plants can also be created at will or afresh. Breeding efforts are aimed at creating genotypes in form of a suitable gene combination that have never existed before. There is need for doing something new and novel in the shortest period of time and in limited space, which nature has not been able to do over millions of years. Success rate will improve with the increase of genetic variability. Now-a-days, it becomes tedious and almost unmanageable, as the number of genes/traits increases particularly in rice. The use of biochemical and molecular marker techniques has helped tremendously to increase efficiency of plant genetic improvement with shortest time and with utmost precision. However, crop improvement still awaits practical realization of the potential of these new techniques.

In recent years, the food crops succumb to ecological adversities particularly, extremes of water availability, temperature or mineral supply either transient or lasting

throughout the growing season. Recurrent erratic rainfall and high temperature have drastically reduced the productivity of rice in many states of India. Therefore, the breeding strategies in rice must be reoriented to achieve high productivity under drought stress.

Short duration rice varieties are suitable to combat frequent drought spell spread over seasons. The varieties so far developed are not up to the mark in terms of productivity. The available ruling rice varieties e.g., khandagiri, Parijat, Udaygiri, Naveen, Nilagiri have practically poor genetic potential for seed yield (<20q/ha) and lack abiotic stress tolerance. Therefore, in the present pursuit, a large collection of short duration local land races from different corners of Odisha along with already identified drought tolerant donors from gene bank at CRRI, Cuttack were assessed for genetic variation in terms of DUS characteristics and agro-economic traits including drought tolerance parameters, physiological traits, tolerance to nutritional stresses and diseases. The valuable materials so identified for upland condition based on selection strategies following the concept of pre-breeding; were further characterized for seed storage protein fingerprinting through SDS-PAGE for assessment of *inter se* genetic relationship among genotypes and varietal identification.

## **MORPHOLOGICAL CHARACTERIZATION:**

### **A. Characterization for DUS characteristics:**

Traditional rice varieties once grown and nurtured by indigenous peoples are making a comeback because of the importance of their genes that are necessary in breeding rice for the future. For some time, there was a growing fear that hybrid rice will altogether eliminate traditional rice varieties. Today, current conditions prove that traditional rice varieties are here to stay and are necessary for rice evolution. While traditional rice varieties were forgotten in the past decades, they are the “heart and soul of rice”. They require little fertilizer and no chemical inputs.. Traditional rice varieties are more nutrient-rich, tastier and friendlier to the soil. They allow farmers to protect their soil and ecosystem and have control of the seeds that their forefathers have reared for centuries.

A variety is a breeding line of rice that has successfully undergone tests for distinctiveness, uniformity and stability (DUS). A variety must be distinct from any other variety. In many cases, the distinctness can be associated with morphological characteristics. For instance, breeders inspecting a rice field, use mainly morphological characteristics to identify the variety and also the off-types that may be present within the field. The expression of these characteristics depends on the developmental stage of the plant, i.e. vegetative, reproductive stage, harvest and postharvest stages. It is important to inspect the crop at least once in each of these developmental stages in order to identify and remove off-types.

The stable morphological traits are the priori for varietal identification. A systematic and meticulous effort was therefore, undertaken to score all the relevant stable morphological traits as per DUS testing guideline of DRR, Hyderabad and IRRI, Philippines. The example varieties help in assessing DUS characteristics particularly those which showed close homology for stable morphological character expression. Chakravaty *et al.* (2012) listed a set of example varieties for each of the DUS characteristics which serves as proper reference for DUS assessment. The traits included 54 out of 62 DUS parameters recommended for varietal identification based on the concept of varietal distinctiveness, uniformity and stability over environments. The data recorded for 54 parameters at different stages of crop growth have been presented in **Table 3**. Further, the data have been extrapolated for analysis of genetic divergence using SPSS software (version 13.0). The DUS criteria further allow elimination of duplicates if any. However, none of the genotypes revealed exactly similar DUS characteristics indicating that the set of material studied in the present investigation have tremendous variability.

## **B. Characterization for agro-economic traits:**

### **Analysis of variance:**

Analysis of variance (**Table-4**) revealed significant difference among 4 standard check varieties for all agro-economic characters including grain yield. Genotypic difference was found to be statistically significant at even 1% level of significance for all the characters. Whereas, block effect was found to be non-significant for all agro-economic traits except

Table 3. DUS characteristics of a set of local land races including promising rice genotypes for upland condition.

SL. No.	Morphological DUS characteristics	1	2	3	4	5	6	7	8	9	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	
1	Coleoptile: colour	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	2
2	Basal leaf: sheath colour	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	
3	Leaf: intensity of green colour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	
4	Leaf: anthocyanin colouration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
5	Leaf sheath: anthocyanin colouration	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	1	9	1	
6	Leaf sheath : intensity of anthocyanin colouration	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	-	7	-	
7	Leaf: pubescence of blade surface	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	3	
8	Leaf : auricles	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
9	Leaf: anthocyanin colouration of auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
10	Leaf: collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
11	Leaf: anthocyanin colouration of collar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
12	Leaf: ligule	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
13	Leaf: shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
14	Leaf: colour of ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
15	Leaf: length of blade	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	3	3	
16	Leaf: width of blade	5	7	5	7	5	5	5	7	5	5	7	5	7	5	7	7	5	7	5	7	5	5	5	5	5	7	5	5	7	5	5	7	5	7
17	Culm: attitude	5	5	5	5	5	5	5	1	3	3	3	5	1	3	5	5	3	1	5	3	3	3	3	5	1	1	5	5	5	3	5	1		
18	Time of heading (50% of plants with panicles)	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5		
19	Flag leaf: attitude of blade (early observation)	5	3	1	1	5	7	3	1	1	1	1	1	1	1	5	3	1	1	1	1	3	5	1	1	3	5	5	1	5	1				
20	Spikelet: density of pubescence of lemma	3	3	3	3	5	5	3	5	3	3	5	3	5	3	5	3	3	3	5	5	5	3	3	5	5	3	5	5	5	5	5			
21	Male sterility	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
22	Lemma: anthocyanin colouration of keel	5	5	1	7	5	5	5	7	7	7	3	7	3	7	7	5	7	5	9	7	7	1	1	5	1	1	7	7	7	9	5	5		
23	Lemma: anthocyanin colouration of area below apex	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1		
24	Lemma: anthocyanin colouration of apex	1	1	1	1	1	5	1	1	1	1	1	5	3	9	1	1	1	1	1	1	5	5	5	1	1	1	5	5	1	1	1	1		
25	Spikelet: colour of stigma	1	1	1	1	1	9	.	9	1	1	1	9	5	9	1	1	1	1	1	1	9	1	9	1	1	1	9	9	9	1	1	1		
26	Stem: thickness	7	7	5	5	7	5	7	7	3	5	5	5	5	3	5	7	5	3	3	5	5	5	3	3	5	7	5	3	5	7	3	5		
27	Stem: length (excluding panicle; excluding floating rice)	5	3	3	5	3	5	5	5	5	5	9	7	.	5	5	5	5	3	7	9	7	5	3	5	7	9	9	9	5	7	3	7		

28	Stem: anthocyanin colouration of nodes	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	9	1	1	1	1	1	9	1	1	1	
29	Stem : intensity of anthocyanin colouration of nodes	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3	-	-	-	-	-	5	-	-	-	
30	Stem: anthocyanin colouration of internodes	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
31	Panicle: length of main axis	5	7	5	5	3	5	5	5	5	5	5	3	5	3	5	5	3	3	3	5	5	5	3	5	5	3	5	5	5	3	9	
32	Flag leaf: attitude of blade (late observation)	5	3	1	1	5	7	3	1	1	1	1	1	1	1	5	3	1	1	1	1	1	3	5	1	1	3	5	5	1	5	1	
33	Panicle: curvature of main axis	3	3	5	1	3	3	5	1	3	3	1	3	1	3	5	5	3	5	3	3	5	3	5	3	3	5	5	3	1	3	3	3
34	Panicle: number per plant	5	5	5	5	5	3	3	5	5	5	5	5	5	3	3	5	5	3	5	3	5	5	5	3	5	3	3	5	5	5	3	3
35	Spikelet : colour of tip of lemma	2	2	1	2	3	4	3	3	4	4	2	3	5	3	3	2	5	2	2	4	5	1	1	2	1	1	3	3	3	2	3	1
36	Lemma and Palea: colour	4	4	1	8	4	6	4	9	9	8	5	6	8	6	8	4	8	1	5	8	8	1	1	4	1	1	8	8	8	5	4	1
37	Panicle : awns	1	9	1	1	1	1	9	9	9	1	1	1	9	9	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	9	1	1
38	Panicle: colour of awns (late observation)	.	5	.	.	.	.	1	1	1	.	.	.	8	8	.	.	.	-	1	.	.	.	.	.	.	.	.	.	.	1	.	.
39	Panicle: length of longest awn	.	5	.	.	.	.	7	7	7	.	.	.	3	3	.	.	.	.	7	.	.	.	.	.	.	.	.	.	.	7	.	.
40	Panicle: distribution of awns	.	1	.	.	.	.	3	5	5	.	.	.	1	3	.	.	.	.	5	.	.	.	.	.	.	.	.	.	5	.	.	
41	Panicle: presence of secondary branching	1	1	9	1	1	1	1	1	1	1	9	9	1	9	1	1	1	1	1	9	9	1	1	9	9	9	9	9	9	1	1	1
42	Panicle: secondary branching	.	.	3	.	.	.	.	.	.	.	.	3	3	.	2	.	.	.	.	2	2	.	.	2	2	2	2	3	2	.	.	.
43	Panicle: attitude of branches	3	3	5	1	3	3	5	1	3	3	1	3	1	3	5	5	3	3	3	3	5	3	5	3	3	5	5	3	1	3	3	3
44	Panicle: exertion	3	7	3	7	7	7	3	7	7	7	7	7	7	7	5	7	5	5	7	7	7	3	5	7	7	5	5	5	7	3	3	
45	Time of maturity	3	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	5	
46	Leaf: sence	3	5	7	7	7	5	5	5	5	5	5	7	3	3	3	5	3	5	5	3	7	3	3	3	3	3	3	5	3	5	5	5
47	Grain: weight of 1000 fully developed grains	5	9	1	1	7	1	7	3	3	1	1	1	1	5	1	9	1	1	3	1	1	1	1	5	5	5	1	1	5	5	5	1
48	Grain: length	9	9	9	1	9	3	9	9	9	9	9	9	9	1	9	5	1	5	9	7	9	5	9	9	9	7	7	9	9	7	1	
49	Grain: width	9	9	9	5	5	5	7	5	7	3	5	5	1	7	5	7	3	1	5	5	5	2	5	2	7	7	5	7	5	7	5	
50	Decorticated grain: length	3	5	3	1	3	1	3	5	5	5	5	5	3	5	1	3	1	1	1	3	1	5	1	3	5	5	1	7	5	5	3	1
51	Decorticated grain: width	5	5	2	3	3	3	5	3	3	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
52	Decorticated grain: shape (in lateral view)	3	3	3	2	3	3	3	3	3	5	3	5	3	3	2	3	1	1	3	3	5	5	4	3	3	3	3	3	5	3	3	3
53	Decorticated grain: colour	6	5	5	1	6	1	6	6	6	1	1	5	1	1	1	6	1	1	6	1	1	1	1	6	1	1	1	6	1	6	6	1
54	Decorticated grain: aroma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 3. (contd.....)

SL. No	Morphological DUS characteristics	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6
		3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	
1	Coleoptile: colour	2	3	3	3	2	3	3	2	2	2	2	2	2	3	2	2	2	3	3	3	2	2	3	3	3	3	2	2	2	2	3	2	
2	Basal leaf: sheath colour	2	3	3	3	2	3	3	2	2	2	2	2	2	3	2	2	2	3	3	3	2	2	3	3	3	3	2	2	2	2	3	2	
3	Leaf: intensity of green colour	3	5	3	3	5	7	7	5	3	5	7	5	3	5	3	3	3	3	7	5	3	3	3	3	3	3	7	5	5	3	3	3	
4	Leaf: anthocyanin colouration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
5	Leaf sheath: anthocyanin colouration	1	9	9	9	1	9	9	1	1	1	1	1	1	9	1	1	1	9	9	9	1	1	9	9	9	9	9	1	1	1	1	9	1
6	Leaf sheath : intensity of anthocyanin colouration	7	7	7	7	-	7	7	-	-	-	-	-	-	7	-	-	-	7	9	5	-	-	7	7	7	7	-	-	-	-	9	-	
7	Leaf: pubescence of blade surface	1	1	5	3	5	3	3	5	3	3	1	3	5	1	3	5	5	1	1	1	3	3	3	5	5	5	5	3	5	3	5	5	
8	Leaf : auricles	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
9	Leaf: anthocyanin colouration of auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
10	Leaf: collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
11	Leaf: anthocyanin colouration of collar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
12	Leaf: ligule	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
13	Leaf: shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
14	Leaf: colour of ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
15	Leaf: length of blade	5	5	5	5	3	5	5	5	5	3	5	5	5	5	5	5	5	7	5	5	5	5	5	5	3	5	5	5	5	5	5		
16	Leaf: width of blade	7	5	5	5	7	7	5	5	5	7	7	7	5	5	7	7	7	5	5	5	3	3	5	5	7	7	7	7	3	3	5	7	
17	Culm: attitude	5	5	5	3	1	3	5	3	5	5	1	1	5	5	3	3	3	5	3	1	1	3	5	5	3	3	1	1	1	5	3	5	
18	Time of heading (50% of plants with panicles)	3	3	3	3	3	3	3	3	3	5	3	3	3	3	3	3	5	3	3	5	5	3	3	3	3	3	5	5	5	3	3	3	
19	Flag leaf: attitude of blade (early observation)	3	5	5	1	1	5	5	1	7	1	1	1	5	1	1	1	1	7	7	1	1	1	7	1	1	1	1	1	1	5	1	5	
20	Spikelet: density of pubescence of lemma	3	3	3	3	3	5	5	5	5	3	5	3	5	3	5	5	5	3	3	5	5	5	5	7	5	5	3	5	5	7	5		
21	Male sterility	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
22	Lemma: anthocyanin colouration of keel	1	5	5	7	3	7	7	7	5	1	1	1	1	7	7	5	1	9	5	1	1	5	5	3	7	7	1	1	1	1	9		
23	Lemma: anthocyanin colouration of area below apex	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
24	Lemma: anthocyanin colouration of apex	1	5	1	5	1	5	5	1	1	1	1	1	5	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1		
25	Spikelet: colour of stigma	7	9	1	9	1	9	9	1	1	1	1	1	9	1	1	1	7	9	1	1	1	1	1	1	1	1	1	1	1	9	7		
26	Stem: thickness	7	5	5	5	5	7	7	5	5	3	3	7	5	5	5	5	5	3	5	7	5	5	7	7	7	5	3	5	5	7	7		
27	Stem: length (excluding panicle; excluding floating rice)	3	3	1	5	3	5	5	5	5	3	5	3	9	7	5	7	5	7	1	7	5	5	7	5	5	5	5	5	5	7	5		



Table 3. (contd.....)

SL. No	Morphological DUS characteristics	65	66	67	68	69	70	71	72	73	74	75	IR 87707-445	CR-143-2-2	CR- Dhan 40	Kalakeri	Safri 17	IR 20	Anjali	Browngora	Salampikit	Sadabahar	Sahbhagidhan	Saria	Annada	Sneha	Zhu 11-26	Heera	Vanaprabha	N 22	Vandana	Khandagiri	Mandakini
1	Coleoptile: colour	2	2	3	2	2	2	2	3	2	2	2	2	3	3	2	2	2	2	3	2	2	2	3	2	2	2	2	3	2	2	2	2
2	Basal leaf: sheath colour	2	2	3	2	2	2	2	3	2	2	2	2	3	3	2	2	2	2	3	2	2	2	3	2	2	2	2	2	3	2	2	2
3	Leaf: intensity of green colour	3	5	5	3	3	5	3	5	3	3	3	5	3	3	3	5	7	3	3	5	5	5	3	7	7	5	7	5	3	3	7	5
4	Leaf: anthocyanin colouration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	Leaf sheath: anthocyanin colouration	1	1	9	1	1	1	1	9	1	1	1	1	9	9	1	1	1	1	9	1	1	1	9	1	1	1	1	1	9	1	9	1
6	Leaf sheath : intensity of anthocyanin colouration	-	-	7	-	-	-	-	7	-	-	-	-	7	7	-	-	-	-	7	-	-	-	7	-	-	-	-	-	7	-	3	-
7	Leaf: pubescence of blade surface	3	5	5	3	3	5	5	3	3	3	1	3	5	5	5	1	1	3	1	3	1	5	3	5	3	3	3	1	5	5	3	3
8	Leaf : auricles	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
9	Leaf: anthocyanin colouration of auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Leaf: collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
11	Leaf: anthocyanin colouration of collar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	Leaf: ligule	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
13	Leaf: shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
14	Leaf: colour of ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	Leaf: length of blade	5	5	5	5	5	5	5	5	5	3	3	3	3	3	5	5	5	5	3	3	5	5	3	3	3	3	3	5	5	5	3	5
16	Leaf: width of blade	3	7	5	7	5	5	5	5	7	5	5	7	5	5	7	7	7	5	5	5	5	7	5	5	5	5	5	3	5	5	7	5
17	Culm: attitude (late stage)	5	5	5	5	5	5	3	3	5	3	1	1	3	5	1	1	5	5	1	3	1	5	1	1	1	1	1	5	5	3	1	
18	Time of heading (50% of plants with panicles)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	5	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3
19	Flag leaf: attitude of blade (early observation)	3	5	2	5	5	5	5	1	5	5	3	1	1	5	5	1	1	3	1	1	3	1	7	1	3	3	3	1	3	5	3	3
20	Spikelet: density of pubescence of lemma	7	7	5	5	5	3	5	3	5	5	3	5	5	3	5	5	5	3	5	5	3	3	5	5	5	5	3	5	3	3	3	3
21	Male sterility	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	Lemma: anthocyanin colouration of keel	1	7	1	3	5	5	5	5	5	3	1	1	3	3	9	1	1	1	5	1	1	5	5	1	1	1	7	1	1	1	1	
23	Lemma: anthocyanin colouration of area below apex	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1
24	Lemma: anthocyanin colouration of apex	1	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1



Table 4 . ANOVA of RBD for check varieties.

Sl. No.	Character	Source of variation	df	M.S.S.	F-value	CVe (%)
1.	Days to 50% flowering	Block	3	0.063	0.011	3.40
		Check var.	3	109.563	19.895**	
		Error	9	5.507		
2.	Days to maturity	Block	3	0.917	0.347	1.64
		Check var.	3	106.417	40.326**	
		Error	9	2.639		
3.	Leaf rolling	Block	3	0.563	1.246	13.36
		Check var.	3	26.063	57.738**	
		Error	9	0.451		
4.	Drought Recovery	Block	3	0.563	1.653	12.14
		Check var.	3	25.729	785.612**	
		Error	9	0.340		
5.	Leaf drying	Block	3	0.750	2.455	12.23
		Check var.	3	25.583	83.727**	
		Error	9	0.306		
6.	Leaf area (cm <sup>2</sup> )	Block	3	0.305	1.662	1.04
		Check var.	3	92.809	506.109**	
		Error	9	0.183		
7.	Chlorophyll Index	Block	3	0.020	1.054	1.49
		Check var.	3	8.864	467.783**	
		Error	9	0.019		
8.	Zinc tolerance	Block	3	0.250	1.000	24.11
		Check var.	3	6.250	25.000**	
		Error	9	0.250		
9.	BLB tolerance	Block	3	0.250	1.000	11.89
		Check var.	3	14.917	59.667**	
		Error	9	0.250		
10.	Plant height	Block	3	3.750	2.755	1.03
		Check var.	3	818.083	601.041**	
		Error	9	1.361		
11.	Ear bearing tillers	Block	3	9.417	0.703	1.14
		Check var.	3	6596.417	492.678**	
		Error	9	13.389		
12.	Panicle length(cm.)	Block	3	0.275	1.488	1.94
		Check var.	3	5.724	31.014**	
		Error	9	0.185		
13.	Panicle weight(g)	Block	3	0.005	0.691	5.50
		Check var.	3	0.751	106.440**	
		Error	9	0.007		
14.	Grains/Panicle	Block	3	2.417	0.540	3.24
		Check var.	3	1130.083	252.689**	
		Error	9	4.472		

Sl. No.	Character	Source of variation	df	M.S.S.	F-value	CVe (%)
15.	1000-Grain weight(g)	Block	3	0.134	1.277	1.56
		Check var.	3	78.849	749.149**	
		Error	9	0.105		
16.	Grain length(cm.)	Block	3	0.007	0.866	1.07
		Check var.	3	5.855	776.420**	
		Error	9	0.008		
17.	Grain breadth(cm.)	Block	3	0.001	0.331	2.63
		Check var.	3	0.270	97.434**	
		Error	9	0.003		
18.	Grain length/Grain breadth	Block	3	0.013	0.628	3.54
		Check var.	3	3.076	147.122**	
		Error	9	0.021		
19.	Kernel length(cm.)	Block	3	0.005	8.468**	0.40
		Check var.	3	2.965	5533.367**	
		Error	9	0.001		
20.	Kernel breadth(cm.)	Block	3	0.002	1.359	1.86
		Check var.	3	0.230	207.951**	
		Error	9	0.001		
21.	Kernel length/Kernel breadth	Block	3	0.005	0.828	2.40
		Check var.	3	2.987	475.048**	
		Error	9	0.006		
22.	Fertility(%)	Block	3	1.180	1.922	1.10
		Check var.	3	490.125	798.620**	
		Error	9	0.614		
23.	Seed yield (qtl/ha)	Block	3	0.440	1.609	1.82
		Check var.	3	49.903	182.576**	
		Error	9	0.273		

kernel length which was significant at 1% level of significance indicating environmental influence on the character.

#### **Coefficient of variation (CV):**

The estimate of coefficient of variation was less than 5% in most of the traits and around 12-13%(Table 4) in case of three drought tolerance parameters e.g., leaf rolling, drought recover, and leaf drying score except tolerance to zinc(24.03%). (14.11%). The low CV estimates in most of the characters justify the experimental precision. Whereas, the characters relating tolerance to drought and zinc which revealed relatively high CV estimates, are highly versatile under micro changes of environment.

#### **Mean performance of genotypes**

##### **a) Flowering and maturity duration:**

Varieties with maturity duration within 90-100 days are *in vogue* preferred for suitability in upland condition. In the present investigation, days to 50% flowering and days to maturity (physiological maturity) ranged from 48.9 to 106.9 days and 78.3 to 135.3days respectively(Table 5). Among the local land races, Setka-1, Danisaria, Podasankara, Dangaradhan, Bhogi, Dasaharadhan, Paradhan, Nandigiri, Dhalashree-B, Saria-B and Zhu 11-26 recorded significantly early flowering at around 60days and attained maturity even within 90 days. Among these, 45 took just 78days to mature and it can be a valuable material for breeding of rice varieties for upland situation.

##### **b) Drought tolerance:**

Farmers used to cultivate early rice varieties in the upland situation which are sown at onset of monsoon and harvested latest before 15<sup>th</sup> October. Cultivation of these varieties entirely depends upon seasonal rainfall during Kharif. Therefore, genotypes chosen for upland situation need to have drought tolerance to combat frequent drought spell during kharif season. In recent years, vagaries of monsoon warrant selection of rice varieties for suitability of cultivation with minimum water availability. Therefore, in the present pursuit, an attempt was taken to screen the available local land races and popular upland rice varieties for rigorous screening for drought tolerance. Drought tolerance was assessed based on three criteria/parameters e.g., leaf rolling, drought recovery

**Table 5. Mean values (adjusted) of upland land races of rice for different agro economic traits.**

Sl. no	Genotype	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	SY
1	Koliha	58.9	89.8	4.8	4.6	7.6	35.6	9.2	3.1	2.8*	112.8*	232.1	21.9	1.3	58.6	20.4	8.5	2.2*	3.7	5.7	2.1*	2.6	76.8*	21.8
2	Kantadumer	61.9	91.8	5.8	5.6	4.6	35.0	15.7*	5.1	4.8	102.8*	182.1	27.4*	2.9*	92.6	29.3*	8.8	2.5*	3.4	6.1*	2.1*	2.8	78.5*	26.6
3	Bastul	64.9	93.8	2.8	2.6	4.6	31.3	15.2*	1.1	2.8*	92.8*	318.1	21.4	1.1	88.6	18.2	8.5	2.4*	3.5	5.7	2.0*	2.8	83.0*	34.8
4	Biramani	66.9	97.8	2.8	0.6*	2.6	29.6	11.3*	1.1	8.8	121.8	306.1	21.9	0.6	31.6	15.7	6.0	1.9	3.0	4.2	1.8	2.2	20.3	19.7
5	Setka-1	52.9*	82.8*	6.8	6.6	7.6	53.4*	14.1*	3.1	4.8	101.8*	327.1	18.9	0.9	34.6	25.0*	8.6	1.7	4.9*	5.6	1.4	3.9*	64.8	28.1
6	Kharkoili	66.9	96.8	4.8	4.6	1.6*	45.1*	10.4*	1.1	6.8	115.8	287.1	24.4*	1.3	65.6	16.6	6.9	1.8	3.6	4.9	1.7	2.8	60.2	20.5
7	Kuliha	59.9	89.8	4.8	4.6	5.6	46.5*	11.2*	3.1	4.8	119.8	228.1	23.9*	1.3	49.6	25.1*	8.2	2.1	3.8	5.6	2.0*	2.7	67.5	29.2
8	Chinger-1	75.9	104.8	4.8	4.6	1.6*	27.3	10.3*	1.1	2.8*	119.8	242.1	22.9	0.9	60.6	23.2*	8.5	1.9	4.4	6.0	1.6	3.3	59.5	20.1
9	Kusuma	76.9	105.8	8.8	8.6	5.6	39.2	11.1*	1.1	8.8	115.8	187.1	23.9*	1.1	50.6	18.6	8.2	2.1	3.9	6.0	1.9*	3.1	63.6	17.7
10	Hiran	74.9	103.8	2.8	2.6	2.6	49.6*	11.1*	1.1	4.8	123.8	326.1	23.9*	1.3	67.6	21.0	8.8	1.6	5.4*	6.0	1.3	4.4*	89.0*	28.3
11	Ninibudhi	79.9	108.8	4.8	4.6	3.6	48.1*	10.1*	3.1	2.8*	137.8	408.1*	20.4	0.9	49.6	16.6	8.4	1.8	4.6*	6.1*	1.6	3.6*	80.4*	33.4
12	Badi	67.9	98.8	6.8	6.6	2.6	52.0*	9.2	1.1	4.8	131.8	487.1*	21.4	0.8	44.6	15.4	8.8	1.7	4.9*	6.1*	1.6	3.6*	59.7	33.2
13	Rangahazari	85.9	116.8	4.8	4.6	4.6	30.4	9.2	1.1	4.8	114.8	408.1*	19.9	0.6	35.6	18.4	8.0	1.3	5.9*	5.7	1.0	5.2*	52.9	29.7
14	Lalubadikaberi	73.9	103.8	8.8	8.6	8.6	42.4*	6.9	1.1	4.8	122.8	248.1	24.4*	2.6*	110.6*	21.1	9.4*	2.2*	4.2	6.2*	2.0*	3.0	75.8	34.7
15	Dangarchudi	66.9	97.8	4.8	4.6	8.6	28.2	8.3	5.1	2.8*	108.8*	377.1	18.9	0.6	49.6	15.1	6.1	1.8	3.3	4.1	1.6	2.4	71.5	28.1
16	Danisaria	54.9*	85.8*	8.8	8.6	4.6	37.1	10.1*	5.1	2.8*	111.8*	261.1	22.9	1.8	67.6	26.5*	8.0	2.0	3.9	5.7	1.8	3.1	95.2*	28.9
17	Sunamukhi	68.9	99.8	4.8	4.6	4.6	34.0	6.4	5.1	4.8	119.8	203.1	20.4	1.0	77.6	11.5	7.0	1.7	4.1	5.0	1.5	3.2	68.8	18.2
18	Baspatri	91.9	121.8	8.8	8.6	8.6	50.1*	11.1*	1.1	2.8*	109.8*	191.1	21.9	0.8	56.6	20.6	5.9	1.3	4.3	4.0	1.2	3.3	64.7	18.9
19	Karni	61.9	92.8	4.8	1.6	4.6	36.4	12.2*	3.1	2.8*	120.8	264.1	19.9	0.8	37.6	20.0	7.4	1.7	4.1	5.0	1.5	3.1	58.2	19.8
20	Harisankar	75.9	104.8	8.8	8.6	4.6	28.6	11.0*	1.1	4.8	134.8	445.1*	19.4	0.8	36.6	16.7	8.2	1.8	4.4	5.7	1.6	3.3	45.6	32.9
21	Dengabari	68.9	97.8	2.8	1.6	1.6*	49.9*	8.4	1.1	4.8	124.8	394.1	21.4	0.5	35.6	15.5	7.8	1.7	4.3	5.1	1.5	3.2	57.8	21.6
22	Kahnei	75.9	106.8	4.8	4.6	3.6	36.4	11.9*	1.1	2.8*	120.8	417.1*	21.4	0.9	34.6	16.6	9.1*	1.9	4.6*	6.3*	1.8	3.5	66.9	33.7
23	Pugukal	65.9	96.8	4.8	4.6	6.6	65.2*	8.6	3.1	4.8	104.8*	238.1	21.6	1.0	70.6	13.3	7.4	1.7	4.2	5.3	1.5	3.3	76.4*	22.4
24	Butasori	66.9	97.3	9.3	9.4	5.6	59.0*	8.4	9.1	5.3	117.1	312.4	20.1	1.0	42.3	20.3	8.4	1.9	4.2	5.9	1.7	3.3	67.2	27.1
25	Pandeydhan	77.9	106.3	5.3	5.4	5.8	37.9	1.3	1.1	5.3	137.1	315.4	23.7*	2.0	81.3	19.9	8.4	2.0	4.2	6.1*	1.8	3.3	82.3*	35.3
26	khursudi	73.9	104.3	3.3	3.4	1.8*	18.0	11.7*	1.1	5.3	139.1	328.4	22.3	1.5	52.3	20.9	8.3	2.0	4.1	5.9	1.7	3.5	90.0*	36.3*
27	Kinari	68.9	97.3	5.3	1.4	0.8*	43.4*	13.4*	1.1	9.3	133.1	504.4*	18.3	0.8	37.3	15.0	7.8	1.8	4.1	5.4	1.6	3.2	88.5*	34.3
28	Jirkubanji	67.9	96.3	9.3	9.4	7.8	41.2	8.3	1.1	5.3	138.1	335.4	21.3	1.1	49.3	16.7	7.7	2.1	3.7	6.4*	1.7	3.7*	56.8	28.0
29	Dular	60.9	89.3	2.3	1.4	1.8*	48.9*	11.5*	3.1	3.3*	122.1	297.4	23.8*	2.3*	100.3*	21.7	8.7	1.9	4.6*	6.1*	1.6	3.6*	90.6*	34.7
30	Kusuma	74.9	104.3	2.3	1.4	1.8*	49.7*	10.5*	1.1	3.3*	123.1	216.4	23.3	1.0	40.3	20.1	8.7	2.1	4.1	5.9	1.8	3.2	68.3	17.8
31	Podasankara	52.9*	81.3*	5.3	5.4	7.8	33.1	11.7*	5.1	9.3	101.1*	244.4	17.3	0.8	35.3	20.7	7.7	2.0	3.7	5.5	1.8	2.9	75.0	18.1
32	Merlo	83.9	112.3	3.3	3.4	2.8	19.7	12.6*	1.1	3.3*	134.1	407.4*	31.3*	1.4	53.3	12.0	6.2	1.9	3.3	4.1	1.7	2.4	41.3	28.0
33	Pankapota	63.9	94.3	1.3	1.4	3.4	33.1	10.8*	3.1	5.3	101.1*	299.4	22.8	2.4*	149.3*	14.8	6.7	1.9	3.4	4.5	1.8	2.5	89.2*	33.6

Sl. no	Genotype	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	SY
34	Rasakadali	66.9	97.3	9.3	9.4	4.4	58.3*	7.4	5.1	3.3*	107.1*	324.4	27.8*	1.2	77.3	19.5	8.2	2.0	4.0	5.8	1.8	3.2	72.6	34.4
35	Setka-2	65.9	95.3	5.3	5.4	4.8	49.0*	10.2*	1.1	3.3*	88.1*	199.4	21.5	1.0	50.3	16.7	8.0	2.2*	3.6	5.5	2.0*	2.7	71.9	17.0
36	Damaraphuli	68.9	97.3	3.3	2.4	1.8*	41.7*	10.7*	1.1	5.3	119.1	180.4	26.3*	1.0	62.3	15.6	7.7	1.4	5.2*	5.1	1.1	4.4*	52.2	15.9
37	Chinger-2	77.9	108.3	1.3	1.4	0.8*	21.3	8.6	1.1	3.3*	106.1*	240.4	23.3	0.8	39.3	17.6	7.2	2.9*	2.5	5.1	1.6	3.1	45.1	14.9
38	Biramani	66.9	95.3	7.3	7.4	3.8	33.2	9.2	1.1	9.3	124.1	245.4	25.8*	2.0	91.3	19.5	7.5	1.9	3.9	5.3	1.6	3.1	80.3*	21.1
39	Dangaradhan	52.9*	83.3*	7.3	7.4	2.8	39.2	10.4*	1.1	5.3	119.1	185.4	22.3	1.9	73.3	23.7*	6.9	2.0	3.4	4.9	1.8	2.7	83.4*	14.7
40	Kalakusuma	77.9	106.3	7.3	7.4	3.8	35.0	10.3*	1.1	3.3*	119.1	239.4	25.8*	1.5	55.3	23.9*	8.0	2.1	3.8	5.6	1.8	3.0	58.4	29.7
41	Sarian	57.9	87.3	5.3	5.4	3.8	55.0*	10.7*	5.1	5.3	109.1*	326.4	24.7*	3.6*	127.3*	25.0*	7.3	2.2*	3.3	5.2	2.0*	2.5	79.4*	34.3
42	Dal	106.9	135.3	7.3	7.4	4.8	38.6	15.5*	1.1	3.3*	119.1	328.4	22.3	1.3	53.3	20.3	8.5	1.8	4.6*	5.8	1.6	3.5	79.3*	32.6
43	Jhulipuagi	69.9	98.3	9.3	9.4	5.8	18.2	14.0*	1.1	5.3	94.1*	427.4*	20.3	1.5	36.3	17.7	9.1*	2.0	4.4	6.6*	1.7	3.7*	75.0	34.3
44	Litipiti	75.9	106.3	7.3	7.4	4.8	34.2	15.2*	1.1	3.3*	113.1*	293.4	22.8	1.5	55.3	21.5	8.4	2.0	4.2	5.8	1.8	3.2	70.1	30.6
45	Bhogi	48.9*	78.3*	3.3	3.4	7.8	40.1	13.0*	1.1	5.3	92.1*	273.4	18.3	1.0	49.3	18.3	7.7	1.8	4.2	5.2	1.6	3.2	83.5*	25.0
46	Barei	66.9	96.3	5.3	5.4	4.8	45.5*	10.4*	1.1	3.3*	131.1	438.4*	16.3	0.5	38.3	15.8	7.1	2.2*	3.1	5.2	2.0*	2.5	34.5	26.9
47	Kutiarasi	76.1	105.3	5.3	5.1	3.6	32.5	9.8*	0.6*	4.8	127.3	502.4*	15.0	0.9	38.1	15.0	8.0	1.9	4.0	5.6	1.7	3.2	74.0	30.0
48	Kusuma	70.1	101.3	4.3	4.1	5.6	25.0	9.5	0.6*	4.8	111.3*	262.4	21.5	0.5	36.1	20.8	7.9	1.9	3.9	5.5	1.8	3.0	43.6	19.6
49	Pora	86.1	115.3	5.3	5.1	5.6	36.8	8.6	0.6*	2.8*	131.3	270.4	23.4	0.8	71.1	20.9	8.2	1.9	4.2	5.9	1.7	3.4	63.2	29.4
50	Karanga	59.1	89.3	5.3	5.1	5.6	65.8*	10.4*	0.6*	2.8*	117.3	306.4	23.0	2.0	70.1	27.1*	8.9*	2.1	4.1	6.3*	1.8	3.3	85.7*	34.0
51	Kenduphula	80.1	110.3	9.3	9.1	6.6	55.3*	12.9*	0.6*	4.8	129.3	359.4	22.7	1.2	73.1	19.7	7.7	1.8	4.1	5.2	1.8	2.8	88.7*	34.2
52	Malkadua	82.1	111.3	9.3	9.1	3.6	50.5*	9.0	2.6	2.8*	90.3*	272.4	20.5	0.7	36.1	15.6	7.6	1.9	3.9	5.3	1.6	3.1	45.9	26.4
53	Ambajhuka	85.1	116.3	1.3	1.1	1.6*	61.2*	11.4*	0.6*	2.8*	134.3	188.4	20.0	0.8	50.1	22.3	7.2	1.5	4.6*	5.0	1.2	3.9*	60.8	16.6
54	Kunor	75.1	106.3	7.3	7.1	3.6	52.6*	10.1*	0.6*	4.8	114.3*	349.4	21.5	1.5	52.1	21.3	8.5	1.7	4.8*	5.1	1.4	3.5	88.9*	34.0
55	Haladigundi	58.1	87.3	5.3	5.1	5.6	60.0*	7.3	4.6	2.8*	122.3	313.4	25.0*	1.9	62.1	26.4*	8.4	2.0	4.0	5.9	1.9*	3.1	80.5*	35.0
56	Padarabank	67.1	98.3	3.3	3.1	4.6	61.6*	8.8	0.6*	4.8	133.3	315.4	26.5*	3.0*	142.1*	19.5	8.7	2.1	3.9	6.3*	1.8	3.4	86.4*	32.2
57	Mahulakunchi	69.1	98.3	5.3	5.1	6.6	20.2	8.8	4.6	2.8*	112.3*	329.4	25.5*	1.7	64.1	23.2*	7.7	2.2*	3.3	5.2	2.0*	2.5	71.5	34.1
58	Somo	68.1	99.3	3.3	1.1	2.6	36.9	8.0	4.6	2.8*	124.3	347.4	28.0*	2.4*	81.1	26.6*	7.8	2.2*	3.3	5.4	2.0*	2.6	81.3*	32.0
59	Jhitipiti	83.1	112.3	1.3	1.1	2.6	30.7	11.0*	0.6*	4.8	118.3	333.4	23.5	1.4	36.1	22.2	8.8	2.0	4.1	6.1*	1.8	3.2	37.8	29.6
60	Ras	86.1	116.3	3.3	3.1	2.6	33.5	6.1	0.6*	4.8	101.3*	280.4	22.0	0.8	38.1	14.9	8.5	2.0	4.1	5.9	1.8	3.2	31.3	15.8
61	Sanarasi	85.1	115.3	1.1	1.1	2.6	69.1*	7.0	0.6*	4.8	111.3*	341.4	19.5	0.9	44.1	17.0	8.4	1.7	4.7*	5.8	1.5	3.8*	68.0	25.5
62	Dasaharadhan	54.1*	85.3*	5.3	5.1	4.6	75.0*	6.7	0.6*	4.8	110.3*	195.4	23.5	1.5	76.1	17.9	9.4*	2.0	4.5	6.7*	1.7	3.8*	66.4	25.9
63	Bhatasakli	79.1	108.3	5.3	5.1	5.6	41.3	5.9	2.6	4.8	122.3	381.4	19.5	0.7	33.1	21.5	8.4	1.9	4.2	5.9	1.6	3.6*	40.7	26.7
64	Paradhan	57.1*	86.3*	6.3	6.1	6.6	32.8	7.3	6.6	2.8*	118.3	315.4	20.5	1.2	50.1	26.4*	8.3	2.2*	3.6	5.7	2.0*	2.7	82.3*	28.5
65	Kandasuri	68.1	98.3	7.3	7.1	6.6	52.6*	7.3	2.6	4.8	123.3	329.4	20.0	1.5	53.1	28.0*	8.7	2.2*	3.8	6.3*	2.0*	3.1	91.7*	34.1
66	Kanding	67.1	99.3	6.3	6.1	2.6	28.6	6.8	2.6	2.8*	131.3	381.4	25.0*	2.0	89.1	21.4	8.5	2.0	4.2	6.0	1.7	3.3	93.0*	34.6
67	Asumakunda	68.1	96.3	7.3	7.1	5.6	50.5*	8.5	4.6	4.8	132.3	340.4	29.3*	3.0*	98.1*	26.6*	8.7	1.9	4.5	6.2*	1.5	3.9*	90.0*	36.6*
68	Nandigiri	54.1*	83.3*	9.3	9.1	5.6	27.5	9.0	8.6	2.8*	101.3*	330.4	20.5	2.9*	114.1*	23.4*	7.9	2.2*	3.5	5.5	2.0*	2.7	90.1*	34.0

Sl. no	Genotype	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	SY
69	Dhobasaria	57.1*	88.3	2.3	1.1	2.6	56.2*	7.3	4.6	2.8*	91.3*	327.4	23.0	3.7*	128.1*	28.8*	8.0	2.2*	3.5	5.6	2.0*	2.8	95.9*	35.1
70	Dhalashree -B	56.9*	86.3*	8.3	8.6	3.8	53.0*	7.5	5.1	4.8	101.6*	236.9	20.7	2.2	76.8	26.3*	8.1	2.2*	3.6	5.7	2.1*	2.6	90.2*	29.4
71	Saria-B	52.9*	84.3*	0.5*	0.6*	1.8*	42.4*	9.3	5.1	2.8*	104.6*	306.9	18.7	1.6	65.8	23.7*	7.6	2.1	3.6	5.3	1.9*	2.7	86.5*	30.1
72	Hiran	77.9	109.3	4.5	4.6	2.8	53.1*	7.3	1.1	2.8*	143.6	461.9*	18.2	1.1	37.8	19.2	8.5	1.9	4.3	6.1*	1.7	3.4	87.6*	34.1
73	Mahularani	69.9	101.3	4.5	4.6	4.8	36.4	4.5	1.1	4.8	120.6	291.9	24.5*	1.8	63.8	23.3*	8.5	2.1	3.9	5.9	1.9*	3.1	70.2	31.2
74	Kapaanthi	56.9*	88.3	5.5	5.6	4.8	54.8*	8.3	1.1	2.8*	112.6*	205.9	21.7	2.5*	86.8	27.2*	8.4	2.2*	3.8	5.8	2.0*	2.8	75.2	24.5
75	Pustak	63.9	93.3	6.5	6.6	4.8	30.6	7.9	1.1	2.8*	122.6	319.9	25.7*	3.1*	148.8*	19.0	7.1	2.4*	2.9	5.8	2.0*	2.3	90.4*	33.3
76	IR 87707-445-B-B	73.9	103.3	2.5	2.6	3.8	25.1	7.2	1.1	4.8	101.6*	303.9	22.6	1.2	55.8	18.3	9.1*	2.0	4.5	6.4*	1.8	3.5	57.3	30.5
77	CR 143-2-2	71.9	102.3	4.5	4.6	2.8	35.3	7.3	1.1	2.8*	101.6*	499.9*	16.0	0.9	66.8	16.4	7.3	2.1	3.4	5.2	1.9*	2.6	90.2*	35.0
78	CR Dhan 40	67.9	99.3	0.5*	0.6*	3.8	38.7	5.9	1.1	2.8*	117.6	347.9	23.5	3.0*	98.8*	24.3*	8.0	2.1	3.7	5.8	1.9*	2.9	64.9	35.0
79	Kalakeri	63.9	95.3	3.5	0.6*	1.8*	31.3	7.9	5.1	2.8*	104.6*	392.9	23.2	1.4	40.8	27.7*	9.3*	2.1	4.4	6.5*	1.9*	3.3	75.9	31.6
80	Safri -17	86.9	115.3	3.5	3.6	4.8	31.3	7.1	1.1	2.8*	99.6*	343.9	24.2*	1.1	60.8	19.6	8.0	2.4*	3.3	6.7*	2.1	3.1	66.0	30.0
81	IR 20	84.9	114.3	3.5	3.6	4.8	22.7	8.0	1.1	2.8*	89.6*	369.9	20.5	1.0	31.8	17.3	8.2	1.8	4.3	5.8	1.7	3.3	35.8	26.3
82	Anjali	62.9	93.3	1.5	1.6	3.8	38.0	11.1*	1.1	2.8*	104.6*	313.9	26.7*	3.5*	121.8*	25.4*	8.3	2.1	3.8	6.0	1.9*	3.0	71.0	33.7
83	Browngora	65.9	95.3	2.5	0.6*	1.8*	44.8*	6.2	1.1	2.8*	109.6*	381.9	19.5	1.8	61.8	26.8*	9.5*	2.0	4.5	6.6*	1.9*	3.4	51.3	30.1
84	Salampikit	79.9	110.3	2.5	1.6	1.8*	40.0	7.1	1.1	0.8*	152.6	281.9	19.7	2.8*	63.8	26.4*	7.0	2.1	3.3	5.5	1.9*	2.8	72.4	33.8
85	Sadabahar	66.9	98.3	6.5	6.6	2.8	32.3	6.7	1.1	2.8*	102.6*	244.9	24.2*	2.0	91.8	18.6	8.5	1.7	5.0*	6.0	1.5	3.8*	52.0	28.4
86	Sahbhagi dhan	73.9	105.3	2.5	2.6	3.8	39.2	7.4	1.1	2.8*	100.6*	291.9	17.2	1.5	67.8	19.8	8.9*	1.7	4.9*	5.7	1.6	3.4	79.8*	35.0
87	Saria	58.9	89.3	6.5	6.6	5.8	52.0*	6.1	1.1	2.8*	115.6	183.9	23.5	0.5	80.8	29.4*	8.3	2.2*	3.6	5.8	2.0*	2.8	86.0*	19.3
88	Annada	65.9	97.3	4.5	4.6	5.8	21.6	10.6*	1.1	4.8	90.6*	373.9	21.7	2.2	79.8	24.5*	8.1	1.9	4.2	5.7	1.7	3.3	75.2	33.8
89	Sneha	64.9	95.3	3.5	3.6	5.8	23.0	9.0	1.1	4.8	92.6*	327.9	21.7	2.2	68.8	19.9	8.0	2.1	3.7	5.7	2.0*	2.7	72.4	34.1
90	Zhu 11-26	55.9*	86.3*	1.5	1.6	4.8	37.3	9.9*	1.1	4.8	101.6*	383.9	20.2	1.9	76.8	22.9*	8.2	2.0	4.0	5.6	1.9*	2.9	88.1*	31.8
91	Heera	57.9	87.3	8.5	8.6	4.8	27.8	10.1*	1.1	6.8	75.6*	322.9	18.7	1.3	56.8	17.9	8.3	1.8	4.5	5.7	1.6	3.4	62.5	25.0
92	Vanaprava	69.9	99.3	6.5	6.6	3.8	63.2*	7.0	1.1	4.8	113.6*	279.9	21.7	2.2	71.8	25.8*	9.4*	2.0	4.5	7.0*	1.9*	3.6*	67.1	28.4
93	N 22	65.2	98.2	2.5	2.0	2.5	48.9*	6.0	1.0	1.0*	103.5*	334.5	20.5	1.2	50.5	15.2	6.0	1.9	3.0	5.5	1.8	2.9	85.5*	26.8
94	Vandana	64.0	91.7	2.7	2.7	4.0	40.1	9.2	1.0	5.0	117.5	384.0	22.3	2.0	90.5	21.9	8.6	2.0	4.1	6.0	1.8	3.3	73.7	34.8
95	Khandagiri	64.5	95.5	8.0	7.5	7.5	38.1	7.7	3.5	4.5	86.2*	400.7*	21.3	1.0	64.7	21.9	8.0	1.5	5.2*	7.0*	1.4	4.9*	58.5	27.9
96	Mandakini	75.0	104.0	5.0	5.5	7.5	40.2	9.0	1.0	5.0	115.2	314.5	23.2	1.1	62.7	25.9*	8.6	2.0	4.1	7.4*	1.9*	3.7*	73.7	29.4
Grand mean		69.3	99.2	5.1	4.8	4.4	40.9	9.4	2.1	4.2	114.5	314.8	22.1	1.5	65.1	20.7	8.1	2.0	4.1	5.7	1.7	3.2	70.7	28.5
CD		6.6	4.5	1.8	1.6	1.5	1.2	0.3	1.4	1.4	3.2	10.3	1.2	0.2	5.9	0.9	0.2	0.1	0.4	0.06	0.09	0.2	2.2	1.4

\*- significant at P<sub>0.05</sub> and P<sub>0.01</sub> respectively, Best check-Vandana.

(% of plants recovered due to irrigation just after a period of drought stress) and leaf drying which were scored on 0-9 scale as recommended by IRRI. Saria-B and CR Dhan 40 (Table 5) were identified to have high degree of drought tolerance compared to the best check variety Vandana based on leaf rolling score (LRS). Whereas, Biramani, Dhanisaria, Padarabank, Saria B, CR Dhan 40, Kalakeri and Browngora were found highly tolerant to drought stress based on drought recovery score (DRS) and Kharkoili, Chinger-1, Dengabari, Khursudi, Kinari, Dular, Kusuma, Damaraphuli, Chinger-2, Saria-B, Kalakeri, Browngora and Salampikit sustained drought tolerance based on leaf drying score (LDS) (Table 6). Considering above three criteria/parameters for assessment of drought tolerance, Saria-B, Kalakeri, Browngora, CR Dhan 40 and N22 were worth to have high degree of drought tolerance. Varieties screened were noted to be different depending upon the parameter used for selection of genotypes for drought tolerance. The differential response of rice varieties might be due to different mechanisms they harbour to combat the limited water stress condition. For instance, rice varieties which scored tolerant based on leaf rolling score, might have a strong mechanism to restrict transpiration loss of water plausibly by equipped functioning of guard cells. Similarly, varieties responded well for drought recovery might have efficient root characteristics while, those scored tolerant for leaf drying might have efficient mechanism for stability for synthesis of chlorophyll in the green foliage.

**c) Physiological traits:**

Physiological parameters pertaining to drought stress e.g., leaf area and chlorophyll index (measured by chlorophyll meter) were assessed in present set of materials. Very high leaf area ( $\geq 60\text{cm}^2$ ) was recorded in case of Sanarasi, followed by Pugakal, Karanga, Ambajhuka, Haladigundi, Padarabank, while, chlorophyll index was shown to be significantly high in case of Kantadumer, Bastul, Litipiti, Dal, Jhulipuagi and Setka-1 (Table 5). It is worth to note that the genotypes recorded high in leaf area did not have high chlorophyll index. Considering both leaf area and chlorophyll index, Kinari and Setka-1 had significantly higher value which is required for efficient synthesis of photosynthates under water stress.

**Table 6. Promising upland land races of rice in relation to specific stable morphological traits**

Characters	Range	Promising upland rice Genotypes
<b>Days to 50% flowering</b>	48.9-106.9	Early flowering : Setka-1, Danisaria, Podasankara, Dangaradhan, Bhogi, Dasaharadhan, Paradhan, Nandigiri, Dhobasaria , Dhalashree –B, Saria-B, Kapaanthi, Zhu 11-26
<b>Days to maturity</b>	78.3-135.3	Early maturity :Setka-1, Danisaria, Podasankara, Dangaradhan, Bhogi, Dasaharadhan, Paradhan, Nandigiri, Dhalashree –B, Saria- B, Zhu 11-26
<b>Leaf rolling</b>	0.5-9.3(0-9 scale)	Saria-B, CR Dhan 40
<b>Drought Recovery</b>	0.6-9.4(0-9 scale)	Biramani, Saria-B , Browngora ,CR Dhan 40, Kalakeri
<b>Leaf drying</b>	0.8-8.6(0-9 scale)	Kharkoili, Chinger-1, Dengabari, khursudi, Kinari, Dular, Kusuma, Damaraphuli, Chinger-2, Ambajhuka, Saria-B, Kalakeri, Browngora, Salampikit
<b>Leaf area (cm<sup>2</sup>)</b>	18.0-75.0	Setka-1, Kharkoili , Kuliha, Hiran , Ninibudhi, Badi, Baspatri, Kusuma, Pugukal, Butasori ,Kinari, Dular, Dengabari Rasakadali, Setka-2, Damaraphuli, Sarian, Barei, Karanga, Kenduphula, Malkadua, Ambajhuka, Kunor, Haladigundi, Asumakunda, Sanarasi, Dasaharadhan, Kandasuri, Padarabank, Dhalashree –B, Dhobasaria, N 22, Vanaprava , Saria, Browngora, Kapaanthi, Hiran, Saria-B
<b>Chlorophyll Index</b>	1.3-15.7	Kantadumer , Bastul , Biramani , Setka-1 , Kharkoili , Kuliha , Chinger-1 , Kusuma , Hiran , Ninibudhi , Danisaria , Setka-2 Kahnei , khursudi , Kinari , Dular , Kusuma , Podasankara , Merlo , Pankapota , Baspatri , Karni , Harisankar, Damaraphuli , Dangaradhan , Kalakusuma , Sarian , Dal , Jhulipuagi ,Litipiti , Bhogi , Barei , Kutiarasi , Karanga , Kenduphula , Ambajhuka , Kunor , Jhitipiti , ,Anjali , Annada , Heera , Zhu 11-26
<b>Zinc tolerance</b>	0.6-8.6(0-9 scale)	Kusuma , Sanarasi , Dasaharadhan , Kutiarasi , Karanga , Kenduphula , Ambajhuka , Kunor , Padarabank , Jhitipiti , Ras , Pora
<b>BLB tolerance</b>	0.8-9.3(0-9 scale)	Koliha , Bastul , Chinger-1 , Ninibudhi , Dangarchudi , Danisaria , Baspatri , Karni , Kahnei , Dular , Setka-2 , Merlo , Rasakadali , Kusuma , Chinger-2 , Kalakusuma , Kapaanthi , Litipiti , Barei , Pora , Karanga , Malkadua , Ambajhuka Haladigundi , Mahulakunchi , Somo , Paradhan , Kanding , Nandigiri , Dhobasaria , Saria-B , Hiran , Dal , Pustak CR 143-2-2 , N 22 , CR Dhan 40 , Kalakeri , Safri -17 , IR 20 , Anjali , Browngora , Salampikit , Sadabahar , Sahbhagi Dhan , Saria
<b>Plant height</b>	88.1-138.1	Dwarf plant types: Khandagiri, Setka-2, Dhobasaria, Malkadna, Bhogi, and Jhulipuagi Tall plant types: Ninibudhi, Badi, Harisankar, Pandeydhan, Khursudi, Kinari, jhirkabanji, Merlo, Barei, Pora, Ambajhuka, Padarabank, kandig, Asumakanda and Hiran Intermediate plant types : Rest of the genotypes
<b>Ear bearing tillers</b>	180.4-461.9	More no of tillers : Khandagiri , Harisankar , Kahnei , Kinari , Merlo , Jhulipuagi , Barei , Kutiarasi , Hiran , CR 143-2-2 , Ninibudhi , Badi , Rangahazari
<b>Panicle length(cm.)</b>	15.0-28.0	Long panicle length : Kantadumer , Kharkoili , Sadabahar , Kusuma , Hiran , Lalubadikaberi , Pandeydhan , Dular , Merlo , Rasakadali , Damaraphuli , Biramani , Kalakusuma , Sarian , Haladigundi , Padarabank , Mahulakunchi , Somo , Kanding , Asumakunda , Mahularani , Pustak , Safri -17 , Anjali, Kuliha

Characters	Range	Promising upland rice Genotypes
Panicle weight(g)	0.5-3.7	Higher panicle weight : Salampikit , Lalubadikaberi , Dular , Pankapota , Sarian , Padarabank , Somo , Asumakunda , Nandigiri , Dhobasaria , Kapaanthi , Pustak , CR Dhan 40 , Anjali , Kantadumer
Grains/Panicle	31.6-149.3	More no. of grains per panicle : Dhobasaria , Anjali , Dular , Pankapota , Sarian , Padarabank , Nandigiri , Asumakunda , Pustak , CR DHAN 40 , Lalubadikaberi
1000-Grain weight(g)	11.5-29.4	Kantadumer , Setka-1 , Kuliha , Chinger-1 , Danisaria , Dangaradhan , Kalakusuma , Sarian , Karanga , Haladigundi , Mahulakunchi , Somo , Paradhan , Kandasuri , Asumakunda , Nandigiri , Dhobasaria , Dhalashree –B , Saria-B , Mahularani , Kapaanthi , CR Dhan 40 , Mandakini , Anjali , Browngora , Salampikit , Saria , Annada , Zhu 11-26 , Vanaprava , Kalakeri
Grain length(cm.)	5.9-9.5	Lalubadikaberi , Kahnei , Jhulipuagi , Karanga , Dasaharadhan , IR 87707-445-B-B
Grain breadth(cm.)	1.3-2.9	Saria , Safri -17 , Pustak , Kapaanthi , Dhalashree –B , Dhobasaria , Nandigiri , Kandasuri , Paradhan , Somo , Mahulakunchi , Barei , Sarian , Chinger-2 , Setka-2 , Lalubadikaberi , Kantadumer , Bastul , Koliha
Grain length/Grain breadth	2.5-5.9	Setka-1 , Hiran , Ninibudhi , Badi , Rangahazari , Kahnei , Dular , Damaraphuli , Dal , Ambajhuka , Kunor , Sanarasi , Sadabahar , Khandagiri , Sahbhagi dhan
Kernel length(cm.)	4.1-7.4	Kahnei , Kantadumer , Ninibudhi , Mandakini , Lalubadikaberi , Pandeydhan , Jirkubanji , Dular , Jhulipuagi , Karanga , Padarabank , Jhitipiti , Dasaharadhan , Kandasuri , Asumakunda , Hiran , IR 87707-445-B-B , Kalakeri , Safri -17 , Browngora , Vanaprava , Khandagiri , Badi
Kernel breadth(cm.)	1.0-2.1	Koliha , Kantadumer , Bastul , Kuliha , Kusuma , Lalubadikaberi , Setka-2 , Sarian , Barei , Haladigundi , Mahulakunchi , Somo , Paradhan , Kandasuri , Nandigiri , Dhobasaria , Dhalashree –B , Saria-B , Mahularani , Kapaanthi , Pustak , CR 143-2-2 , CR Dhan 40 , Kalakeri , Anjali , Browngora , Salampikit , Saria , Mandakini , Zhu 11-26 , Vanaprava , Sneha
Kernel length/Kernel breadth	2.2-5.2	Setka-1 , Mandakini , Ninibudhi , Badi , Rangahazari , Jirkubanji , Dular , Damaraphuli , Jhulipuagi , Ambajhuka , Dasaharadhan , Bhatasakli , Asumakunda , Sanarasi , Sadabahar , Vanaprava , Khandagiri , Hiran
Fertility(%)	20.3-95.9	Koliha , Kantadumer , Bastul , Hiran , Ninibudhi , Danisaria , Pugukal , Pandeydhan , khursudi , Kinari , Dular , Pankapota , Biramani , Dangaradhan , Sarian , Dal , Bhogi , Karanga , Kenduphula , Kunor , Haladigundi , Padarabank , Somo , Paradhan , Kandasuri , Kanding , Asumakunda , Nandigiri , Dhobasaria , Dhalashree –B , Saria-B , Hiran , Pustak , CR 143-2-2 , N 22 , Saria , Zhu 11-26 , Sahbhagi dhan
Seed yield (qtl/ha)	14.7-36.6	Khursudi , Asumakunda

**Table 7. Estimates of variability for seed yield and its component traits.**

Estimates of variability	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	SY
Mean	69.3	99.2	5.1	4.8	4.4	40.9	9.4	2.1	4.2	114.5	314.81	22.1	1.5	65.1	20.7	8.1	2.0	4.1	5.7	1.7	3.2	70.7	28.5
Range	48.9-106.9	78.3-135.3	0.5-9.3	0.6-9.4	0.8-8.6	18.0-75.0	1.3-15.7	0.6-8.6	0.8-9.3	88.1-138.1	180.4-461.9	15.0-28.0	0.5-3.7	31.6-149.3	11.5-29.4	5.9-9.5	1.3-2.9	2.5-5.9	4.1-7.4	1.0-2.1	2.2-5.2	20.3-95.9	14.7-36.6
Phenotypic variance	104.1	100.9	5.6	6.7	3.6	153.1	6.2	3.4	2.6	207.7	5934.8	8.1	0.5	722.4	17.7	0.6	0.06	0.33	0.36	0.05	0.27	265.9	36.9
CV (%)	14.7	10.1	46.8	53.6	42.8	30.2	26.5	86.5	38.5	12.5	24.4	12.8	49.6	41.3	20.3	9.4	11.8	14.0	10.4	12.2	15.9	23.0	21.2
CD <sub>5%</sub> between two check variety means	3.7	2.5	1.0	0.9	0.8	0.6	0.2	0.7	0.7	1.8	5.8	0.6	0.1	3.3	0.5	0.1	0.08	0.2	0.03	0.05	0.12	1.25	0.83
CD <sub>5%</sub> between adjusted values of two test varieties in the same block	7.5	5.1	2.1	1.8	1.7	1.3	0.4	1.5	1.5	3.7	11.6	1.3	0.2	6.7	1.0	0.2	0.1	0.4	0.07	0.1	0.2	2.5	1.6
CD <sub>5%</sub> between adjusted values of two test varieties in different blocks	8.3	5.8	2.4	2.0	1.9	1.5	0.4	1.7	1.7	4.1	13.0	1.5	0.3	7.5	1.1	0.3	0.1	0.5	0.08	0.1	0.2	2.7	1.8
CD <sub>5%</sub> between adjusted values of a test variety and a check variety mean	6.6	4.5	1.8	1.6	1.5	1.2	0.3	1.4	1.4	3.2	10.3	1.2	0.2	5.9	0.9	0.2	0.1	0.4	0.06	0.09	0.2	2.2	1.4

**d) Tolerance to nutritional stress:**

Among the nutritional stresses, zinc deficiency many often become a serious problem. Zinc deficiency was scored on 0-9 scale at early seedling stage (within 10-12days). Vandana-the best check variety was tolerant to zinc stress but susceptible to BLB (to be discussed later). Among the test genotypes, Kutiarasi, Kusuma, Pora, karanga, Kenduphula, Ambajhuka, Kunor, Padarabank Jhitipiti, Raas, Sanarasi and Dasaharadhan had shown significantly high degree of tolerance to zinc deficiency even compared to Vandana (Table 5).

**e) Tolerance to diseases:**

Among the diseases, bacterial leaf blight (BLB) is a serious problem in upland rice cultivation. Most of the test genotypes succumb to BLB infestation. Among the check varieties, N22 scored tolerant to BLB while Vandana was highly sensitive. In the present investigation, Salampikit and N22 were identified to be nearly free from BLB (0.8-1.0 score) whereas, almost all other genotypes showed moderate to high susceptibility (Table 5).

**f) Morpho-economic traits:**

Varieties with plant height around 100-110cm. is usually suitable for drought stress upland condition. Three popular check varieties included under study e.g., N22, Vandana and Mandakini had moderate plant height(around 115cm.) whereas, Khandagiri, was significantly shorter in height (86.2cm.) as compared to the best standard check variety Vandana(Table 5).

. Among the test genotypes, Bastul, Setka-2, Dhubasaria, Malkadna, Jhulipuagi and Bhogi had shown plant height around 90-95cm. In contrast, Ninibudhi, Badi, Harishankar, Pandeydhan, Khursudi, Kinari, Jhirkabanji, Merlo, Barei, Pora, Ambajhuka, Padarabank, Kanding, Asumakunda and Hiran exhibited tall stature (>130cm.) which may not be suitable for upland situation. Rest of the test genotypes had recorded moderate plant height.

Kutiarasi, Badi, Hiran, Kinari and CR Dhan 143-2-2 revealed significantly high tillering ability(around 500/m<sup>2</sup>) among the test entries as compared to Vandana. Besides, Harishankar, Kahnei, Merlo, Jhulipuagi and Barei had shown high tillering ability. Panicle features including length, weight, compactness and grains per panicle seem to be

important factors for high productivity. Merlo, Rasakadali and Anjali excelled in panicle length ( $\geq 27.0$ cm.) among the test genotypes under study. Sarian, Padarabank, Asumakunda, Dhubasaria, Pustak, CR Dhan 40 and Anjali had shown significantly higher panicle weight ( $\geq 3.0$ g). In this context, some of the land races e.g., Pankapota, Padarabank and Pustak had significantly very high number of grains per panicle.

Grain dimension determines the test weight of a genotype. A few of the test genotypes e.g., Kantadumer, Dhubasaria, Saria and Kandasuri in the present pursuit had bold grains and therefore, recorded significantly high 1000-grain weight (28-29g) among the test genotypes.

Flowering to grain filling seems to be a critical stage for upland rice for recovery of high grain yield under drought stress. Therefore, genotypes with high grain fertility status under drought stress is an important criterion for screening of genotypes for drought tolerance. Among the test genotypes, Khursudi, Dular, N22, Asumakunda, Pustak, Kandasuri, kanding, Dhanisaria, Nandigiri, Dhubasaria, Dhalashree-B and CR 143-2-2 had recorded significantly high grain fertility percentage even more than 90% and each of them excelled over the best standard check variety Vandana.

Grain yield of a genotype is an artifact which resulted from a combination of yield contributing ancillary traits and it depends upon the interaction with ecological/environmental condition under which the genotype is grown. Genotypes with combination of desirable ancillary traits along with drought tolerance would certainly excel in productivity under drought stress. In the present investigation, the overall mean grain yield/ha revealed a spectacular wide array of variation ranging from 14.7 to 36.6q/ha among the test genotypes. In the present pursuit, Asumakunda and Khursudi performed well with significantly high grain yield ( $>36.0$ q/ha) than the best standard check variety Vandana.

#### **g) Quality traits:**

Besides, test weight; the grain dimension seems to be important criteria for determining physical quality traits. In the present investigation, grain and kernel dimension with regard to length, breadth and length/breadth ratio have been studied to sort out a few test genotypes for acceptable quality traits. Kernel dimension is important for consumer's preference. Genotype with kernel length greater than 7.0 and L/B ratios

greater than 3.5 usually attracts consumer's preference and fetch market value. Lalubadikaberi, Kahnei, Jhulipuagi, Karanga, Dasaharadhan, IR 87707-445-B-B, Vanaprabha and Khandagiri recorded high grain and kernel length. Grain length/grain breadth and kernel length/kernel breadth give a an idea of relative slenderness of grain and kernel of a genotype. In this context, Hiran, Rangahazari, Setka-land Damaraphuli had shown higher value for GL/GB as well as KL/KB(**Table 5**).

### **Genetic variability**

Variation and selection are the two basic requirements of genetic improvement in any crop. Without variation, selection becomes ineffective. Therefore, knowledge on the extent of genetic variability with regards to various traits and their nature of transmission to the succeeding generations is indispensable for reliable selection.

The present set of upland rice genotypes revealed a wide range of variation for all agro-economic traits including drought tolerance, physiological traits, zinc tolerance and tolerance to BLB. The overall mean value, range, phenotypic variance, co-efficient of variability and critical differences(CDs) between two check variety means, between adjusted value of a test variety & a check variety mean, between adjusted values of two genotypes in the same block and that in different blocks have been presented in **Table 7**. The range of each agro-economic traits was tremendously high. The phenotypic variance may not give useful information for comparison among agro-economic traits as it is not unit free. However, co-efficient of variation which is expressed in percentage may be suitably used for comparison and selection of trait(s) with high variability. In the present pursuit, 9.4% in case of grain length to 86.5% in case of zinc tolerance. Among the agro-economic traits, all the three drought tolerance parameters (LRS, DRS and LDS), zinc tolerance, BLB tolerance, panicle weight and grains per panicle had shown high CV ( $\geq 40\%$ ). Hence, there could be tremendous scope for selection of drought tolerant genotypes with desirable yield contributing traits( panicle weight & grains/panicle) along with zinc tolerance and tolerance to BLB. In the present investigation, Vandana yielded highest among the check varieties. Hence, Vandana was considered best standard check variety. Significant difference of adjusted mean values of a test genotype from that of Vandana was therefore, considered for varietal comparison.

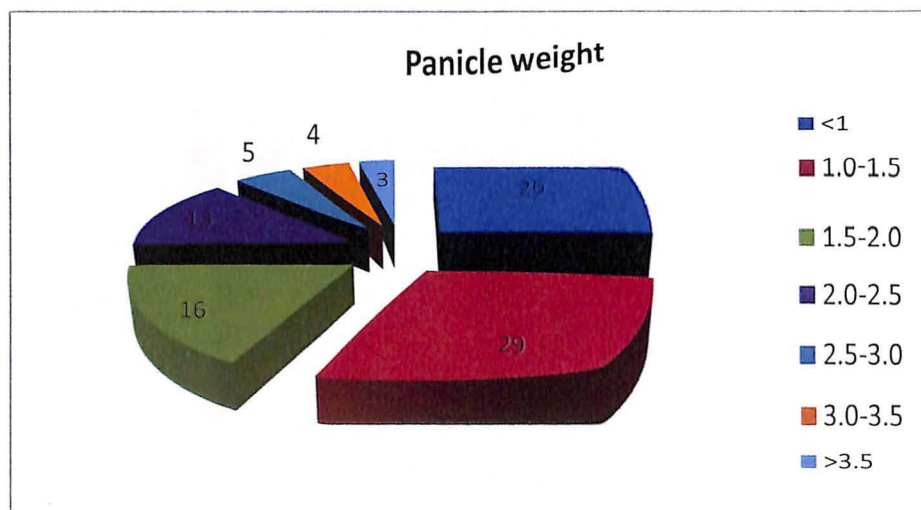
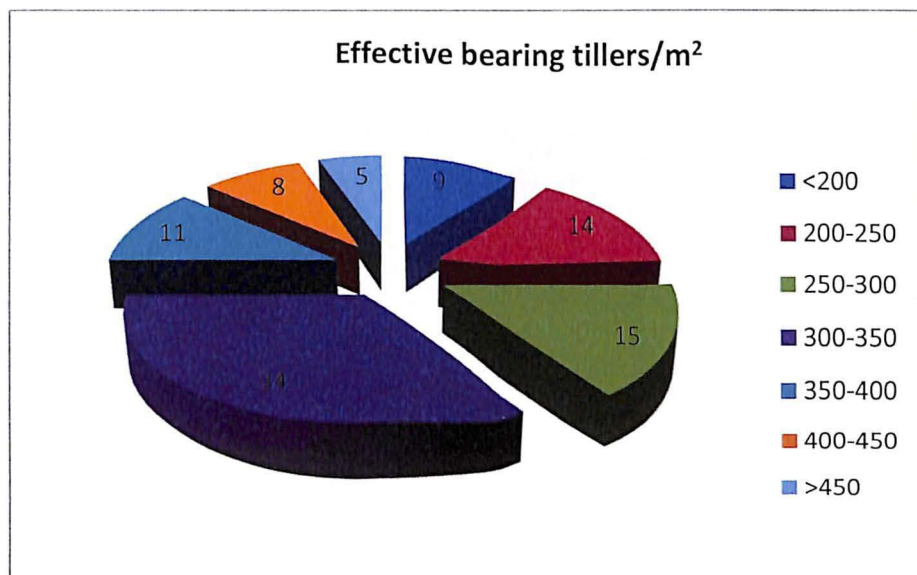
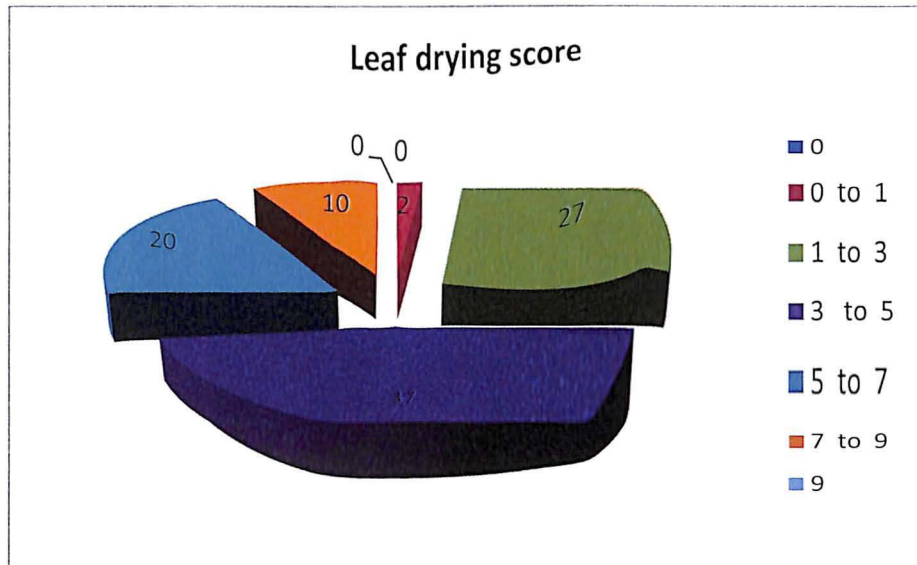
Gomathinayagam *et al* (1990) studied the genetic variability in 40 upland rice genotypes. The coefficient of variation was high for number of effective tillers per plant, grain yield per plant and weight of roots. High heritability estimate and genetic advance were observed for the characters which may be considered reliable for selection under rain fed condition. Sharma *et al.* (1990) measured awning, pigmentation, length: breadth (L/B)ratio and 1000-grain weight in 43 traditional rice varieties. Grain length ranged from 5.7 to 9.9 mm, breadth from 1.8-3.0 mm and L/B ratio from 2.4 to 4.3. A range of 8.4 to 25.5g was recorded for 1000-grain weight. Rao (2000) observed highest genetic variation in grain yield followed by percentage of filled spikelets in very early rice genotypes under upland condition. High heritability estimates coupled with high genetic advance was also observed for 1000-grain weight suggesting the dominant role of additive gene effects in expression of the trait.

Frequency distribution of test genotypes in different class intervals is presented in **Table 8 and Fig. 1-4**. Only two genotypes e.g., Kinari and Chinger -2 out of 96 test entries showed high degree of drought tolerance based on leaf drying score. Five genotypes (Harisankar, CR Dhan 143-2-2, Kinari, Hiran, Badi and Kutiarasi) for EBT/m<sup>2</sup> and three varieties each for panicle weight (>3.5g, (Sarian, Dhobasaria, and Anjali) and grains per panicle(>140, Padarabank, Pustak and Pankopoot) were shown to occur in the extreme boundary in the frequency distribution. Similarly, a few test genotypes had shown excellent performance for grain weight and quality traits. For instance, a single land race Chinger -2 was shown to have very bold grains(GB=>2.75mm) and three popular upland rice genotypes e.g., Khandagiri, Vanaprabha and Mandakini revealed kernel length even more than 7mm. However, in the present set of germplasm, twenty three genotypes had recorded >85% grain fertility percentage among which Dhanisaria and Dhobasaria revealed highest fertility status(>95%). With regard to productivity, eight test genotypes e.g., Pandeydhan, Khursudi, Haladigundi, Asumakunda, Dhobasaria, CR 143-2-2, CR Dhan 40 and Sahbhagidhan were placed in the extreme class interval with grain yield  $\geq 35.0$ q/ha. The above genotypes sorted out by way of frequency distribution, may serve as valuable material for genetic improvement of upland rice through recombination breeding.

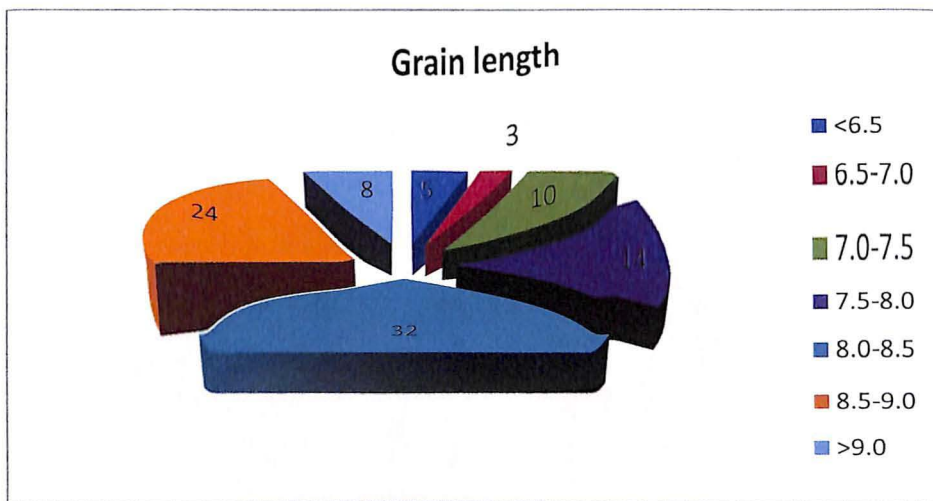
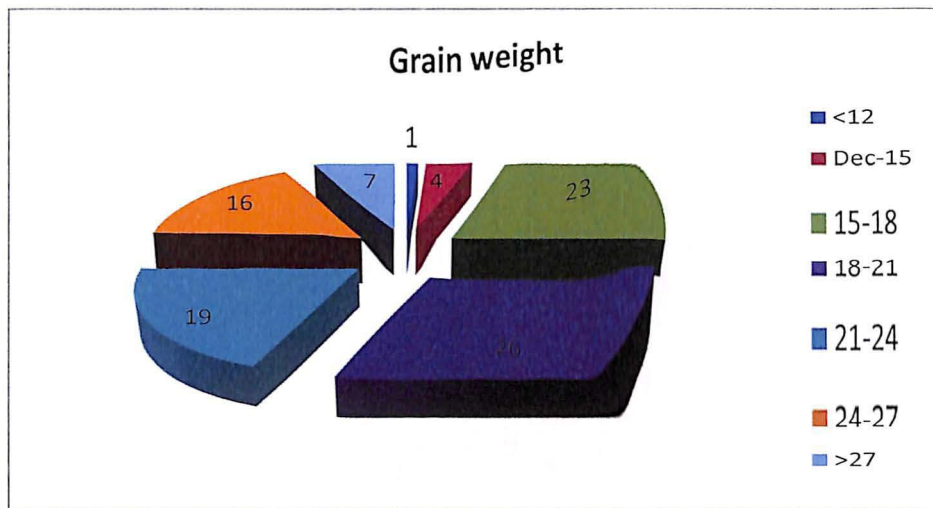
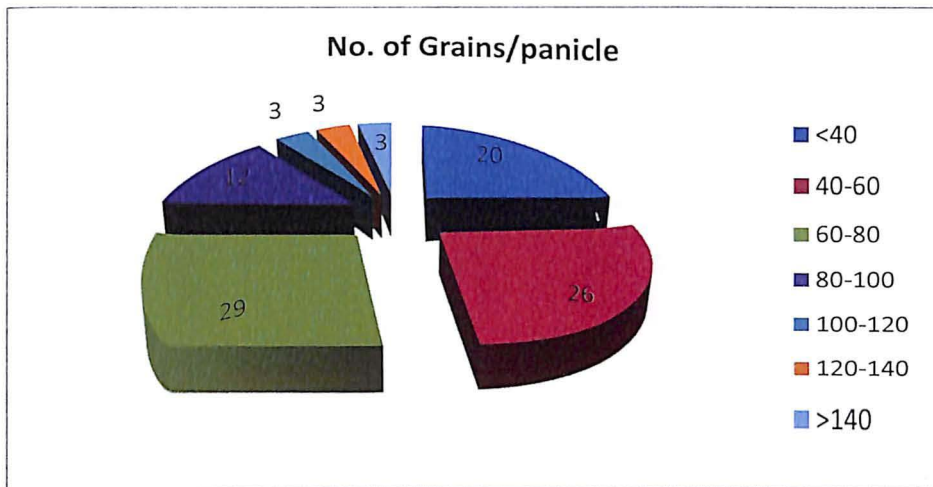
**Table 8. Frequency distribution of 96 germplasm lines in different class intervals.**

LDS		EBT		PW		G/P		GW		GL		GB		KL		KB		F%		SY	
C.I.	f	C.I.	f	C.I.	f	C.I.	F	C.I.	f	C.I.	f	C.I.	f	C.I.	f	C.I.	f	C.I.	f	C.I.	f
0	0	<200	9	<1	26	<40	20	<12	1	<6.5	5	<1.50	3	<4.5	4	<1.0	0	<35	3	<15	2
0-1	2	200-250	14	1.0-1.5	29	40-60	26	12-15	4	6.5-7.0	3	1.50-1.75	6	4.5-5.0	3	1.0-1.2	2	35-45	5	15-20	13
1-3	27	250-300	15	1.5-2.0	16	60-80	29	15-18	23	7.0-7.5	10	1.75-2.00	3	5.0-5.5	19	1.2-1.4	3	45-55	7	20-25	7
3-5	37	300-350	34	2.0-2.5	13	80-100	12	18-21	26	7.5-8.0	14	2.00-2.25	4	5.5-6.0	40	1.4-1.6	10	55-65	16	25-30	28
5-7	20	350-400	11	2.5-3.0	5	100-120	3	21-24	19	8.0-8.5	32	2.25-2.50	9	6.0-6.5	22	1.6-1.8	28	65-75	21	30-35	38
7-9	10	400-450	8	3.0-3.5	4	120-140	3	24-27	16	8.5-9.0	24	2.50-2.75	1	6.5-7.0	5	1.8-2.0	33	75-85	21	>35	8
9	0	>450	5	>3.5	3	>140	3	>27	7	>9.0	8	>2.75	1	>7.0	3	>2.0	20	>85	23	-	-

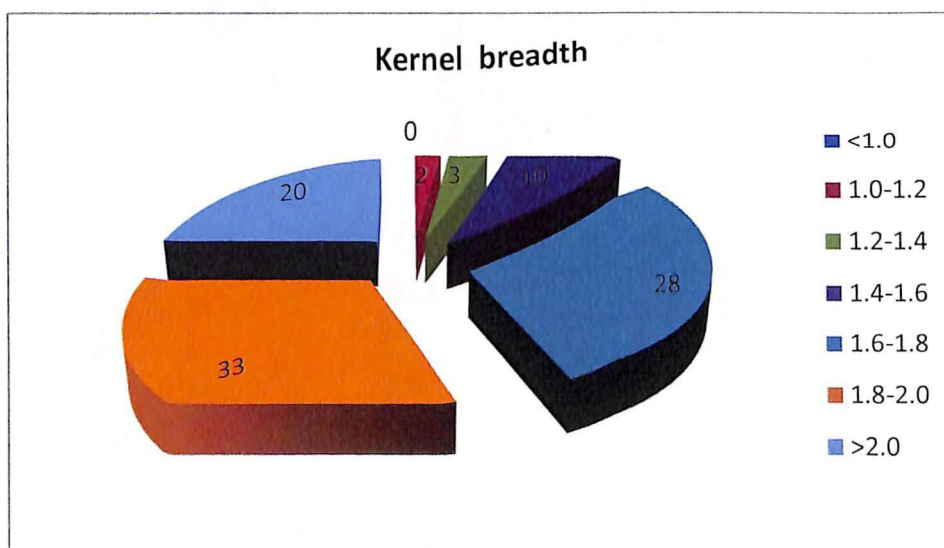
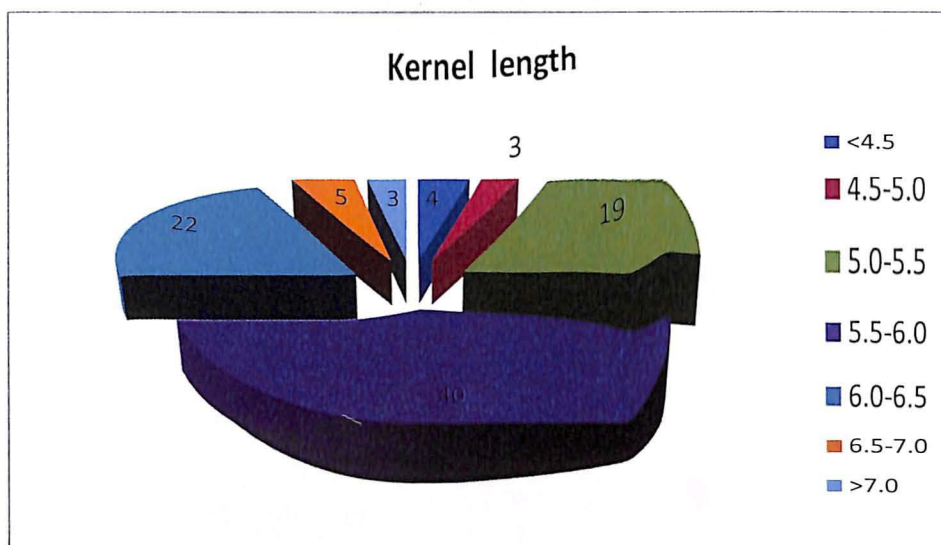
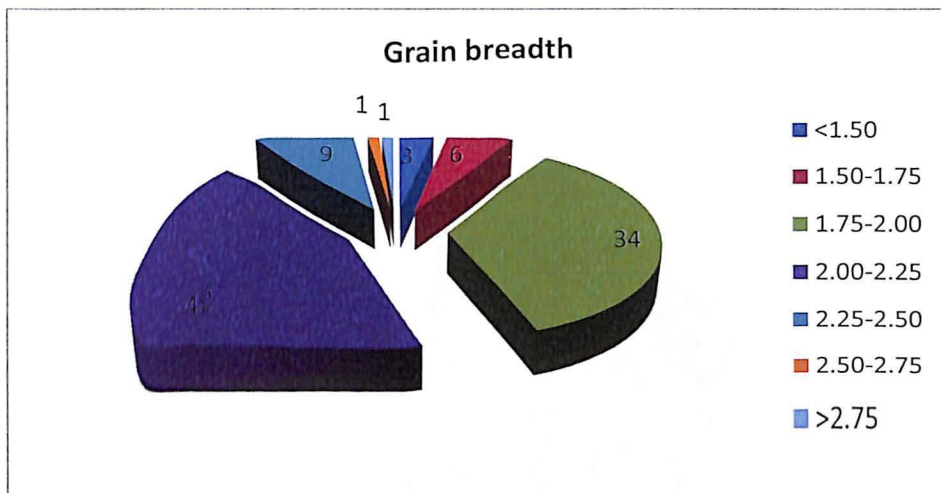
**N.B.: C.I.- Class interval      f- Frequency of genotypes in the class interval**



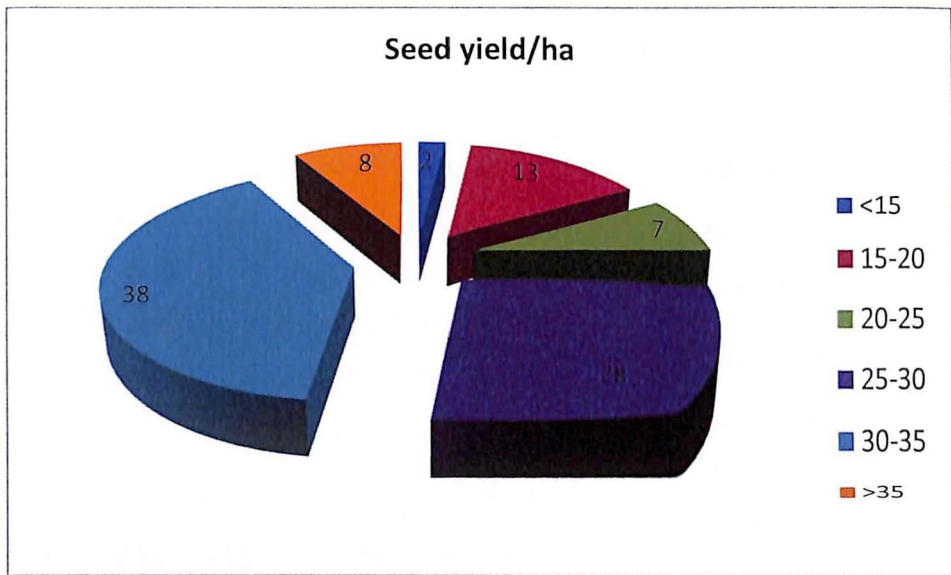
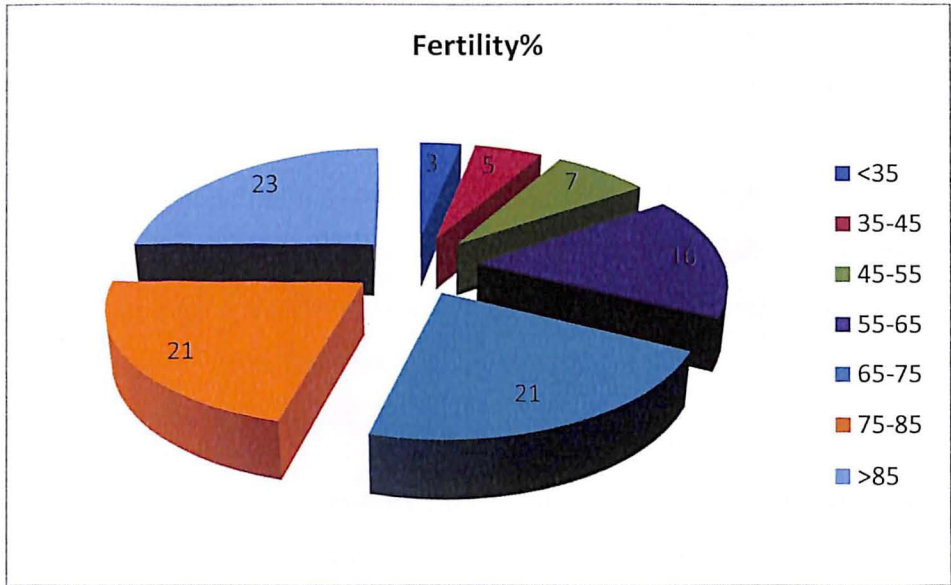
**Fig 1.** Frequency of genotypes for leaf drying score, effective bearing tillers/m<sup>2</sup> and Panicle weight in different class intervals.



**Fig 2. Frequency of genotypes for no. of grains/panicle, grain weight and grain length in different class intervals.**



**Fig 3. Frequency of genotypes for grain breadth, kernel length and kernel breadth in different class intervals.**



**Fig 4. Frequency of genotypes for fertility percentage and seed yield/ha in different class intervals.**

### **Character association of yield and yield attributing characters:**

Estimation of phenotypic correlations between grain yield and component characters as well as *inter se* association provides information for choice of characters in selection programme. The strength of character association as measured by estimates of co-efficient of correlation depends upon the composition of the test materials, characters studied, previous selection history and the environment under which the breeding materials were tested. A perusal of **Table-9** indicated phenotypic correlation co-efficients between agro-economic traits including drought tolerance parameters, physiological traits, tolerance to nutritional stresses and BLB.

In the present investigation, number of ear bearing tillers/m<sup>2</sup>, panicle weight, grains/panicle, 1000-grain weight, grain length, kernel length, kernel breadth and fertility percentage correlated significantly with grain yield/ha. All these component traits except 1000-grain weight recorded significant value at even 1% level of significance. Among these traits, the strength of positive association of number of ear bearing tillers/m<sup>2</sup> ( $r = 0.595^*$ ) and panicle weight ( $r = 0.485^{**}$ ) and fertility percentage ( $r = 0.458^{**}$ ) with grain yield were very high indicating their importance for genetic improvement of productivity in upland rice. The latter two component traits were also shown to have very high *inter se* significant positive correlation ( $r = 0.476^{**}$ ) at even 1% level of significance. Therefore, selection for any one of these characters automatically selects the other trait and thus, together could result recovery of high grain yield. It was worth to note that the said two component traits had strong negative association with days to 50% flowering and days to maturity. This suggests that there is ample scope for selection of early maturing genotypes with high panicle weight and grain fertility status. The merit of these two characters is further ascribed to their positive correlated response through other component traits e.g., grains/panicle, 1000-grain weight, and kernel breadth. Besides, grains per panicle and 1000-grain weight had also shown significant negative correlation with the above flowering and maturity traits indicating more scope for selection of early maturing plant types with high grain number and bold seeds. Basavaraja *et al.* (1997) stressed the importance of panicle weight for selection of grain yield. Nath and Talukdar (1997) reported significant association of number of grains per panicle with grain yield.

**Table 9. Phenotypic correlation between agro-economic traits in a set of 96 upland genotypes of rice.**

CHAR.	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%
DM	0.994**																					
LRS	-.027	-.049																				
DRS	-.003	-.025	0.963**																			
LDS	-.153	-.167	0.494**	0.549**																		
LA	-.136	-.125	0.082	0.069	0.00																	
CI	0.014	-.013	.085	.054	.016	-.151																
ZTS	-.426**	-.429**	.242*	0.195	.210*	.045	-.065															
BLB	-.118	-.153	.185	.146	.008	-.043	.211*	-.086														
PHT	.297**	.289**	.005	-.036	-.207*	.158	-.067	-.153	.008													
EBT	.160	.157	-.046	-.088	-.130	-.176	-.001	-.062	-.023	.158												
PL	.040	.029	.012	.024	-.077	-.015	.000	.041	-.041	.159	-.317**											
PW	-.334**	-.330**	-.075	-.046	-.059	.080	-.114	.202*	-.133	-.027	-.106	.459**										
G/P	-.331**	-.327**	-.054	-.003	.034	.118	-.141	.157	-.110	-.066	-.193	.468**	.825**									
GW	-.328**	-.324**	.049	.037	.110	.164	-.079	.290**	-.234*	-.021	-.233*	.231*	.523**	.288**								
GL	-.026	.039	.077	.086	.011	.169	-.084	-.072	-.016	-.035	.073	.061	.159	.012	.377**							
GB	-.283**	-.293**	-.086	-.049	-.016	-.144	-.106	.148	-.155	-.085	-.113	.199	.369**	.272**	.337**	.141						
GL/GB	.246*	.247*	.102	.083	-.007	.205*	.048	-.191	.111	.024	.130	-.128	-.227*	-.230*	-.052	.522**	-.746**					
KL	.009	-.003	.114	.136	.083	.139	-.229*	-.099	-.060	-.012	.125	.071	.176	.058	.358**	.825**	.197	.373**				
KB	-.355**	-.360**	-.012	.009	.113	-.082	-.149	.215*	-.150	-.106	-.032	.116	.407**	.294**	.389**	.148	.832**	.675**	.210*			
KL/KB	.291**	.290**	.076	.073	-.023	.178	-.017	-.213*	.098	.057	.111	-.061	-.235*	-.228*	-.070	.419**	-.609	.866**	.473**	-.734**		
F%	-.375**	-.369**	.096	.105	.097	.239*	.014	.283**	-.108	.032	.014	.046	.476**	.512**	.367**	.137	.171	-.083	.126	.245*	-.133	
Seed Yield	-.018	-.019	.042	.051	.059	-.021	-.059	.116	-.228*	.126	.595**	.072	.485**	.356**	.249*	.336*	.127	.075	.339**	.262**	.001	.458**

However, Mishra and Verma (2002), Goswami *et al.* (2000), Choudhury and Motiramani (2003) observed significant association of number of effect bearing tillers per plant with grain yield. Fertile grains per panicle registered positive significant association with panicle length (Gupta *et al.*1999), number of productive tillers per plant (Satish *et al.*2003) and total spikelets per panicle. Goswami *et al.*(2000) observed positive association between panicle number per unit area, grain numbers per panicle and filled grain percent with grain yield per plant.

Plant height seems to have significant positive correlation with flowering and maturity traits. But each of these seems to have no association with grain yield. Besides, plant height maintained no significant correlation with any of the yield contributing traits indicating that there is less likely to improve seed yield through selection for comparatively reduced culm length.

#### **Character association of quality traits:**

In the present investigation, efforts have been taken to study and implicate the relationship of some easily observable physical quality traits with yield. In the present investigation, grain and kernel dimension were assessed in terms of length, breadth and length/breadth ratio. Among these physical quality traits, Kernel length and kernel breadth had strong positive relationship with grain yield at even 1% level of significance(**Table 9**). While, grain length correlated positively with grain yield at 5% level of significance. Slender kernel types attract consumer's preference. But, such genotypes in general, are observed to have low yield potential. Length and breadth of grain and kernel individually revealed significant positive association with 1000-grain weight. Breadth of grain and kernel had positive significant relationship with panicle weight. Besides, bold kernel type was shown to have significant positive association with grain fertility percentage which is considered as one of the most important criterion for yield improvement in upland rice. Thus, selection of long bold grain or kernel types may certainly pave the way for genetic improvement of seed yield in upland rice.

In the present investigation, kernel length exhibited significant positive correlation with grain length, grain L/B ratio and kernel L/B ratio as also reported by

Vivekanandan and Giridharan (1998). Chouhan (1996) reported significant positive association of kernel length and kernel L/B ratio but negative association with kernel breadth in some crosses of aromatic x non-aromatic varieties.

#### **Path analysis of yield and yield attributing characters:**

The correlation co-efficients of component traits with grain yield were partitioned into their direct and indirect effects on grain yield following path co-efficient analysis to ascertain further conclusive information on choice of characters required for selection of high yielding genotypes for upland condition. Number of ear bearing tillers/m<sup>2</sup> (EBT), panicle weight (PW), grain length (GL) followed by grain fertility percentage (F%) and kernel breadth (KB) exhibited high direct effects on grain yield (**Table10**) as well as significant positive association with grain yield. Whereas, kernel length had revealed negative direct effect indicating limited scope for genetic improvement of grain yield based on selection through longer kernel. It is worth to note that grains per panicle had negligible direct effect, but was shown to have high indirect effect on grain yield via panicle weight(PW). Similar was the case for kernel length which had no noticeable direct effect on grain yield, but contributed much towards high productivity indirectly via grain length. Hence, for improving grain yield, emphasis must be given for selecting high tillering plant types having heavy panicle with more number of fertile grains. Gupta *et al.* (1999) carried out path analysis and revealed that panicle weight, panicles per plant and grains per panicle were important traits influencing the grain yield. However, several other workers also reported positive direct effects of effective bearing tillers per plant on grain yield (Nayak *et al.* 2001, Singh *et al.* 2002, Choudhury and Motiramani 2003, Khedikar *et al.* 2003, Behera 2007).

Lower the score value on 0-9 scale, more is the degree of tolerance for drought and BLB infestation. It is worth to note that BLB score had significant negative correlation with grain yield. Further, the present set of materials revealed negative direct effect of these two stress related traits suggesting ample scope for selection of drought tolerant and BLB tolerant genotypes based on leaf rolling score and BLB score.

**Table 10. Path analysis for agro-economic traits in a set of 96 upland genotypes of rice.**

CHAR.	DF	DM	LRS	DRS	LDS	LA	CI	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	r(x,y)
DF	0.060	0.062	0.003	0.000	-.023	0.005	0.001	-.013	0.016	0.015	0.100	0.002	-.123	-.018	0.000	-.008	0.057	-.023	-.001	-.069	.021	-.080	-.018
DM	.060	.062	.005	-.003	-.025	.004	-.001	-.013	.020	.014	.099	.002	-.122	-.018	.000	-.012	.059	-.023	.000	-.070	.020	-.078	-.019
LRS	-.002	-.003	-.110	.116	.073	-.003	.003	.008	-.025	.000	-.029	.001	-.028	-.003	.000	.024	.017	-.010	-.012	-.002	.005	.020	.042
DRS	.000	-.002	-.106	.120	.081	-.002	.002	.006	-.020	-.002	-.055	.001	-.017	.000	.000	.027	.010	-.008	-.014	.002	.005	.022	.051
LDS	-.009	-.010	-.054	.066	.148	.000	.001	.007	-.012	-.010	-.082	-.004	-.022	.002	.000	.003	.003	.001	-.009	.022	-.002	.021	.059
LA	-.008	-.008	-.009	.008	.000	-.034	-.006	.001	.006	.008	-.111	-.001	.030	.007	.000	.053	.029	-.019	-.015	-.016	.013	.051	-.021
CI	.001	-.001	-.009	.006	.002	.005	.040	-.002	-.028	-.003	-.007	.000	-.042	-.008	.000	-.027	.021	-.005	.024	-.029	-.001	.003	-.059
ZTS	-.026	-.027	-.027	.023	.031	-.002	-.003	.031	.012	-.008	-.039	.002	.075	.009	.000	-.023	-.030	.018	.010	.042	-.015	.060	.116
BLB	-.007	-.010	-.020	.018	.013	.001	.008	-.003	-.134	.000	-.014	-.002	-.049	-.006	.000	-.005	.031	-.011	.006	-.029	.007	-.023	-.228*
PHT	.018	.018	-.001	-.004	-.031	-.005	-.003	-.005	-.001	.050	.099	.009	-.010	-.004	.000	-.011	.017	-.002	.001	-.021	.004	.007	.126
EBT	.010	.010	.005	-.011	-.019	.006	.000	-.002	.003	.008	.628	-.017	-.039	-.011	.000	.023	.023	-.012	-.013	-.006	.008	.003	.595**
PL	.002	.002	-.001	.003	-.011	.001	.000	.001	.005	.008	-.199	.054	.170	.026	.000	.019	-.040	.012	-.007	-.022	-.004	-.010	.072
PW	-.020	-.021	.008	-.006	-.009	-.003	-.005	.006	.018	-.001	-.067	.025	.369	.046	.000	.050	-.070	.022	-.019	.079	-.017	.101	.485**
G/P	-.020	-.020	.006	.000	.005	-.004	-.006	.005	.015	-.003	-.121	.025	.305	.056	.000	.004	-.055	.022	-.006	.057	-.016	.109	.356**
GW	-.020	-.020	-.005	.004	.016	-.006	-.003	.009	.031	-.001	-.146	.012	.193	.016	.001	.119	-.068	.005	-.038	.075	-.005	.078	.249*
GL	-.002	-.002	-.008	.010	.002	-.006	-.003	-.002	.002	-.002	.046	.003	.059	.001	.000	.316	-.028	-.050	-.087	.029	.030	.029	.336**
GB	-.017	-.018	.009	-.006	-.002	.005	-.004	.005	.021	-.004	-.071	.011	.136	.015	.000	.045	-.021	.071	-.021	.169	-.043	.036	.127
GL/GB	.015	.015	-.011	.010	-.001	-.007	.002	-.006	-.015	.001	.082	-.007	-.084	-.013	.000	.165	.150	-.095	-.039	-.131	.061	-.018	.075
KL	.001	.000	-.013	.016	.012	-.005	-.009	-.003	.008	-.001	.078	.004	.065	.003	.000	.261	-.040	-.035	-.105	.041	.033	.027	.339**
KB	-.021	-.022	.001	.001	.017	.003	.006	.007	.020	-.005	-.020	.006	.150	.016	.000	.047	-.167	.064	-.022	.194	-.052	.052	.262**
KL/KB	.018	.018	-.008	.009	-.003	-.006	-.001	-.007	-.013	.003	.070	-.003	-.087	-.013	.000	.133	.123	-.082	-.050	-.142	.070	-.028	-.001
F%	-.023	-.023	-.011	.013	.014	-.008	.001	.009	.014	.002	.009	.002	.176	.028	.000	.043	-.034	.008	-.013	.047	-.009	.213	.458**

**Residual effect(R) =0.437      R SQR(PC) =80.926**

### **Selection strategy:**

The knowledge of genetic variability and imposing proper selection strategy is imperative for efficient sampling and utilization of germplasm. Plant Breeders always look for combining desirable genes from diverse germplasm into a single genotype. This warrants immediate genotypic characterization followed by pre-breeding to select valuable germplasm lines resulting the development of a core germplasm collection. Morphological characterization of germplasm collections is an important step in this regard. In the present investigation, genotypes with regard to stable morphological traits have been identified and assessed for DUS characteristics. Such a preliminary knowledge would serve for better planning and use of genetic stocks in crop improvement programme. Besides, promising genotypes have been identified in relation to specific traits associated with productivity as well as biotic and abiotic stress tolerance. The productivity *per se* is a complex character. Many often direct selection based on *per se* grain yield led to missing of valuable breeding materials which otherwise have potential genotypic worth for some specific traits. Therefore, it needs proper selection strategy often with the help of suitable statistical method. In this investigation, a few genetically potential genotypes have been isolated through evaluation of 96 germplasm lines including four standard checks in an augmented design. Adjusted values were derived from observed value of each agro-economic traits following standard statistical procedure. Each character was critically examined against the best standard check and promising genotypes with respect to specific traits have been sorted out and presented in **Table 6** for ease of better comprehension. The best entries with regard to specific traits may be utilized as donors in future breeding programme.

Among the standard check varieties, Vandana was the highest yielder (34.8q/ha) and hence could be considered as best standard check variety in terms of productivity. Genotypes exceeding 34.8q/ha in seed yield could be considered as significantly higher yield as compared to best standard check variety (Vandana). These include Khursudi and Bastul. Besides, a few genotypes seem to be comparable or recorded marginally increased seed yield were Bastul, Lalubadikaberi, Pandeydhan, Kinari, Dular, Rasakadali, Sarian, Jhulipuagi, karanga, Kenduphula, Kunor, Haladigundi, Mahulakunchi, Kandasuri,

Kanding, Nandigiri, Dhubasaria, Hiran, CR 143-2-2, CR Dhan 40, Sahbhagidhan and Sneha. The best standard check variety "Vandana" showed early flowering (64.0 days) and days to maturity (91.7 days). Bhogi was identified as the earliest in flowering and maturity duration (48.9\* and 78.3\*) and as expected it has low yield potential (25q/ha). However, some of the important early flowering genotypes with moderately high seed yield have been sorted out were Nandigiri, Dhubasaria, Setka-1, Dhanisaria, Saria -B and Zhu 11-26. Khursudi – a local land race was selected as comparatively dwarf plant type among the local land races. Besides, N22, Salampikit, Browngora, CR Dhan 40, Kalakeri, Saria-B to name a few were identified to have high degree of drought tolerance. These germplasm lines could be of immense value for future breeding programme for augmentation of productivity *per se*.

Direct selection based on mean performance often led to statistical error and lack of precision. Besides, regression analysis of yield component traits on seed yield could establish a basis of selection based on important yield contributing characters simultaneously. In the present set of test materials, the partial regression co-efficients for yield determining traits were worked out (Table 11) with a view to fit the regression equation  $Y(\text{Seed yield}) = -12.365 + 0.420X_1 + 0.051X_2 + 2.978X_3 + 0.014X_4 + 0.046X_5 + 1.773X_6 - 2.550X_7 - 0.438X_8 + 2.736X_9 + 0.067X_{10}$ ; where  $X_i$  – represents independent variables e.g., leaf drying score(LDS), number of ear bearing tillers/m<sup>2</sup>(EBT), panicle weight(PW), grains/panicle(G/P), 1000-grain weight(GW), grain length(GL), grain breadth(GB), kernel length(KL), kernel breadth(KB) and grain fertility percentage(F%). Regression co-efficients of panicle weight (PW) and kernel breadth (KB) were found significant at 1% level of significance indicating that these two component traits have definitely predominant influence on seed yield in upland rice. In contrast, grain breadth had also significant b-value but acted negatively (b=-2.550\*). However, such a comparison of characters based on estimates of the partial regression co-efficients has no meaning as b-values are in fact not unit free. Therefore, overall mean value of each component traits was multiplied with respective b-value and expressed in terms of yield units. The relative magnitude of these products may be considered for logistic comparison among component characters towards contribution for seed yield. On verification of the product values, it was revealed that number of ear bearing tillers/m<sup>2</sup>

**Table 11. Regression analysis of yield component traits on seed yield.**

Estimates of regression	a-value	LDS (X1)	EBT (X2)	PW (X3)	G/P (X4)	GW (X5)	GL (X6)	GB (X7)	KL (X8)	KB (X9)	F (10)
Partial regression coefficient(b)	-12.365	0.420	0.051	2.978**	0.014	0.046	1.773	2.550*	-0.438	2.736**	0.067
SE of partial regression coefficient	4.719	0.184	0.005	0.938	0.025	0.111	0.762	2.531	0.986	2.856	0.025
t- value of partial regression coefficient	-	2.28	10.2	3.17	0.56	0.41	2.32	1.00	0.44	0.95	2.68
Regression equation	Y(Seed yield)= -12.365+ 0.420X1 + 0.051X2 + 2.978X3 + 0.014X4 +0.046X5 + 1.773X6 - 2.550X7 -0.438X8 + 2.736X9 +0.067X10										

**Coefficient of partial determination (R<sup>2</sup>) = 0.7633**

and grain length followed by grain breadth, fertility percentage, kernel breadth and panicle weight have high contribution for expression of seed yield.

### **Genetic divergence:**

Assessment of genetic diversity in a set of breeding materials is a pre-requisite to distinguish the genotypes into genetically close and divergent types. Further, it is also possible to assess the contribution of different component traits to the total divergence. The genotypes which are genetically distant enough with regard to traits contributing sizeable genetic divergence are expected to generate wide range of genetic variation in recombination breeding and pave the way for greater scope for recovery of transgressive segregants (Murty and Arunachalam 1996, Mohapatra *et al.* 1995, Kole 2000, Das *et al.* 2004, Shukla *et al.* 2006 and Sharma *et al.* 2008).

Genetic diversity is the diversity of the sets of genes carried by different genotypes of a species. Information of genetic resources with broad genetic diversity is a pre-requisite for accelerated genetic improvement of crops. This helps to distinguish the genotypes into genetically close and divergent types. The extent of genetic diversity can be accessed through morphological characterization and through genetic markers or considering both simultaneously. Variances of relatively highly heritable quantitative genetic markers provide an estimate of genetic diversity. Various techniques including numerical taxonomic approaches have been found quite efficient to assess genetic diversity in a set of germplasm lines. Researchers can use these information on genetic dissimilarity to make decisions for selection of superior genotypes for genetic improvement or for use as parents in hybridization programme. In addition, it helps in design of populations for genome mapping experiments. Therefore, an attempt has been made to quantify the magnitude of genetic divergence between each pair of genotypes following Euclidian method of genetic distance analysis for both DUS characteristics and morpho-economic traits using SPSS computer software programme (version 13.0). Besides, the data were analysed for principal component analysis to estimate relative contribution of first three principal components for different agro-economic traits and to assess the characters for relative contribution for genetic divergence.

### ❖ Contribution of characters to genetic divergence:

Selection and choice of parents mainly depends upon contribution of characters towards divergence (De et al. 1988). The relative contribution of different characters to the total divergence was assessed (Table-10) in terms of the magnitude of the coefficients of first three Eigen vectors estimated using Principal component analysis. Among the three Eigen vectors (PC-1, PC -2 and PC-3), Eigen vector -1 contributed maximum to the extent of 79.05% of total variation, while Eigen vector-2 and Eigen vector-3 contributed just 11.05% and 3.26% only (Table 12). In this context, ear bearing tillers/ m<sup>2</sup> (EBT) concerning to Eigen vector-1, recorded as the single most important yield component trait which contributed maximum (0.9945%) to genetic divergence. Besides, seed yield and maturity traits seem to have some role towards genetic divergence in the present set of materials. Mishra and Das (1997) and Pradhan and Mani (2005) reported higher contribution of days to 50% flowering to the total divergence. Several other workers also reported relative contribution of days to flowering and different other characters to total divergence (Kole 2000, Motiramani and Khan 2003, Nayak *et al.* 2004, Singh *et al.* 2005). With regard to Eigen vector -2, grains/panicle revealed considerable contribution towards genetic divergence. Hence, both number of ear bearing tillers/m<sup>2</sup> and grains per panicle could be used for isolation of genotypes with desirable traits. Roy *et al.* (2002) revealed that days to 50% flowering, grain length, kernel breadth and grain yield/plant were the major component characters contributing towards genetic diversity. Joshi *et al.* (2008) studied the genetic divergence among rice varieties in Punjab and found days to 50% flowering and plant height contributing to 61% of total divergence. Bhardwaj *et al.* (2001) concluded that length: breadth ratio contributed maximum towards genetic divergence followed 100-grain weight and grain yield per plant accounting to 80% of total divergence.

### ❖ Nature of genetic divergence

For ease in identification of genotypes and genotype combinations; ten top ranking genotypes for mean genetic distance and ten top ranking genotypic combinations for *inter se* genetic distance using DUS Characteristics, agro-economic traits and both DUS and agro-economic traits have been presented in Table 13(a), 13(b) and 13(c) respectively.

**Table 12 :** Principal component analysis based on variance, co-variance of 23 agroeconomic characters of 96 upland rice varieties.

Character	Eigen vector (1)	Eigen vector-(2)	Eigen vector-(3)
Days to 50% flowering	0.0231	-0.1551	0.3719
Days to maturity	0.0224	-0.1513	0.3647
Leaf rolling score(LRS)	-0.0014	-0.0020	-0.0006
Drought recovery score(DRS)	-0.0029	0.0008	-0.0014
Leaf drying score (LDS)	-0.0032	0.0030	-0.0249
Leaf area (LA)	-0.0290	0.0671	0.2286
Chlorophyll index	-0.0002	-0.0104	-0.0170
Zinc tolerance score (ZTS)	-0.0016	0.0150	-0.0309
Bacterial leaf blight tolerance	-0.0005	-0.0064	-0.0098
Plant height (PHT)	0.0307	-0.0259	0.8092
Ear bearing tillers/ m <sup>2</sup> (EBT)	0.9945	0.0725	-0.0277
Panicle length (PL)	-0.0120	0.0325	0.0508
Panicle weight (PW)	-0.0013	0.0216	0.0021
Grains per panicle (G/P)	-0.0761	0.8766	0.1173
1000-grain weight	-0.0132	0.0475	-0.0100
Grain length (GL)	0.0007	0.0020	-0.0004
Grain breadth (GB)	-0.0004	0.0022	-0.0022
Grain length / grain breadth (GL/GB)	0.0010	-0.0040	0.0039
Kernel length (KL)	0.0009	0.0024	0.0008
Kernel breadth (KB)	-0.0001	0.0025	-0.0026
Kernel length / kernel breadth (KL/KB)	0.0008	-0.0040	0.0049
Fertility (F%)	-0.0003	0.3967	0.0295
Seed YIELD /ha (SY)	0.0458	0.1121	0.0393
Lamda value	Lamda(1)=5985.986	Lamda(2)=837.091	Lamda(3)=246.493
Contribution to PC value (%)	PC 1 =79.05	PC 2 =11.05	PC 3 =3.26

**N.B.:** Sum of other eigen or lamda values : 502.965  
Sum of all eigen or lamda values : 7572.535

**Table 13(a) . Ten top ranking genotypes for mean genetic distance and ten top ranking genotypic combinations for genetic distance using DUS Characteristics.**

Sl. No.	Top ranking genotypes for mean genetic distance	Genetic distance	Sl. No.	Top ranking genotypic pairs for genetic distance	Genetic distance
1.	V18- Baspatri	201.85	1.	V18 X V93 : Baspatri X N22	280.21
2.	V36- Damaraphuli	126.37	2.	V18 X V79 : Baspatri X Kalakeri	259.41
3.	V46- Barei	124.88	3.	V18 X V14 : Baspatri X Lalubadikaberi	256.79
4.	V7- Kuliha	111.58	4.	V18 X V46 : Baspatri X Barei	254.16
5.	V38- Biramani	108.36	5.	V18 X V40 : Baspatri X Kalakusuma	252.58
6.	V21- Dengabari	107.45	6.	V18 X V64 : Baspatri X Paradhan	249.23
7.	V39- Dangaradhan	106.65	7.	V18 X V62 : Baspatri X Dasaharadhan	247.42
8.	V37- Chinger-2	103.93	8.	V18 X V65 : Baspatri X Kandasuri	240.10
9.	V23- Pugukal	102.86	9.	V18 X V29 : Baspatri X Dular	239.19
10.	V61- Sanarasi	101.30	10.	V18 X V39 : Baspatri X angaradhan	238.36

**Table 13(b) . Ten top ranking genotypes for mean genetic distance and ten top ranking genotypic combinations for genetic distance using agro-economic traits.**

Sl. No.	Top ranking genotypes for mean genetic distance	Genetic distance	Sl. No.	Top ranking genotypic pairs for genetic distance	Genetic distance
1.	V5-Setka-1	86.56	1.	V32 x V5 : Rangahazari x Setka-1	156.79
2.	V13- Rangahazari	80.54	2.	V13 x V 75 : Rangahazari x Pustak	153.83
3.	V18-Baspatri	80.30	3.	V13 x V2 : Rangahazari x Kantadumer	153.39
4.	V4- Biramani	73.70	4.	V13 x V69 : Rangahazari x Dhobasaria	149.01
5.	V32- Merlo	71.16	5.	V5 x V84 : Setka-1 x Salampikit	147.47
6.	V37- Chinger-2	70.73	6.	V13 x V68 : Rangahazari x Nandigiri	146.95
7.	V53- Ambajhuka	64.59	7.	V13 x V41 : Rangahazari x Sarian	145.67
8.	V68- Nandigiri	62.31	8.	V37 x V5 : Chinger-2 x Setka-1	142.38
9.	V42- Dal	61.62	9.	V18 x V69 : Baspatri x Dhobasaria	138.63
10.	V95- Khandagiri	60.27	10.	V4 X V5 : Biramani x Setka-1	132.10

**Table 13(c) . Ten top ranking genotypes for mean genetic distance and ten top ranking genotypic combinations for genetic distance using both DUS and agro-economic traits.**

Sl. No.	Top ranking genotypes for mean genetic distance	Genetic distance	Sl. No.	Top ranking genotypic pairs for genetic distance	Genetic distance
1.	V18- Baspatri	282.14	1.	V18xV79 : Baspatri X Kalakeri	382.94
2.	V93-N22	197.94	2.	V18x V2 : Baspatri X Kantadumer	362.44
3.	V36- Damaraphuli	190.96	3.	V18xV69 : Baspatri X Dhobasaria	359.88
4.	V14- Lalubadikaberi	186.88	4.	V18xV93 : Baspatri X N22	356.05
5.	V32- Merlo	180.28	5.	V18xV62 : Baspatri X Dasaharadhan	350.11
6.	V37- Chinger-2	174.63	6.	V18xV14 : Baspatri X Lalubadikaberi	347.28
7.	V46- Barei	174.56	7.	V18xV64 : Baspatri X Paradhan	345.54
8.	V53- Ambajhuka	162.65	8.	V18xV29 : Baspatri X Dular	342.06
9.	V5 - Setka-1	159.28	9.	V18xV68 : Baspatri X Nandigiri	340.50
10.	V2 - Kantadumer	157.36	10.	V18xV46 : Baspatri X Barei	336.85

Using 54 assessable DUS characteristics, Baspatri, Damaraphuli, Barei, Kuliha, Brahmani, Dengabari, Dangardhan, Chinger-2, Pugakal and Sanarasi were found to have high average genetic distance from rest of the test genotypes. In this context, Baspatri happens to be one of the genotype in the ten top ranking genotype combinations showing high inter se genetic divergence.

Genetic divergence based on agro-economic traits may be useful for identification of genetically distant genotypes. In the present pursuit, Setka-1, Rangahazari, Baspatri, Biramani, Merlo, Chinger-2, Ambajhuka, Nandigiri, Dal and Khandagiri were shown to be highly divergent genotypes. It is worth to note that Baspatri, Biramani and Chinger-2 had also revealed high genetic divergence for DUS characteristics. Hence, these three test genotypes certainly would serve as valuable materials for breeding of upland rice. Among the genotypic combinations, Rangahazari with Setka-1, Pustak, Kantadumer and Dhobasaria; and Setka-1 with Salampikit and Chinger-2 had shown very high inter se genetic distance. Hence, such genetic combination would have some merit for recovery of transgressive segregants following recombination breeding.

Morphological diversity of genotypes could be more comprehensive, if the test genotypes are assessed for genetic divergence using both morphologically stable DUS characteristics and agro-economic traits. In the present pursuit, Baspatri, N22, Damaraphuli, Lalubadikaberi, Merlo, Chinger-2, Barei, Ambajhuka, Setka-1 and Kantadumer were recorded as top ten ranking genotypes for average genetic distance with rest of the test genotypes. Baspatri in combination with Kalakeri, Kantadumer, Dhobasaria, N22, Dasaharadhan, Lalubadikaberi, Paradhan, Dular, Nandigiri or Barei was identified to have high *inter se* genetic divergence.

#### ❖ **Group constellation and Characteristic features of clusters for agro-economic traits**

Grouping of test genotypes into different clusters was made based on Euclidian genetic distance between all possible pairs of genotypes following SPSS analysis (version 13.0). In the present investigation, the total 96 test genotypes including standard check varieties were grouped into 12 distinct clusters (Table-14). Cluster I, Cluster-II, Cluster-VI, Cluster-VII and Cluster VIII were mono-genotypic which included V5 (Setka-1), V37 (Chinger-2), V42(Dal), V18 (Baspatri) and V27 (Kinari). While, Cluster-III, Cluster-IV

**Table 14. Cluster composition and cluster mean of local land races for agro-economic characters.**

Cluster No.	SI No. of the genotypes	DF	DM	LRS	DRS	LDS	LA	C.I.	ZTS	BLB	PHT	EBT	PL	PW	G/P	GW	GL	GB	GL/GB	KL	KB	KL/KB	F%	SY
I	5	52.9	82.8	6.8	6.6	7.6	53.4	14.1	3.1	4.8	1.8	327.1	18.9	0.9	34.6	25	8.6	1.7	4.9	5.6	1.4	3.9	64.8	28.1
II	37	77.9	108.3	1.3	1.4	0.8	21.3	8.6	1.1	3.3	106.1	240.4	23.3	0.8	39.3	17.6	7.2	2.9	2.5	5.1	1.6	3.1	45.1	14.9
III	4,32	75.4	105.0	3.0	2	2.7	24.65	11.95	1.1	6.05	127.9	356.75	26.6	1.0	42.45	13.85	6.1	1.9	3.15	4.15	1.75	2.3	30.8	23.85
IV	13,95	75.2	106.15	6.4	6.0	6.0	34.2	8.4	2.3	4.6	100.5	404.4	20.6	0.8	50.1	20.1	8.0	1.4	5.5	6.3	1.2	5.0	55.7	28.8
V	36, 53	77.0	106.8	2.3	1.7	1.7	51.4	11.0	0.8	4.0	126.7	184.4	23.1	0.9	56.2	18.9	7.4	1.5	4.9	5.0	1.1	4.1	56.5	16.2
VI	42	106.9	135.3	7.3	7.4	4.8	38.6	15.5	1.1	3.3	119.1	328.4	22.3	1.3	53.3	20.3	8.5	1.8	4.6	5.8	1.6	3.5	79.3	32.6
VII	18	91.9	121.8	8.8	8.6	8.6	50.1	11.1	1.1	2.8	109.8	191.1	21.9	0.8	56.6	20.6	5.9	1.3	4.3	4.0	1.2	3.3	64.7	18.9
VIII	27	68.9	97.3	5.3	1.4	0.8	43.4	13.4	1.1	9.3	133.1	504.4	18.3	0.8	37.3	15.0	7.8	1.8	4.1	5.4	1.6	3.2	88.5	34.3
IX	15,46,77, 47,9,84	71.2	101.7	4.2	3.9	4.0	38.4	8.2	1.7	2.6	120.8	405.7	17.7	1.2	51.2	17.3	6.9	2.0	3.4	5.2	1.8	2.7	71.4	30.1
X	45,31,39, 38,6	57.7	87.0	5.6	5.6	4.8	38.1	10.9	1.9	7.2	110.4	247.1	21.6	1.4	63.0	19.8	7.3	1.9	3.8	5.2	1.7	2.9	76.5	19.9
XI	Noted below	63.6	93.7	4.8	4.6	4.6	42.9	8.6	2.7	3.7	111.8	301.4	23.2	2.1	83.4	23.9	8.3	2.1	3.8	5.9	1.9	3.0	79.0	31.1
XII	Noted below	74.5	104.4	5.4	5.2	4.1	40.0	9.4	1.7	4.3	116.4	323.7	21.7	1.1	51.7	18.7	8.3	1.9	4.3	5.8	1.6	3.3	64.3	27.7

**N.B. :Cluster –XI : Entry Nos. 71, 3, 89, 88, 94, 90, 82, 78, 58, 57, 83, 79, 96, 65, 55, 50, 92, 62, 14, 66, 73, 25, 67, 56, 29, 2, 68, 64, 70, 16, 35, 7, 1, 87, 74, 33, 75, 69, 41, 52. Cluster-XII : Entry Nos. 8, 9, 10, 11,12, 17, 19, 20, 21, 22, 23, 24, 26, 28, 30, 34, 40, 43, 44, 48, 49, 51, 54, 59, 60, 61, 63, 72, 6,80, 81, 85, 86, 91**

and Cluster-V contained two test genotypes each, e.g., Biramani & Merlo, Rangahazari & Khandagiri, and Damaraphuli & Ambajhuka respectively. Cluster -IX and Cluster-X included six (V15, V46, V77, V47, V9 and V84) and five (V45, V31, V39, V38 and V6) test genotypes. Rest of the genotypes in the present set of germplasm were distributed into two large multivariety clusters e.g., Cluster -XI and Cluster -XII which contained 40 and 34 genotypes respectively. The genotypes included under Cluster -XI and Cluster-XII maintain close *inter se* homology while, those included under rest of the clusters definitely have genetic dissimilarity in agro-economic traits. However, for breeding point of view, genotypes designated under Cluster I to Cluster-VIII may be sorted out as highly divergent (Fig. 6).

Some of the clusters have characteristic features expressed in terms of cluster mean value for different agro-economic traits. Cluster mean of days to 50% flowering and days to maturity ranged from 52.9 to 106.9 days and 82.8 to 135.3 days. V5 included in Cluster- I had shown very early maturity. Chinger -2(V37) comprising Cluster-II recorded highest degree of drought tolerance based on all three criteria(LRS, DRS and LDS). Among the clusters, Cluster-I revealed characteristically high chlorophyll content (revealed from high chlorophyll index measured at PI stage) as well as more leaf area. However, Cluster-VI recorded highest chlorophyll index with intermediate leaf area. Among the clusters, Cluster-V comprising V36 and V53 revealed very high degree of field tolerance to zinc stress, whereas, genotypes included under Cluster-IX had shown moderately tolerant to BLB infestation. Among the agro-economic traits, EBT/m<sup>2</sup>, grains/panicle, panicle weight and fertility percentage are more important than other ancillary traits. Cluster VIII recorded highest tillering ability ( $\geq 500$ ) and Cluster-III had shown longer panicles(26.6cm.). while, Cluster-XI emerged as the most important cluster for panicle weight, grains/panicle, 1000-grain weight, grain length and kernel breadth. Among the genotypes, V27(Kinari) was separated from rest of the genotypes to constitute Cluster VIII which recorded highest in terms of grain fertility percentage and grain yield/ha. The above promising clusters and genotypes identified in this pursuit may serve as good information relating to the level of genetic divergence in relation to agro-economic traits and this would help in planning for genetic improvement in upland rice.

### ❖ Construction of dendrogram and clustering pattern

The whole set of 96 test genotypes comprising early duration local land races of Odisha, popular drought tolerant donors and high yielding upland varieties of rice were distributed into twelve clusters based on average Euclidian genetic distance using SPSS software programme (version 13.0) for 23 agro-economic traits including grain yield (Fig.6). Roy *et al.*(2002) revealed the nature and magnitude of genetic diversity in 50 high yielding varieties and traditional germplasm of rice. The genotypes were grouped into 10 clusters. Vivekanandan and Subramanian(1993) also assessed genetic divergence in twenty -eight genotypes of rain fed rice and identified few highly divergent rice genotypes suitable for upland rice breeding.

In the present investigation, Baspatri was found most divergent based on DUS characteristics (Fig. 5) while, Setka-1 and Rangahazari revealed high genetic distance from rest of the varieties based on agro-economic traits(Fig.6). Considering both DUS and agro-economic traits; Baspatri, N22, Damaraphuli and Lalubadikaberi may be sorted out as highly divergent genotypes(Fig.7). Further, it was revealed that Baspatri with either Kalakeri, Kantadumer, Dhobasaria, N22 or Dular had shown very high genetic divergence. Inter-varietal crossing between Baspatri with above genotypes may result useful transgressive segregants for desirable selection of plant types suitable for upland situation.

### BIOCHEMICAL CHARACTERIZATION:

Since, seed is the edible portion of cereal crops and it contains major nutritive contents. Variation in seed storage proteins either in terms of the amount of crude protein in seeds or its characteristic polypeptide banding pattern have become major concerns for cereal breeders and geneticists. Besides, some of the seed characteristics e.g., seed colour happens to be common among genotypes. Therefore, electrophoresis of seed storage proteins could be a method to investigate genetic variation and to classify plant varieties.

Seed storage proteins have been used as genetic markers in four major areas, e.g., analysis of genetic diversity within and between accessions, plant domestication in relation to genetic resource conservation and breeding, establishing genome relationships,

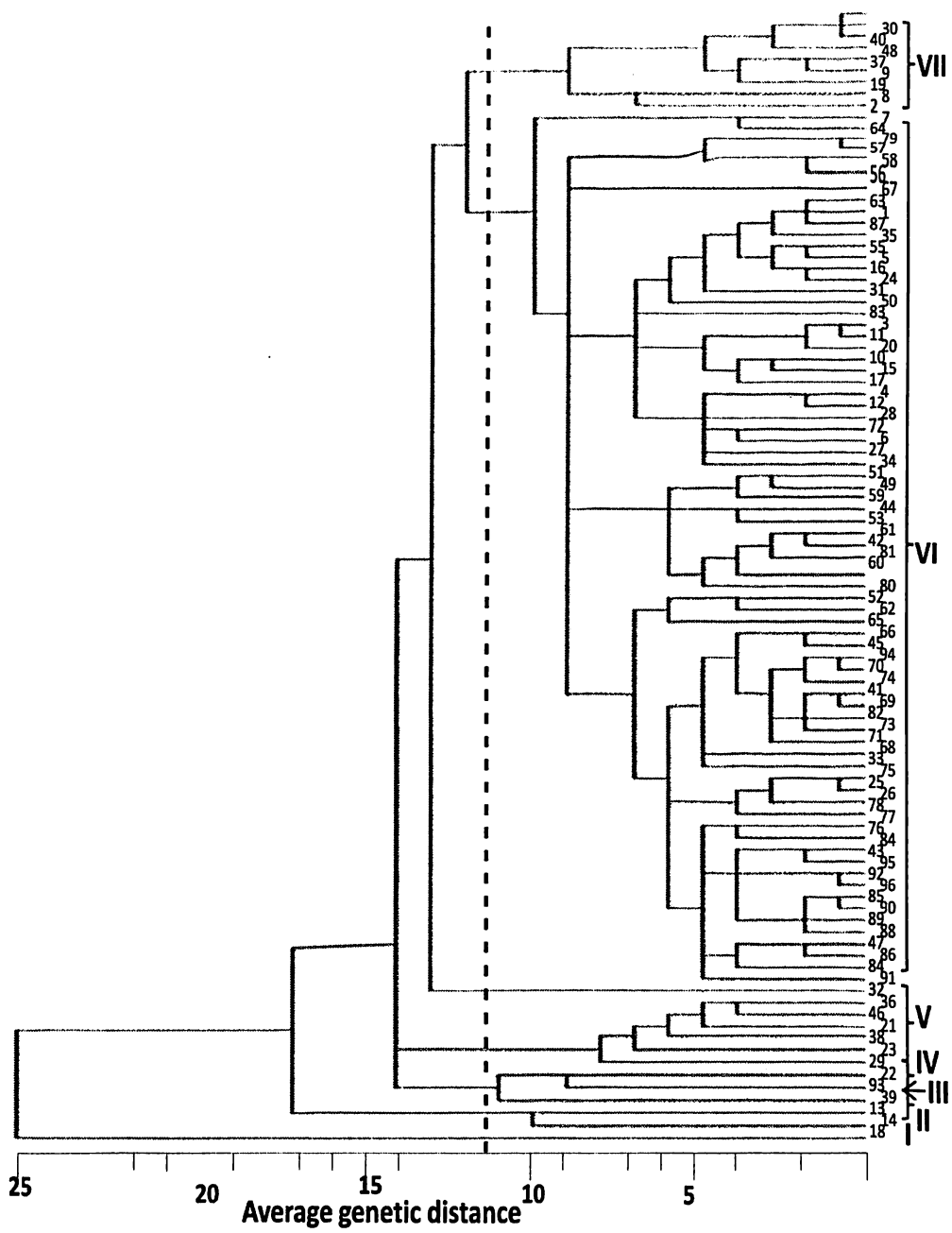


Fig 5. Dendrogram 96 upland rice varieties based on DUS characteristics.

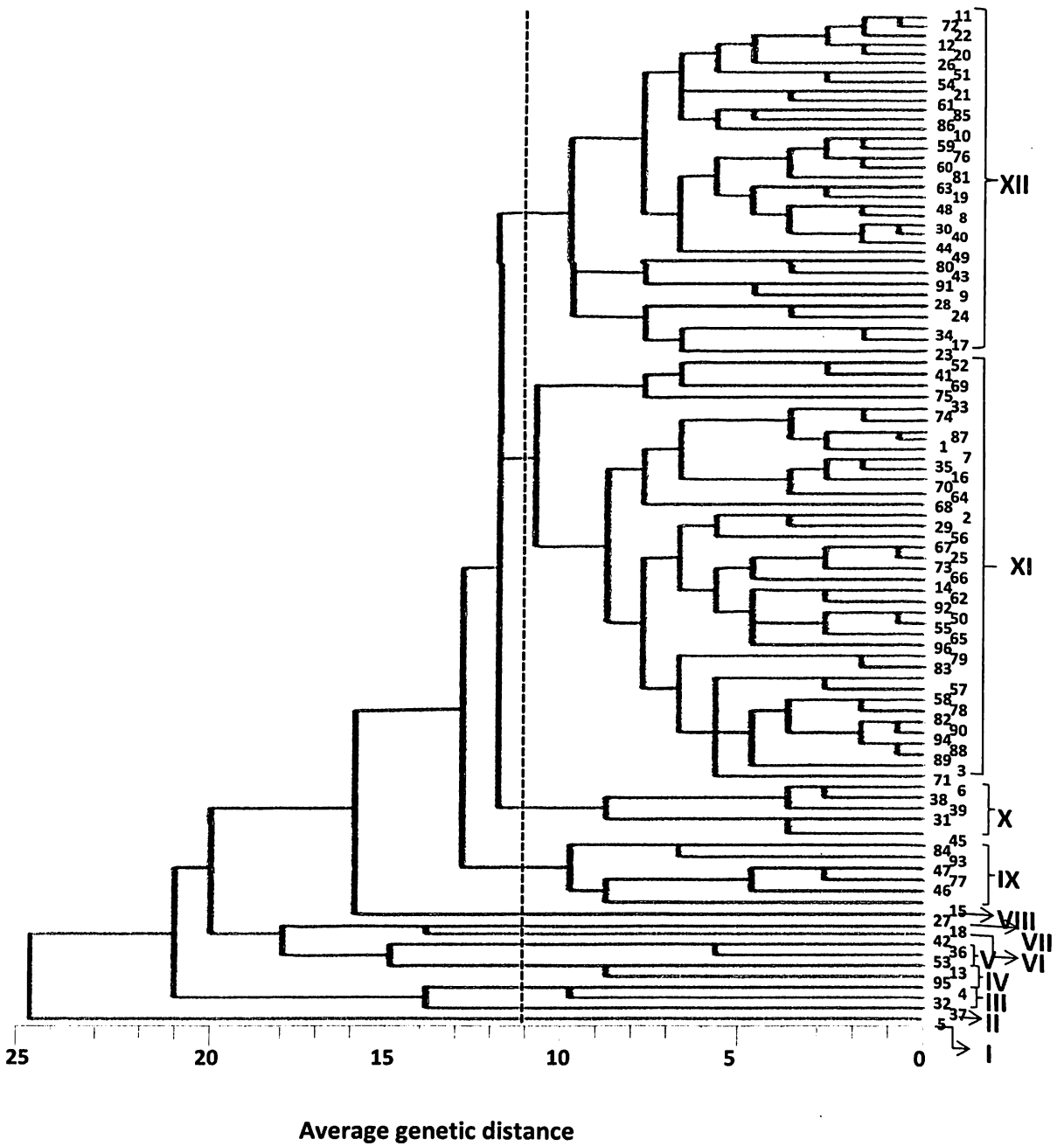


Fig 6. Dendrogram of 96 upland rice varieties based on agro-economic traits.

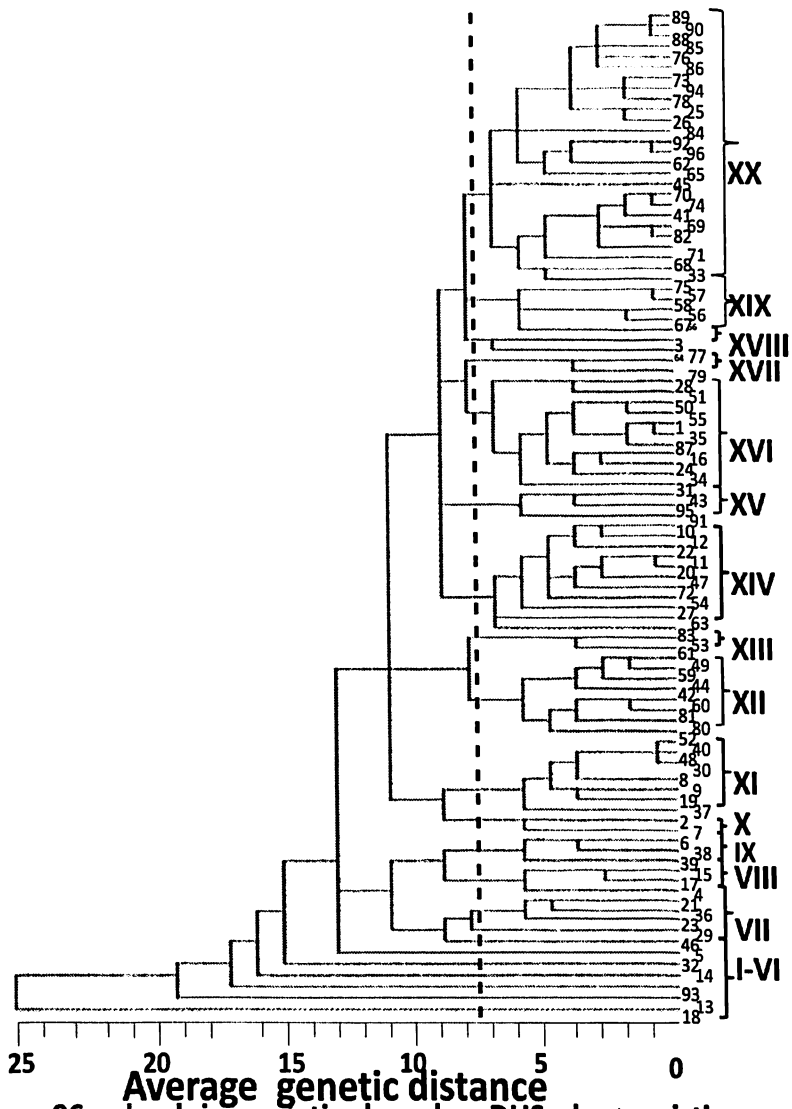


Fig 7. Dendrogram 96 upland rice varieties based on DUS characteristics and agro-economic traits.

and as a tool for crop improvement. SDS-PAGE of seed storage proteins is considered to be a reliable and practical method to study genetic structure of crop germplasm as well as study of genetic variation induced following mutagenesis. Such a technique is often opted because of its ease in experimentation, low cost involved and less influence to environmental fluctuations compared to morphological traits.

SDS-PAGE of seed storage proteins generates different profiles composed of several polypeptide sub-units which migrate in the gel according to their molecular weights. The number of such sub-units indicates the number of multi-gene families involved (de Lumen 1990). Mutation in these gene families or their regulator genes leads to deletion of some or all the genes or production of new alleles which forms the basis of variation in the polypeptide banding patterns involving presence or absence of bands. In addition, polymorphism in terms of intensity of bands could provide information in shutting of some members of the multi-gene family that could produce fewer copies in the genotype exhibiting faint bands. Electrophoregrams of a fairly large number of genotypes may also reveal distinct alleles for gene families comprising two molecular variants of a polypeptide subunit under monogenic control. Such polymorphic polypeptides with varying molecular weights are considered as polypeptide markers.

The polymorphism of polypeptide markers are in vogue used for characterization and categorization of genotypes in addition to its use in hybrid selection, marker assisted selection, elucidation of genetic control of protein expression, linkage of polypeptide bands, stability of polypeptide banding patterns, genome homology, centre of genetic diversity and evolutionary pathways.

Glutelin is the major storage protein which is acid and/or alkaline soluble and accounts for 80% of the total protein. Salt and alcohol soluble proteins (globulin and prolamin respectively) are present in relatively low amounts(5%) in rice endosperm (Cagampang *et al.* 1966). In rice, 60% to 80% of total seed protein is composed of glutelins, encoded by 15 genes in the rice genome, and 20% to 30% of total seed protein are prolamins that are encoded by 34 genes (Kawakatsu *et al.*, 2008; Xu and Messing, 2009). Glutelins can be classified into four groups (GluA, GluB, GluC, and GluD) based on amino acid sequence similarity (Kawakatsu *et al.*, 2008) while, prolamins are

classified into three groups (10, 13, and 16kD) by their molecular mass according to their electro-mobility on SDS-PAGE gels. The glutelin fraction constitutes the 22- to 23- and 37- to 39-kD complexes, and the prolamin fraction is composed mainly of 13-kD polypeptide (Yamagata *et al* 1982).

In rice, storage protein begins to accumulate about 5 days after flowering (Yamagata *et al.* 1982). Two polypeptide groups, 22 to 23 and 37 to 39 kD, the components of glutelin, appeared 5 days after flowering. *In vivo* pulse-chase labeling studies showed the 57kD polypeptide to be a precursor of the 22 to 23 and 37 to 39 kD subunits. The 57kD polypeptide was salt-soluble, but the mature glutelin subunits were almost salt insoluble. *In vitro* protein synthesis also showed that the mRNAs directly coding the 22 to 23 and 37 to 39kD components were absent in developing seeds and that the 57-kilodalton polypeptide was the major product. Thus, it was concluded that 57kD peptide was synthesized at an earlier stage than the glutelin subunits and such two subunits of rice glutelin subunits are formed through post-translational cleavage of the 57-kD polypeptide. A 26kD polypeptide globulin component also appeared 5 days after flowering. While, smaller polypeptides (10- 16kD) including prolamin components, appeared about 10 days after flowering. In contrast, the levels of the 76 and 57kD polypeptides were fairly constant throughout seed development.

#### ❖ **Extent of polymorphism:**

Profiling of total seed storage proteins through SDS-PAGE for differentiating rice genotypes is well established (Saruyama and Shinbasi, 1993; Montalvan *et al.*, 1998; Thanh and Hirata, 2002). In the present investigation, SDS-PAGE of total seed storage protein for twenty nine short duration rice genotypes was carried out simultaneously by running two gels at a time in a vertical slab gel electrophoresis apparatus under similar electrophoretic conditions. The SDS-PAGE revealed altogether 19 scorable polypeptide bands with molecular weights ranging from 26.0 to 123.0kD (**Fig. 8, Table 15**). This envisaged that at least 19 multi-gene families are involved in seed storage protein expression in rice. Out of these, four polypeptide bands e.g., B10(57.0kD), B11(53.0kD), B12(51.2kD) and B16( 37-39kD) were found to be monomorphic and rest of the bands had shown polymorphism to the extent of 78.94% among the test genotypes. As a whole,



Fig.8. Total seed storage protein profile of selected local land races and promising drought tolerant donors., M-Mol. wt. marker, Lane 1-29: var. 1-29 (Asumakunda, Bastul, Brahmanaki, Browngora, Dular, Hiran, Kanding, Kalakeri, Khursudi, Nagina 22, Padaradhan, Salkiana, Salampikit, Somo, Sahbhagidhan, IR 87707-445-B-B, CR 143-2-2, CR Dhan 40, Anjali, Sneha, Annada, Zhu 11-26, vandana, Khandagiri, Mandakini, Kinari, Pustak, Nandigiri and Pandeydhan ).

Table 15. Electrophoretic (SDS-PAGE) polypeptide banding patterns of total seed storage protein of 29 rice genotypes.

Bands	Mol.Wt(kD)	Genotypes																												
		Asumakunda	Bastul	Brahmanaki	Browngora	Dular	Hiran	Kanding	Kalakeri	Khursudi	Nagina 22(N22)	Padaradhan	Salkiana	Salampikit	Somo	Sabhagidhan	IR 87707-445-B-B	CR 143-2-2	CR Dhan 40	Anjali	Sneha	Annada	Zhu 11-26	Vandana	Khandagiri	Mandakini	Kinari	Pustak	Nandigiri	Pandeydhan
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29
B1	123.0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0
B2	116.0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
B3	108.0	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
B4	101.4	1	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
B5	97.2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
B6	93.0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B7	70.0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B8	65.2	0	1	1	0	0	1	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
B9	60.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B10	57.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
B11	53.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B12	51.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B13	49.3	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
B14	47.0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1
B15	45.0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0
B16	39.37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
B17	28.7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B18	27.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
B19	26.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		18	17	19	16	16	18	15	16	9	13	18	16	16	16	16	16	15	16	18	16	16	15	19	10	15	13	15	17	14

N.B.: B10 & B11 : Deep yellow bands except V9 and V29 which are black(underlined), B16: Broad yellow band characteristic of rice.Red marked figures at B17 and B18 indicate bands with specifically yellow colour compared to bands in that position in other vars. are faint black in colour.

the resulting data matrix of the presence and absence of bands resolved a total of 338 polymorphic polypeptide bands out of total 454 bands over all the 29 test genotypes used in the study which reveals 74.44% polymorphism.

Besides, a great array of polymorphism was revealed in terms of presence (1) / absence (0) of polypeptide bands as well as intensity of bands and colour of stain revealed by bands following silver staining technique in different test genotypes. The intensity of bands was given due importance while scoring of bands in different genotypes to reveal degree of quantitative variation in polypeptides dissociated following cleavage of storage protein fractions. The presence of densely stained bands was marked bold to discriminate such bands in a particular protein type or polypeptide banding pattern.

### **Characteristic polypeptide banding pattern**

For ease of detection of bands, four distinct zones of polypeptide migration were arbitrarily assigned in the electrophoregram i.e. A (65.2 - 123.0kD), B (53.0 - 60.0kD), C (45.0 - 51.2kD), D (26.0 - 37,39kD). Zone A was designated by two sets of paired bands i.e., B2 & B3 and B5 & B6; and one single fine band at 70kD (B7) present mostly among the test genotypes (**Fig. 8**). Besides, it has three sparsely distributed faint fine bands at B1, B4 and B8. Zone B starts with a black band at 60kD (B9) followed by two thick prominently monomorphic dense yellow bands (B10: 57kD, B11: 53kD,) clubbed together. Zone C included four faint black bands (B12-B15) occupying the space between yellow band at 53kD (B11) and a broad yellow band at 37-39kD (B16). Among the four bands in Zone-C, B12 is shown to be monomorphic over the test genotypes. Zone D is marked by a characteristic monomorphic broad dense yellow band of rice (B16) with mol. wt. 37-39kD (37 and 39kD clubbed together) followed by three low molecular weight bands (B17-B19) ranging from 28.7-26.0kD in descending order.

Genetic variation in relation to presence of number of polypeptide bands in the electrophoregram ranged from 9 in Khursudi to as high as all 19 bands in Brahmanaki and Vandana followed by 18 bands each in case of Anjali, Asumakunda, Hiran and Padaradhan (**Table 15**). Besides, Nandigiri and Bastul also had higher number (17) of

polypeptide bands in the electrophoregram while, Khandagiri recorded as low as only 10 polypeptide fingerprint at different molecular weight positions..

Out of 19 polypeptide bands revealed in this investigation, B1(123.0kD), B4(101.4kD) and B8(65.2kD) were of rare occurrence over varieties in the electrophoregram. The polypeptide band at 123.0kD was present only in seven test genotypes. Whereas, 101.4kD and 65.2kD bands were observed in eight and nine genotypes respectively. Thus, bands at these above molecular weight positions may be considered as most informative and therefore, these may contribute maximum for genetic dissimilarity / genetic divergence among the test genotypes.

In the present investigation, Khursudi can be identified from rest of the test genotypes by absence of polypeptide band B5 (97.2kD) and B6 (93.0 kD). Similarly, Zhu 11-26 and Khandagiri had uniquely absence of band B18(27.5kD) and B17(28.7kD) respectively which were present in all other short duration test genotypes including the popular drought tolerant genotypes Vandana, Sahbhagidhan, Dular and Nagina 22(N22). Khursudi and Nagina 22 had shown absence of a polypeptide band at molecular weight position 70kD. Such a genotype –specific finger printing can be used for varietal identification. Further, the information would safe guard the Farmers' Right over their land races which they used to cultivate over years.

The paired polypeptide finger prints positioned at 57.0kD (B10) and 53kD (B11) was shown to be characteristically deep/dense yellow in all the test genotypes except Khursudi and Pandeydhan which revealed blackish band. Similarly, band B17 (28.7kD) and B18 (27.5kD) which were usually faint black, but few of the test genotypes had shown broad yellow band at these molecular weight positions. For instance, Hiran, Kanding and Khursudi had yellow polypeptide band at both 28.7kD and 27.5kD position.

In contrast, Browngora, Annada and Zhu 11-26 revealed yellow shaded band at 28.7kD; and a few test genotypes e.g., IR 87707-445-B-B, CR 143-2-2, CR Dhan 40, Mandakini and Pandeydhan showed yellow coloured band at 27.5kD only. Such a differential banding pattern could certainly help in varietal identification and certification.

India has a rich heritage of local land races of rice and many of these have been evolved over several years in their native area of cultivation. Some of these local land races might have experienced spontaneous mutation in multi- gene families determining seed storage protein expression. Deletion of any member of these gene family or mutational changes in their regulator genes could certainly have a direct bearing on polypeptide banding pattern. In this context, Asumakunda and Hiran can be differentiated from Brahmanaki by absence of polypeptide band at 65.2kD and 123kD respectively. This signifies spontaneous mutational change in the resulting land race compared to Asumakunda and the mutational changes are hereditary and have direct bearing in total seed protein expression.

The polypeptide band B<sub>9</sub> could be identified in the electrophoregram at mol wt position 60kD. Besides, it gives a characteristic faint blackish brown fingerprint just above the paired yellow bands at mol. wt 53.0kD and 57.0kd position. The 60 kDa seed storage protein is the translation product of waxy (Wx) gene (Sano *et al.* 1985, Sano *et al.* 1986 and Shu *et al.* 1999), which control amylose content. Extent of staining intensity of such 60kDa protein band in different rice varieties can be a reliable parameter to assess amylose content. In this context, Khandagiri and a local upland land race 'Kinari' could not reveal such polypeptide marker indicating very low amylose status in these genotypes. Besides, Asumakunda, Bastul, Brahmanaki, Kanding, Kalakeri, Padaradhan, Salampikit, Somo, CR Dhan 40, Anjali, Vandana and Pandeydhan have been identified as candidate varieties to have intermediate amylose content, while rest of the genotypes revealing very faint band intensity could be sorted out as genotypes with low amylose content. Genotypes with medium amylose content (21-22%) are considered to have better cooking quality. It is estimated that a reduction in the levels of 60-kD polypeptide band resulted in significant reduction of the total amylose content in the endosperm when compared with the wild type. Therefore, the test genotypes with medium staining

intensity of this 60kD band were sorted out for further confirmation and validity of the phenomenon. Kumamaru *et al.* 1988 and Qu *et al.* 2001 emphasized that SDS- PAGE could be effectively employed for screening of mutants for seed storage proteins. In this context, Wei-dong *et al.* (2006) revealed high staining intensity of band 60 kD in the RT series mutants e.g., RT60, RT61, RT62 and RT64 indicating high amylose content compared to the parent variety “Ochikara”. This phenomenon is worthwhile to study further.

❖ **Genetic similarity/distance:**

The genetic distance between genotypes suggests the relationship between the species and within the members of the same species. The crosses between parents with maximum genetic divergence are generally most responsive for genetic improvement as these can result better transgressive segregants through gene shuffling. However, to utilize such parental accessions with maximum genetic divergence, it is necessary to screen and characterize the available germplasm for the nature and extent of genetic diversity included in it. Characterization and cataloguing of germplasm are in vogue carried out on the basis of morpho-agronomic traits. However, use of biochemical and molecular marker in the past two decades have revealed tremendous genetic variation and genetic relatedness found in a population. Among the molecular markers, random amplified polymorphic DNA (RAPD) offer a rapid and reliable tool for identification and characterization of genotypes. This technique generates molecular markers for comparative analysis which is quick, cost effective, easy to use, liable to minimum environmental influence compared to protein and isozyme finger printing, and morphological traits. The RAPD markers are unlimited in number, random with wide coverage of genome and have a relatively higher level of polymorphism. Such a marker system does not require any prior sequence information. Besides, RAPD markers could show pronounced segregation distortion as compared DAF and other five types of molecular markers. On the other hand, polypeptide banding pattern based on SDS-PAGE of seed storage proteins does not require amplification as in case of RAPD or radio-isotope labeling as in case of restriction fragment length polymorphism(RFLP). In spite of the fact that, the degree of intra-specific variation exhibited by polypeptide banding pattern in SDS-PAGE is lower as compared to RAPD or RFLP, this simpler and cheaper

technique could be used to study the species relationship and genetic diversity of genotypes in crop plants. In the present investigation, attempt has been taken to study genetic variation at the level of total seed storage to critically examine genetic diversity among a set of selected genotypes.

Similarity index (S.I.) values (Table 16) between each paired genotypes were estimated as per Jaccard(1908) to reveal genetic relationship in terms of genetic distance/homology for seed storage protein expression. S.I. values ranged from 0.47 to as high as 1.00. Among the test genotypes, Khursudi (Av. S.I.=0.58) followed by Khandagiri (Av. S.I.=0.62) and Nagina 22 (Av. S.I.=0.69) had high genetic dissimilarity from most of the varieties. Khursudi maintained high genetic distance from Bastul, Brahmanaki, Zhu 11-26 and Vandana. Whereas, N22 showed appreciable genetic dissimilarity from Khandagiri and Zhu 11-26. Besides, Khandagiri was shown to be genetically distant from Asumakunda, Brahmanaki, Hiran, Khursudi, N22, Padaradhan, Anjali and Vandana. Thus, the above genotypes may have merit in hybridization programme for upland situation.

In contrast, a few of the test genotypes had revealed inter se genetic homology. Sneha is a promising high yielding upland genotype. It had homology in seed protein expression with popular drought tolerant land races e.g., Browngora, Salampikit, and Somo and a drought tolerant donor IR 87707-445-B-B (received from IRRI) with similarity value 1.00. On the other hand, Vandana - a popular upland variety under drought stress was shown to have 100% homology with a drought tolerant land race Brahmanaki.

#### ❖ Clustering pattern based on total seed storage protein profile

Seed storage protein profiling based on SDS-PAGE could be employed for various purposes, such as characterization of germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005), varietal identification (Song *et al.* 1996 and Zhang *et al.* 1998), hybrid seed purity test (Wei-dong *et al.* 2006), germplasm resource analysis (Sun *et al.* 2000 and Yu *et al.* 2003), genetic diversity analysis (Gan *et al.* 1999 and Yang *et al.* 2005), determination of phylogenetic relationship between different species and generation of pertinent

**Table 16. Similarity coefficients between paired test genotypes for total seed storage protein banding pattern in upland rice(Contd.).**

Sl.No	Genotype	Sahbh agidh an	IR 87707 -445- B-B	CR 143-2- 2	CR Dhan 40	Anjali	Sneha	Anna da	Zhu 11-26	Vanda na	Khand agiri	Mand akini	Kinari	Pusta k	Nandi giri
15		15	16	17	18	19	20	21	22	23	24	25	26	27	28
16.	IR 87707-445-B-B	0.89	1.00												
17.	CR 143- 2-2	0.94	0.94	1.00											
18.	CR Dhan 40	0.89	1.00	0.94	1.00										
19.	Anjali	0.89	0.89	0.84	0.89	1.00									
20.	Sneha	0.89	1.00	0.94	1.00	0.89	1.00								
21.	Annada	0.78	0.89	0.84	0.89	0.89	0.89	1.00							
22.	Zhu 11-26	0.73	0.84	0.78	0.84	0.73	0.84	0.84	1.00						
23.	Vandana	0.84	0.84	0.78	0.84	0.94	0.84	0.84	0.78	1.00					
24.	Khandagiri	0.68	0.68	0.73	0.68	0.57	0.68	0.68	0.63	0.52	1.00				
25.	Mandakini	0.84	0.94	0.89	0.94	0.84	0.94	0.94	0.89	0.78	0.73	1.00			
26.	Kinari	0.73	0.84	0.78	0.84	0.73	0.84	0.84	0.78	0.68	0.84	0.89	1.00		
27.	Pustak	0.84	0.94	0.89	0.94	0.84	0.94	0.84	0.78	0.78	0.73	0.89	0.89	1.00	
28.	Nandigiri	0.84	0.94	0.89	0.94	0.94	0.94	0.94	0.78	0.89	0.63	0.89	0.78	0.89	1.00
29.	Pandeydhan	0.89	0.89	0.94	0.89	0.78	0.89	0.78	0.73	0.73	0.68	0.84	0.73	0.84	0.84

information to complement evaluation (Sammour, 1991; Isemura *et al.*, 2001; Ghafoor *et al.*, 2002). There have been a substantial number of studies that have used SDS-PAGE to profile seed storage proteins in rice (Saruyama and Shinbasi, 1993; Montalvan *et al.*, 1998; Thanh and Hirata, 2002; Netra and Prasad, 2007). Hence, it becomes highly imperative to assess genetic diversity within and between rice landraces for varietal improvement, evaluation and modification, using better methods of germplasm evaluation and characterization strategies. It is also required to investigate the present gene pool for selection of diverse parent cultivar and to broaden the germplasm base of rice breeding programs in the future for sustainable management of the genetic resources.

Electrophoretic analysis of the seed storage proteins had direct relationship to the genetic background of the proteins that reveal genetic diversity. Such analysis can be used to certify the genetic makeup of germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005). Such a technique could be used to create additional data base as a supplement to the existing variation at morpho-economic characteristics of genotypes for more precise and a holistic approach for study of genetic diversity.

The dendrogram showing genetic relationship among twenty nine test genotypes for total seed storage protein expression is presented in **Fig 9**. Initially, the genotypes were distributed into three clusters. V10 (N22) and V9 (Khursudi) constituting first cluster; and V26 (Kinari) and V24(Khandagiri) grouped together in second cluster were initially separated from rest of the genotypes which were clubbed together in third cluster at about 71.5% phenon level. However, for ease in varietal discrimination and characterization; the test genotypes were grouped into nine clusters at 90.5% phenon level. Cluster I to Cluster V were characterized as mono-genotypic as these contained single genotype each. V10 (N22), V9 (Khursudi), V26 (Kinari), V24 (Khandagiri) and V22 (Zhu 11-26) formed cluster I, II, III, IV and V in sequence. Thus, these genotypes could be sorted out as highly divergent genotypes for seed storage protein expression. Rest of the test genotypes were distributed over four clusters (Cluster VI - Cluster IX). Cluster VI and Cluster VII contained three (V8- Kalakeri, V5- Dular and V2- Bastul) and four (V29- Pandeydhan, V17- CR 143-2-2, V15- Sahbhagidhan and V12- Salkiana) genotypes respectively. While, Cluster -VIII and Cluster-IX were large multivariety

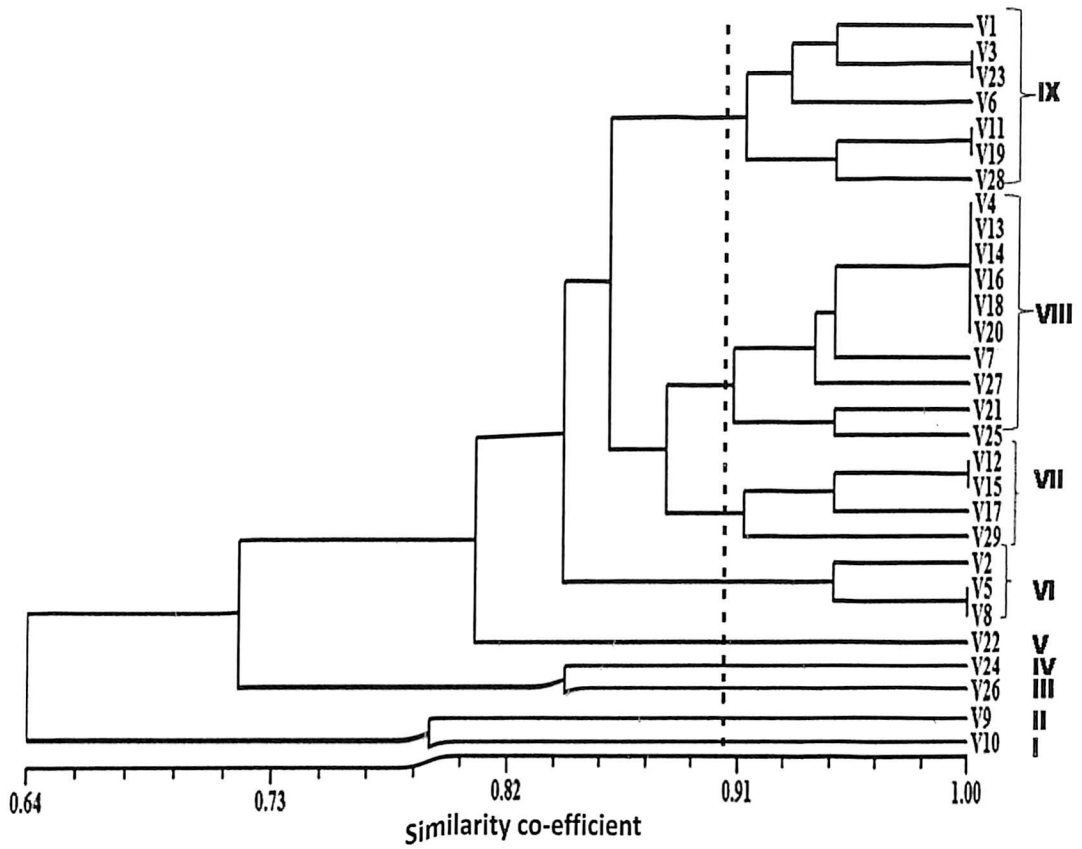


Fig 9. Dendrogram showing genetic diversity of a set of upland rice varieties based on total seed storage protein fingerprinting.

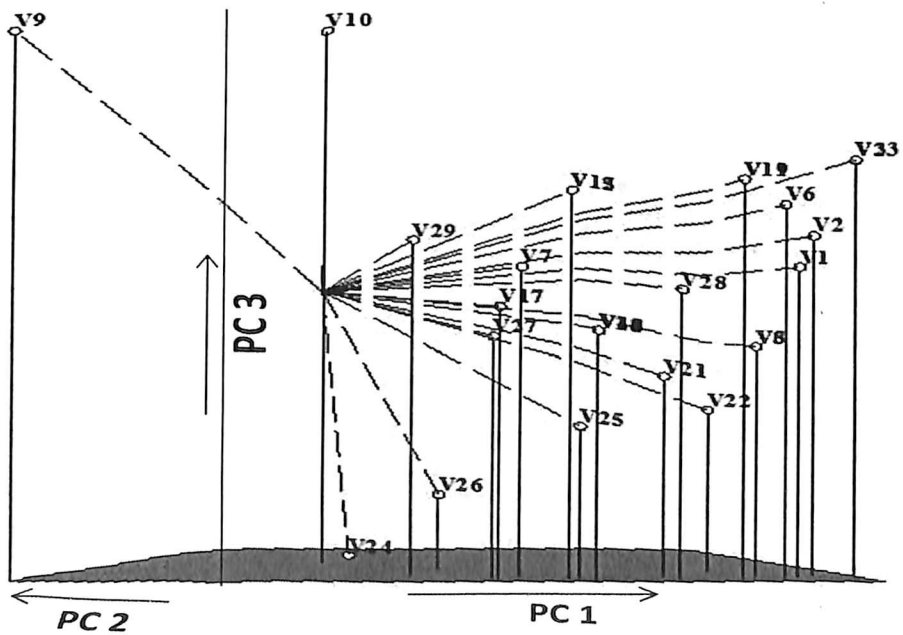


Fig 10. 3D Scaling of principal co-ordinates 1, 2 and 3 using seed storage protein markers with vectors.

clusters which included ten and seven genotypes respectively. Few of the test genotypes could not be discriminated from each other at even 100% phenon level. V8 (Kalakeri) & V5 (Dular) in Cluster-VI, V15 (Sahbhagidhan) & V12 (Salkiana) in Cluster-VII and V23 (Vandana) & V3 (Brahmanaki) in Cluster IX had shown common protein type owing to 100% homology in seed protein expression. It is worth to note that each of the above groups contained at least either Dular, Sahbhagidhan and Vandana which are known to have high degree of drought tolerance and most often opted as parents for hybridization programme in many research centres. This envisaged that Kalakeri, Salkiana and Brahmanaki might be related in some way with the above respective drought tolerant genotypes and could have similar mechanism for drought tolerance. Wei-dong *et al.* (2006) revealed that seed storage protein polymorphism in rice can not distinguish different japonica varieties. Although they had got 19 types of profile in varieties studied, the profiles of 80% varieties were similar. Jahan *et al.* (2003) reported nine variations of glutelin in Bangladeshi rice varieties. Aung *et al.* (2003) found there were two varietal types for glutelin, five varietal types for prolamin in Myanmar rice varieties. However, glutelin and prolamin of Pakistani rice varieties had six and four variation types respectively (Sarker and Bose 1984).

The grouping of genotypes using three dimensional scaling based on PCA values (Fig.10) was found to be more or less consistent with that of UPGMA analysis. The three dimensional scaling with vectors represented clear grouping of test genotypes. V9 (Khursudi), V10 (N22), V24 (Khandagiri) and V26 (Kinari) which were initially separated from rest of the test genotypes in case of UPGMA clustering (Fig.9), were also seen to be screened out to diverse extreme positions in PCA analysis.

In retrospect, this investigation revealed negligible polymorphism, with reference to the total seed protein profiles, among the rice genotypes used in the study. Hence, SDS-PAGE in combination with 2-D electrophoresis is further suggested for documenting contrasting variations of isoforms of protein peptides. Some researchers reported that protein composition is similar in rice with a little exception, and the composition of glutelin subunit is stable (Kusama *et al.* 1984, Guo *et al.* 1988 and Zhan *et al.* 1991). Others found there were great variations in the glutelin and/or prolamin (Satoh

*et al.* 1990a, Satoh *et al.* 1990b, Bhowmik *et al.* 1990, Lin *et al.* 1997, Lu *et al.* 2001, Jahan *et al.* 2003, Aung *et al.* 2003 and Siddiqui *et al.* 2003). Besides, electrophoretic bands of globulin seed storage protein fraction can be used as protein markers for rice seed purity identification.

However, the storage proteins of cereals are of immense importance in determining the quality and end use properties of the grain. Understanding the structures of these proteins, their biophysical and functional properties, and the biological mechanisms which determine their synthesis, trafficking and deposition in the grain is important to underline future attempts to improve the end use quality of grain by genetic engineering. Nagina 22 (N22)- local land race popularly used as national check variety in All India Co-ordinated trials for upland condition, was found to be highly divergent in terms of combined DUS and agro- economic traits as well as seed storage protein expression. Besides, rigorous screening following the concept of pre-breeding and using seed storage protein profiling; Khursudi, Kinari, Khandagiri and Zhu 11-26 have been also identified as highly divergent genotypes suitable for upland condition. Among these Khandagiri is known to have wider adaptability and high yield potential with acceptable grain and kernel characteristics, but sensitive to drought stress. Drought tolerance may be introgressed into Khandagiri using highly divergent drought tolerant donors e.g., N22, Khursudi, Kinari and Zhu 11-26 through recombination breeding.

## CHAPTER-V

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# *Summary & Conclusion*

## SUMMARY AND CONCLUSION

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India has large diversity of rice germplasm and in particular the Jaypore tract of Odisha is considered as secondary centre of origin. The availability of large number of land races pertaining to different ecological situation are still amenable for strategical research particularly for adverse climatic situation e.g., drought, cold, high temperature and soil nutritional stresses. Considering the prevailing paradigm shift in agro-climatic situations in different parts of India, plant scientists must be prepared to face the challenging task to provide food and nutrition for the ever-increasing world population and Asia in particular.

Genetic improvement of rice in India and many other rice growing countries has already achieved yield platuae for medium land irrigated rice ecosystems, but areas pertaining to rainfed rice ecosystems particularly lowland and high land situations are constrained with low productivity owing to erratic rainfall distribution. The existing genotypes particularly the short duration rice varieties so far developed are rarely adaptable to pre-monsoon drought situation. It has been contemplated that climatic change will compel rice breeders to reorient the breeding strategies and develop high yielding short duration rice varieties to combat the recurrent occurrence of drought in coming years.

In this context, an attempt was taken to explore desirable genotypes with sufficient level of biotic and abiotic stress resistance to be used as donor for further genetic improvement in upland rice. The present treaties included large collection upland land races of rice from different places of Odisha, popular drought tolerant donors and high yielding widely adaptable upland rice varieties. The genotypes were laid out in augmented design in the field during Kharif,2013 to record DUS characteristics and to assess agro-economic traits including yield and yield components; physiological traits and tolerance to biotic & nutritional stresses. A second field experiment for the same set of material following the above experimental design was necessitated for evaluation of genotypes for drought tolerance. The data were analysed for mean performance, genetic

variability, character association, path analysis and genetic divergence for characterization of the available germplasm lines. Besides, a set of 26 selected high yielding and/or highly drought tolerant land races and donors along with three high yielding widely adaptable upland rice varieties (Khandagiri, Sneha and Mandakini) were analysed for seed storage protein profiling using SDS-PAGE. The result obtained in the present pursuit is summarized below.

Each of the test genotypes differed in terms at least one or the other DUS characteristics and some of the genotypes were characterized as example varieties owing to their characteristic DUS parameters. Kenduphula- a upland land race has shown inherent characteristic of leaf rolling under intense sunlight.

Kinari and Chinger -2 showed high degree of drought tolerance based on leaf drying score. Harisankar, CR Dhan 143-2-2, Kinari, Hiran, Badi and Kutiarasi excelled for EBT/m<sup>2</sup>, Sarian, Dhobasaria, and Anjali had high panicle weight (>3.5g) while, Padarabank, Pustak and Pankopoot) had shown higher number of grains per panicle(>140). Similarly, a few test genotypes had shown excelling performance for grain weight and quality traits. For instance, a single land race Chinger -2 was shown to have very bold grains(GB=>2.75mm) and three popular upland rice genotypes e.g., Khandagiri, Vanaprabha and Mandakini revealed kernel length even more than 7mm. Dhanisaria and Dhobasaria revealed highest fertility status(>95%). Eight test genotypes e.g., Pandeydhan, Khursudi, Haladigundi, Asumakunda, Dhobasaria, CR 143-2-2, CR Dhan 40 and Sahbhagidhan have been identified for high yield potential under drought stress.

Number of ear bearing tillers/m<sup>2</sup> (EBT), panicle weight (PW), grain length (GL) followed by grain fertility percentage (F%) and kernel breadth (KB) exhibited high direct effects on grain yield (Table-9) as well as significant positive association with grain yield indicating their importance for genetic improvement of productivity in upland rice. Bold kernel type was shown to have significant positive association with grain fertility percentage. Regression co-efficients of panicle weight (PW) and kernel breadth (KB) were found significant at 1% level of significance indicating that these two component traits have definitely predominant influence on seed yield in upland rice. A regression

equation  $Y(\text{Seed yield}) = -12.365 + 0.420X_1 + 0.051X_2 + 2.978X_3 + 0.014X_4 + 0.046X_5 + 1.773X_6 - 2.550X_7 - 0.438X_8 + 2.736X_9 + 0.067X_{10}$  ; where  $X_i$  – represents independent variables e.g., leaf drying score(LDS), number of ear bearing tillers/m<sup>2</sup>(EBT), panicle weight(PW), grains/panicle(G/P), 1000-grain weight(GW), grain length(GL), grain breadth(GB), kernel length(KL), kernel breadth(KB) and grain fertility percentage(F%);. can be fitted for effective selection of genotypes for high seed yield among large collection of germplasm lines.

Number of ear bearing tillers/ m<sup>2</sup> (EBT) recorded as the single most important yield component trait which contributed maximum (0.9945%) to genetic divergence. Baspatri was found most divergent based on DUS characteristics while, Setka-1 and Rangahazari revealed high genetic distance from rest of the varieties based on agro-economic traits. Considering both DUS and agro-economic traits; Baspatri, N22, Damaraphuli and Lalubadikaberi may be sorted out as highly divergent genotypes. Further, it was revealed that Baspatri with either Kalakeri, Kantadumer, Dhobasaria, N22 or Dular had shown very high genetic divergence. Inter-varietal crossing between Baspatri with above genotypes may result useful transgressive segregants for desirable selection of plant types suitable for upland situation.

The SDS-PAGE seed storage proteins revealed altogether 19 scorable polypeptide bands with molecular weights ranging from 26.0 to 123.0kD and polymorphism to the extent of 78.94%. Genetic variation in relation to presence of number of polypeptide bands in the electrophoregram ranged from 9 in Khursudi to as high as all 19 bands in Brahmanaki and Vandana followed by 18 bands each in case of Anjali, Asumakunda, Hiran and Padaradhan.

The polypeptide band at 123.0kD was present only in seven test genotypes. Whereas, 101.4kD and 65.2kD bands were observed in eight and nine genotypes respectively. Thus, bands at these above molecular weight positions may be considered as most informative and therefore, these may contribute maximum for genetic dissimilarity / genetic divergence among the test genotypes.

Khursudi can be identified from rest of the test genotypes by absence of polypeptide band B5 (97.2kD) and B6 (93.0 kD). Similarly, Zhu 11-26 and Khandagiri

had uniquely absence of band B18(27.5kD) and B17(28.7kD) respectively which were present in all other short duration test genotypes including the popular drought tolerant genotypes Vandana, Sahbhagidhan, Dular and Nagina 22(N22). Such a genotype - specific finger printing can be used for varietal identification.

The polypeptide band B9 could be identified in the electrophoregram at mol wt position 60kD. Extent of staining intensity of such 60kDa protein band in different rice varieties can be a reliable parameter to assess amylose content. In this context, Khandagiri and a local upland land race 'Kinari' could not reveal such polypeptide marker indicating very low amylose status in these genotypes. Besides, Asumakunda, Bastul, Brahmanaki, Kanding, Kalakeri, Padaradhan, Salampikit, Somo, CR Dhan 40, Anjali, Vandana and Pandeydhan have been identified as candidate varieties to have intermediate amylose content,

Among the test genotypes, Khursudi (Av. S.I.=0.58) followed by Khandagiri (Av. S.I.=0.62) and Nagina 22 (Av. S.I.=0.69) had high genetic dissimilarity from most of the varieties. Khursudi maintained high genetic distance from Bastul, Brahmanaki, Zhu 11-26 and Vandana. Whereas, N22 showed appreciable genetic dissimilarity from Khandagiri and Zhu 11-26. Besides, Khandagiri was shown to be genetically distant from Asumakunda, Brahmanaki, Hiran, Khursudi, N22, Padaradhan, Anjali and Vandana. Thus, the above genotypes may have merit in hybridization programme for upland situation. Besides, Drought tolerance may be introgressed into Khandagiri using highly divergent drought tolerant donors e.g., N22, Khursudi, Kinari and Zhu 11-26 through recombination breeding.

## **CONCLUSION:**

The present investigation on genotypic characterization of ninety six upland land races including popular drought tolerant donors and high yielding widely adaptable upland rice varieties. revealed important and valuable informations for the rice researchers to be utilized for different purposes. These are depicted as below:

- ❖ The high yielding rice genotypes with wide genetic diversity selected in this study could be used immediately as parents in recombination breeding for genetic improvement in productivity.
- ❖ A few of the high yielding drought tolerant donors e.g., Khursudi, Salampikit, Dular, Dhobasaria, CR Dhan 40, Sahbhagidhan and Zhu 11-26; identified in this study may serve as valuable material for introgression of drought tolerance to high yielding genetic background of upland rice through recombination breeding.
- ❖ Number of ear bearing tillers/m<sup>2</sup>, panicle weight, grains per panicle and fertility percentage seems to have more influence on seed yield than other ancillary traits.
- ❖ SDS-PAGE is too simple compared to DNA analysis which requires sophisticated technique and laboratory requirements. SDS-PAGE may be tried first to assess for genetic relationship and for fine tuning, DNA analysis may be opted to maximize the *in-situ* genetic diversity among a set of test genotypes.
- ❖ The genotype-specific protein markers obtained in this pursuit, could specify distinctiveness of test genotypes to be used for species and varietal identification, and elimination of duplicates.

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# *Bibliography*

## REFERENCES

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- Albani D, Hammond-Kosack MCU, Smith C, Conlan S, Colot V, Holdsworth M, Bevan MW (1997). The wheat transcriptional activator SPA: a seed-specific bZIP protein that recognizes the GCN4-like motif in the bifactorial endosperm box of prolamins genes. *The Plant Cell* 9, 171–184.
- Altschuler Y, Galili G (1994). Role of conserved cysteines of a wheat gliadin in its transport and assembly into protein bodies in *Xenopus* oocytes. *Journal of Biological Chemistry* 269, 6677–6682.
- Altschuler Y, Harel R, Galili G (1993). Role of the N- and C-terminal regions of a wheat c-gliadin in its transport via the endoplasmic reticulum of *Xenopus* oocytes. *The Plant Cell* 5, 443–450.
- Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero J-M (1989). Nucleotide sequences of the two high-molecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv. Cheyenne. *Nucleic Acids Research* 17, 461–462.
- Aryan A (2006). Understanding the genetics of rice cooking quality. IREC Farmers' Newsletter, No. 174: 50-52.
- Asghar R (2004). Inter and intra-specific variations in SDS-PAGE of total seed protein in rice (*Oryza sativa* L.) germplasm. *Pak. J. Biol. Sci.* 7: 139-143.
- Aung P.P., Kumamaru T, Satoh H (2003). Genetic variation in storage protein and storage endosperm starch in local rice cultivars of Myanmar. In: Khush G S, Brar D S, Hardy B. eds. *Advance in Rice Genetics*. Manila, Philippines: IRRI., 196-198.
- Babu, V.R.(2006). Study of genetic parameters, correlations, and path coefficient analysis of rice (*Oryza sativa* L.) under saline conditions. *Annals of Agric. Res.*, 17(4):370-374.
- Bagga S, Adams H, Rodriguez FD, Kemp JD, Sengupta-Gopalan C (1997). Co-expression of the maize d- and b-zein genes results in stable accumulation of d-zein in ER-derived protein bodies formed by b-zein. *The Plant Cell* 9, 1683–1696.

- Bagga, S., Adams H, Kemp JD, Sengupta-Gopalan C (1995). Accumulation of 15-kilodalton zein in novel protein bodies in transgenic tobacco. *Plant Physiology* 107, 13–23.
- Bansal, M.P., Panwar, D.V.S., and Naidu, M.R., (1990). Genetic diversity among the breeding lines of diverse crosses of rice. *Research and Development Reporter*, 7 (1-2):90-96.
- Bartels D, Altosaar I, Harberd NP, Barker RF, Thompson RD (1986). Molecular analysis of c-gliadin gene families at the complex Gli-1 locus of bread wheat (*T. aestivum* L.). *Theoretical and Applied Genetics* 72, 845–853.
- Bartels D, Thompson RD (1986). Synthesis of messenger-RNAs coding for abundant endosperm proteins during wheat grain development. *Plant Science* 46, 117–125.
- Basavaraja, P.; Rudradhya, M. and Kulkarni, R.S. (1997). Genetic variability, Correlation and Path-analysis of yield components in F2 population of fine grain rice. *Mysore J. Agric. Sci.*, 31: 1-6.
- Bastia, D., Mishra T.K., Pradhan, B. and Das S.R., (2007). Character association and path coefficient analysis of yield and its components in upland rice genotypes. *Curr. Agric Res.*, 20 (1 & 2): 58-62.
- Beccari (1745). *De Frumento. De Bononiensi Scientiarum et Artium Instituto atque Academia Commentarii*, II. Part I., 122–127.
- Bechtel DB, Wilson JD, Shewry PR (1991). Immunocytochemical localization of the wheat storage protein triticin in developing endosperm tissue. *Cereal Chemistry* 68, 573–577..
- Behera, T. (2007). Genetic basis of yield variation in indigenous aromatic rices of Orissa, M.Sc (Ag.) Thesis, Plant Breeding and Genetics, OUAT, Bhubaneswar.
- Bellon, M.R., Brar D.S, Lu BR and Pham JL, (1998). Rice Genetic Resources. In: Bertin, J., J. Hermardinquer and M. Keul (Eds.), *Atlas of Food Crops*, pp: 73-94.
- Bharadwaj, C., Satyavati, C.T. and Subramanyam, D. (2001). Evaluation of different classificatory analysis methods in some rice (*Oryza sativa* L.) collection. *Indian J. Agric. Sci.*, 71(2):123-125.
- Bhatt, G.M., (1970). Multivariate analysis approach to selection of parents for hybridization aiming at yield improvement in self pollinated crops. *Aust. J. Agric. Sci. Res.*, 22:1-7.

- Bhowmik A, Omura T, Kumamaru T(1990). Screening of rice varieties for endosperm storage proteins. *Plant Breeding*, 105: 101-105.
- BO (1978). Properties of glutelin from mature and developing rice grain. *Phytochemistry* 17: 177-182.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.* 72: 248-254.
- Burgess SR, Shewry PR (1986). Identification of homologous globulins from embryos of wheat, barley, rye and oats. *Journal of Experimental Botany* 37, 1863–1871.
- Cai, J.Z.; Wang, V.L.; He, J .S.; Zcu, L.F; Zou, D.H.; Yuan, H.C.; Ni, J.N.; and Zhu, H.W. (1989). Relationship between yield components and dry matter production and their effects on grain yield. *Journal of Jiangsu agricultural college*, 10(4): 9-10.
- Casey R (1999). Distribution and some properties of seed globulins. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 159–169.
- Chand, S. P., Sarkar, G.B., Senapati, S.K., Roy G.S., Mandal P.D. Mahato and S. Panda (2004). Genetic variability and character association in rainfed lowland, Aman Paddy, *Envir. and Eco.*, 22 (2): 430-434.
- Chakrabarty S.K., Joshi M.A., Singh Y., Dadlani M.(2012). Example varieties for DUS testing in rice (*Oryza sativa*). *The Indian Journal of Agricultural Sciences*, 82(12): 42-49.
- Chaudhuri, M and N.K. Motiramani(2003). Variability and association among yield attributes and grain quality in traditional aromatic rice accessions. *Crop Improvement.*,30(1):84-90.
- Chaudhury, M. and Motiramani, N.k. (2003). Variability and association among yield attributes and grain quality in traditional aromatic rice accessions. *Crop Improv.*, 30(1) :84-90.
- Chen J N, Fu H Y, Lu Z X, Qin H Y, Qu J, Que Q, Cao S Y (1998). Temperature, heat shock proteins and fertility change of sorghum. *Acta Genet Sin*, 25 (4): 356-361.
- Choi S-B, Wang C, Muench DG, Ozawa K, Franceschi VR, Wu Y, Okita TW (2000). Messenger RNA targeting of rice seed storage proteins to specific ER subdomains. *Nature* 407, 765–767.

- Chowdhury MA, Vandenberg B, Warkentin T (2002). Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). *Euphytica*, 127: 317-325.
- Chuan, Z.S.; W.J. Sheing; Li Hong; H.D. Quiang; X.Z.wen; L.D. Cheng (2003). Correlation among characters of core germplasm with good quality Qinglinai No.1 and its pedigree in rice. *Acta agronomica Sinica*. 29(1); 97-104.
- Coleman CE, Herman EM, Takasaki K, Larkins BA (1996). The maize c-zein sequesters a-zein and stabilizes its accumulation in protein bodies of transgenic tobacco endo-sperm. *The Plant Cell* 8, 2335–2345.
- Coleman CE, Larkins BA (1999). The prolamins of maize. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 109–139.
- Colot V, Robert LS, Kavanagh TA, Bevan MW, Thompson RD (1987). Localization of sequences in wheat endosperm protein genes which confer tissue-specific expression in tobacco. *The EMBO Journal* 6, 3559–3564.
- Cook RJ (1984). The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5:59-72.
- Craufurd, P., Dorward, P., Opoku-Apau, A, Dartey, K. Bimpong, I and Marfo, K. (2000). Testing drought-tolerant plant types of upland rice in Ghana using participatory methods-a plant sciences research programme project. Department of International Development (DFID), West African Rice Development Association (WARDA),
- Cuddeford D (1995). Oats for animal feed. In: Welch RW, ed. The oat crop: production and utilization. London: Chapman & Hall, 321–368.
- Das, S.R., Roy, J.K., Kar, M. and Das, S.(2000). Aromatic rices of Orissa. In: *Aromatic rices of India*. Editor Prof. R.K. Singh.
- Das, S. Subudhi, H.N., Reddy, J.N., (2001). Genetic variability in grain quality characteristic and yield in lowland rice genotypes. *Oryza*, 44(4):343-346.
- De, D.K.; Pal, S.K.; Ghosh, M. and Pal, A.K. (2005). Correlation and path analysis in aromatic rice. *Annals of Agricultural research*, 26(3):398-401.
- De, R.N., Reddy, J.N., Rao, A.V.S. and Mohanty, K.K. (1992). Genetic divergence in early rice under two situations. *Indian J. Genet.* 63(1):73-74.

- Ghafoor A, Ahmad Z, Qureshi AS, Bashir M (2002). Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. *Euphytica*, 123: 367-378.
- Gilliand TJ (1989). Electrophoresis of sexually and vegetatively propagated cultivars of allogamous species. *Plant varieties and Seeds* 2: 15.
- Gomathinayagam, P., Papiah, C. M. and Sundar Pradhan, G., (1990). Path coefficient analysis in upland varieties of rice. *Madras Agric. J.*, 75(11-12); 449-450.
- Goodrich WJ., Cook RJ. and Morgan AG (1985). The application of Electrophoresis to the characterization of cultivars of *Vicia faba* L. *FABIS Newsletter*, 13:8.
- Goswami, R. K.; Barua, K.K; Pathak, P.K. and Pathak, A.K. (2000). Evaluation of rice varieties for yield attributes and milling quality. *JASS*, 13(1): 1-7.
- Goswami, R.K., Barua, K.K., Pathak, P.K. and Pathak, A.K. (2000). Evaluation of rice varieties for yield attributes and milling quality. *JASS*, 13(1): 1-7.
- Grist DH (1986). Rice. Tropical Agriculture Series. *Longman Publisher, New York*.
- Guo JC and Dong JW Electrophoresis analysis on seed protein of paddy rice and upland rice. *J Beijing Agric Univ*, 1988, 14(1): 77-81.
- Gupta, K.R; Panswar, D.V.S and Kumar, R. (1999). Character association in segregating population in basmati rice. *Oryza*, 36 (1): 16-19.
- Halford NG, Forde J, Shewry PR, Kreis M (1989). Functional analysis of the upstream regions of a silent and an expressed member of a family of wheat seed protein genes in transgenic tobacco. *Plant Science* 62, 207–216.
- Hammond-Kosack MCU, Holdsworth MJ, Bevan MW (1993). In vivo footprinting of a low molecular weight glutenin gene (LMWG-1D1) in wheat endosperm. *The EMBO Journal* 12, 545–554.
- Heck GR, Chamberlain AK, Ho DT-H (1993). Barley embryo globulin I gene, Beg1: characterization of cDNA chromosome mapping and regulation of expression. *Molecular and General Genetics* 239, 209–218.
- Hong DL, Satoh H, Kumamaru T, Qu LQ, Sadar US (2001). Protein markers of BT type hybrid and its three lines in japonica rice (*Oryza sativa* L.). *Chinese J Rice Sci*, 15(3): 165-168.

- Horikoshi M, Morita Y (1975). Localization of c-globulin in rice seed and changes in c-globulin content during seed development and germination. *Agricultural and Biological Chemistry* 39, 2309–2314.
- Hsia CC, Anderson OD (2001). Isolation and characterization of wheat v - gliadin genes. *Theoretical and Applied Genetics*,103, 37–44.
- Immanuel Selvaraj, C., Nagarajan, P., Thiagarajan, K., Bharath, M. and Rabindran, R.(2011). Genetic parameters of variability, correlation and path coefficient studies for grain yield and other yield attributes among rice blast disease resistant genotypes of rice (*Oryza sativa* L.). *African j. Biotech.*,10 (17):3322-3334.
- Iqbal SH, Ghafoor A, Ayub N (2005). Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. *Pak. J. Bot.* 37: 87-96.
- Isemura T, Shiyo N, Shigeyuki M (2001). Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. *Breed. Sci.* 51: 225-230.
- Jaccard, P. (1908). Nouvelles recherches Sur la distribution florale. *Bulletin Society Vaud Science National* 44 : 223-270.
- Jahan MS, Kumamaru T, Satoh H, Hamid A (2003). Genetic diversity in seed storage proteins of Bangladeshi rice cultivars. In: Khush G S, Brar D S, Hardy B. eds. *Advance in Rice Genetics*. Manila, Philippines: IRRI,. 194-195
- Javid I, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak. J. Bot.* 36: 25-29.
- Jena, B.K. (2002). Genetic basis of yield variation in scented rice . M.Sc (Ag.) Thesis. Deptt. Of Plant breeding and genetics, OUAT, Bhubaneswar.
- Joshi, M.A., Singh, P., Sarao, N.K., Sharma, R.C., Bharaj, T.S.,(2008). Genetic divergence among rice varieties in Punjab. *Oryza*, 45 (4):277-279.
- Kar, A.K.(2008). Morphological characterization of indigenous aromatic rice for high yield and yield stability. Ph.D. Thesis, Department of Plant Breeding and Genetics, OUAT, Bhubaneswar.

- Kawakatsu T, Yamamoto MP, Hirose S, Yano M, Takaiwa, F. (2008). Characterization of a new rice glutelin gene GluD-1 expressed in the starchy endosperm. *J Exp Bot* 59: 4233–4245.
- Kermode AR, Bewley JD (1999). Synthesis, processing and deposition of seed proteins: The pathway of protein synthesis and deposition of the cell. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 807–841.
- Khedikar, V.P.; A.A. Bharose; d. Sharma ; Y.P. khedikar and A.S.khिलारे (2004). Path coefficient analysis of yield components of scented rice. *J. of soils and Crops*. 14(2): 228-342.
- Kochko A (1987). Isozymic variability of traditional rice, *Oryza sativa* L. in Africa. *Theor. Appl. Genet.*, 73: 675-682.
- Kreis M, Forde BG, Rahman S, Miflin BJ, Shewry PR (1985). Molecular evolution of the seed storage proteins of barley, rye and wheat. *Journal of Molecular Biology* 183, 499–502.
- Krishnan HB, Franceschi VR, Okita TW (1986). Immunochemical studies on the role of the Golgi complex in protein body formation in rice seeds. *Planta* 169: 471–480.
- Krishnan HB, Franceschi VR, Okita TW (1986). Immunochemical studies on the role of the Golgi complex in protein body formation in rice seeds *Planta* 169, 471–480.
- Kriz AL (1989). Characterization of embryo globulins encoded by the maize Glb genes. *Biochemical Genetics* 27, 239–251.
- Kriz AL (1999). 7S globulins of cereals. In: Shewry PR, Casey R, eds Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 477–498.
- Kriz AL, Schwartz D (1986). Synthesis of globulins in maize embryos. *Plant Physiology* 82, 1069–1075.
- Kriz AL, Wallace NH (1991). Characterization of the maize Globulin-2 gene and analysis of two null alleles. *Biochemical Genetics* 29, 241–254.
- Kumamaru T, Satoh H, Iwata N, Omura T, Ogawa M, Tanaka K (1988). Mutants for rice storage proteins: Screening of mutants for rice storage proteins of protein bodies in the starchy endosperm. *Theor Appl Genet*, 76:11-16.

- Kusama T, Yangi SO, Iwamoto M (1984). Comparison of rice (*Oryza sativa* L.) seed proteins from several varieties. *Agric Biol Chem*, , 48(6): 1649-1651.
- Laemmli, U.K. (1970). Cleavage of structural protein during the assembly of the head of bacteriophage. *Nature*, **227**: 680-685.
- Lamacchia C, Shewry PR, Di Fonzo N, Forsyth JL, Harris N, Lazzeri PA Napier JA, Halford NG, Barcelo P (2001). Endosperm-specific activity of a storage protein gene promoter in transgenic wheat seed. *Journal of Experimental Botany*.52, 243–250.
- Laxuman, P.M. Salimath, Sashidhar, H.E. Mohankumar, H.D., Patil, S.S., Vamadevaiah, H.M., and Janagoudar, B.S.(2011). Character association and path coefficient analysis among the backcross inbred lines derived from Indica x Nerica cross for productivity traits in rice (*Oryza sativa* L.) *Karnataka J. Agric. Sci.*,24 (5):626-628.
- Leite A, Neto GC, Vettore AL, Yunes JA, Arruda P (1999). The prolamins of sorghum, Coix and millets. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 141–157.
- Lending CR, Chesnut RS, Shaw KL, Larkins BA (1989). Immunolocalization of avenin and globulin storage proteins in developing endosperm of *Avena sativa* L. *Planta* 178, 315–324.
- Lending CR, Larkins BA (1989). Changes in the zein composition of protein bodies during maize endosperm development. *The Plant Cell* 1, 1011–1023.
- Li X, Franceschi V, Okita TW (1993a). Segregation of storage protein mRNAs on the rough endoplasmic reticulum membranes of rice endosperm membrane. *Cell* 72, 869–879.
- Li X, Wu Y, Zhang D-Z, Gillikin JW, Boston RS, Franceschi VR, Okita TW (1993b). Rice prolamins protein body biogenesis: a BiP-mediated process. *Science* 262, 1054–1056.
- Li XX, Wu YJ, Zhang DZ, Gillikin JW, Boston RS, Franceschi VR, Okita TW (1993). Rice prolamins protein body biogenesis: a BiP-mediated process. *Science* 262: 1054–1056.

- Lin JQ, He ZH, Ma ZC, Li DB (1997). Studies on polymorphism of rice prolamines. *Chinese J Rice Sci*, 11(3): 129-135.
- Liu G Q, Zhu B G, Chen J N (2003). Fertile inheritance of the progeny from male sterile line 3197A of Sorghum after heat shock treatment. *J Plant Physiol & Mol Biol*, 29(1): 71-74.
- Lockhart HB, Hurt HD (1986). Nutrition of oats. In: Webster FH, ed. Oats: chemistry and technology. St Paul, Minnesota, USA: *American Association of Cereal Chemists, Inc.*, 297-308.
- Lu ZM, Wang H, Shen Y J. Polymorphism of endosperm storage protein and application in hybrid rice (*Oryza sativa* L.). *J Nanjing Agric Univ*, 2001, 24(2): 6-11.
- Mahalanobis, P.C. (1928). A statistical study of Chinese heads' measurement. *Journal of Asiatic Society* (Bengal), 25: 301-317.
- Mahto, R.N., Yadav, M.S., Mohan, K.S. (2003). Variability, correlation and path analysis in early rice. *J. Res. Birsa Agric. Univ.* 15.(2):261-263.
- Maity S, Datta AK, Chattopadhyay A (2009). Seed protein polymorphism in nine species of Jute (*Corchorus*, Family: *Tiliaceae*). *Indian J. Sci. Technol.* 2: 34-36.
- Manonmani, S. AND Fazlullah Khan, A.K. (2003). Analysis of genetic diversity for selection of parents in rice. *Oryza*, 40, (3&4):54-56.
- Marris C, Gallois P, Copley J, Kreis M (1988). The 59 flanking region of a barley B hordein gene controls tissue and developmental specific CAT expression in tobacco plants. *Plant Molecular Biology* 10, 359-366.
- McMaster TJ, Miles MJ, Wannerberger L, Eliasson A-C, Shewry PR, Tatham AS (1999). The identification of microphases in mixed a- and c-gliadin protein films investigated by atomic force microscopy. *Journal of Agricultural and Food Chemistry* 47, 5093-5099.
- Mennella G, Onofaro SV, Tonini A, Magnifico V (1999). Seed storage protein Characterization of Solanum species and of cultivars and androgenic lines of *S. melongena* L., by SDS-PAGE and AE-hplc. *Seed Sci. Technol.* 27: 23-35.

- Mishra, L.K. and verma, R.K.(2002). Correlation and path coefficient analysis for morphological and quality traits in rice (*Oryza sativa* L.). *Plant archives*, **2**(2): 275-284.
- Montalvan R, Ando A, Echeverrigaray S (1998). Use of seed protein polymorphism for discriminating of improvement level and geographic origin of upland rice cultivars. *Genet. Mol. Biol.* **21**: 531-535.
- Muench DG, Ogawa M, Okita TW (1999). The prolamins of rice. In P Shewry, R Casey, eds, *Seed Proteins*. Kluwer Academic Publishers, *Dordrecht, The Netherlands*, pp 93–108.
- Muench DG, Ogawa M, Okita TW (1999). The prolamins of rice. In: Shewry PR, Casey R, eds. *Seed proteins*. *Dordrecht: Kluwer Academic Publishers*, 93–108.
- Muench DG, Wu Y, Coughlan SJ, Okita TW (1998). Evidence for a cytoskeleton-associated binding site involved in prolamine mRNA localization to the protein bodies in rice endosperm tissue. *Plant Physiology* **116**, 559–569.
- Muller M, Knudsen S. (1993). The nitrogen response of a barley C- hordein promoter is controlled by positive and negative regulation of the GCN4 and endosperm box. *The Plant Journal* **4**, 343–355.
- Mullins, I.M (1999). Phylogeny of *Oryza*, Poaceae, inferred from prolamin (seed storage protein) gene. *International Botanical Congress*. St. Louis, Missouri, pp: 1-7.
- Murty, B.R. and Arunachalam, V. 1966. The nature of genetic divergence in relation to breeding system in some crop plants. *Indian J. Genet.*, **26A**:188-198.
- Nath, N. and Talukdar, P. (1997). Genetic variability and correlation studies in segregating populations of indigenous scented X high yielding non-scented crosses of rice. *Oryza*, **34**(2): 91-93.
- Nayak, A.K.; Chaudhury, D. and Reddy, J.N. (2001). Correlation and path analysis in scented rice. *Indian J. Agric. Res.*, **35**(3) : 86-89.
- Nayak, A.R., Chaudhury, D. and Reddy, J.N.,(2001). Correlation and path analysis in scented rice (*Oryza sativa* L.). *Indian J. Agric. Res.*, **35**(3):186-189.
- Naz F, Farooq S, Arshad R, Afzaal M, Akram M (2006). Discriminating upland and lowland rice genotypes through proteomic approach. *Pak.J. Bot.* **38**: 1731-1738

- Netra N, Prasad S (2007). Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins. *Seed Sci. Technol.* 35: 176-186.
- Panse VG and Sukhatme PV(1985). Statistical methods for agriculture workers. Fourth edition, p 97-123.
- Prajapati, M.K., Singh, C.M., Suresh Babu, G. Rupalavanya, G., and Jadhav,P.(2011).Genetic parameters for grain yield and its component character in rice. *Electronic J. Plant Breed.*, 2 (2):235-238.
- Prasad, G.S.K., Prasad, A.S.R., Sastry, M.V.S., and Srinivasan, T.E.(1998).Genetic relationship among yield components in rice. *Indian J. Agric. Sci.*, 58(6):470-472.
- Qu LQ, Wei XL, Satoh H, Ogawa M, Kumamaru T(2001),. Seed storage glutelin  $\alpha$ -2 subunit lacking mutants in rice. *Acta Bot Sin*, 43(11): 1167 - 1171.
- Quayle T, Feix G (1992). Functional analysis of the - 300 region of maize zein genes. *Molecular and General Genetics* 231, 369–374.
- Rabbani MA, Pervaiz ZH, Masood MS (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electron. J. Biotechnol.* 11: 1-10.
- Ramiah, K. (1953). Rice Breeding and Genetics Scientific Monograph No. 19, Indian Council of Agricultural Research, New Delhi.
- Rao CR (1952). Advanced statistical methods in Biometric Research. *John Wiley and Sons*. New York. P. 1-88.
- Rao, S.S.,(2000). Estimation of grain yield and inter-relationship with yield components in upland rice. *Mysore J. Agric. Sci*, 34:142-146.
- Rather, A.G., Zargar M.A. and Sheikh, F.A.(2001).Genetic divergence in rice (*Oryza sativa* L.) under temperate conditions. *Indian J. Agric. Sci.*,71:344-45.
- Ravi M, Geethanjali S, Sameeyafarheen F and Maheswaran M (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*, 133: 243-252.
- Reddy. J.N. and Mahana, N.K., (1992). Divergence analysis in short duration rice. *Crop Improvement* , 19(1):34-37.

- Reddy. Y.,(2008) Genetic in yield attributes and yield of rice. *Madras Agril. J.*,82(4):310-313.
- Roy , B., Hossin, M. and Hossin, F (2001). Genetic variability in yield components of rice (*Oryza sativa* L.). *Environment & Ecology*, 19(1) : 186-189.
- Sadia M, Malik SA, Rabbani MA, Peace SR (2009). Electrophoretic Characterization and the relationship between some brassica species. *Electron. J. Biol.* 5:1-4.
- Sadia M, Malik SA, Rabbani MA, Peace SR (2009). Electrophoretic Characterization and the relationship between some brassica species. *Electron. J. Biol.* 5:1-4.
- Sammour RH (1991). Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. *J. Islamic Acad. Sci.* 4: 221-226.
- Sano Y, Katsumata M, Okuno K (1986). Genetic studies of speciation in cultivated rice: 5. Inter and intra-specific differentiation in the waxy gene expression of rice. *Euphytica*, 35: 1-9.
- Sano Y, Makekawa M, Kikuchi H (1985). Temperature effects on Wx protein level and amylose content in the endosperm of rice. *Heredity*, 76: 221-222.
- Sarawagi , A.K. and Bisne Rita (2007). Studies on genetic divergence of aromatic rice germplasm for agro morphological and quality characters. *Oryza*, 44(1): 74-76.
- Sarker R and Bose (1984). Electrophoretic characterization of rice varieties using single seed (salt soluble) proteins. *Theor Appl Genet*, 68: 415-419.
- Saruyama H, Shinbashi N (1993). Identification of specific proteins from seed embryos by two-dimensional gel electrophoresis for the discrimination between indica and japonica rice. *Theor. Appl. Genet.* 84: 947-951.
- Satish, Y.; K.V. Seetharamanmaiah; N. Srerama and T.C.M. Naidu (2003). Genetic variability and genetic advance in scented rice. *The Andhra agric. J.* 50(3-4): 232-234.
- Satoh H, Ching'ang'a HM, Ilaila D, Katayama TC. (1990b). SDS-PAGE analysis of storage proteins of cultivated rice collected in Tanzania, 1988. Kagoshima university research center for the south pacific, *Occasional papers*, 18: 114-126.

- Sato H, Ronald RX, Katayama TC(1990a) . SDS-PAGE analysis of storage proteins of cultivated rice collected in Madagascar 1988. Kagoshima University research center for the south pacific, *Occasional papers*, , 18: 101-113.
- Sharma, M.K., Richharia, A.K. and Agarwal, R.K.(1990). Variability heritability, genetic advance and genetic divergence in upland rice. *International Rice Research Notes*, 21(1):25-26.
- Sharma, S. D., Smita Tripathy and J. Biswal.(2000): Origin of O. Sativa and its ecotypes. In: Rice Breeding and Genetics – Research priorities and Challenges (Ed. J.S. Nanda), *Oxford and IBH publishing Co. Pvt. Ltd.*, New Delhi, pp. 349-369.
- Shewry PR (1999). The synthesis, processing and deposition of gluten proteins in the developing wheat grain. *Cereal Foods World* 44, 587–589.
- Shewry PR, Brennan C, Tatham AS, Warburton T, Fido R, Smith D, Griggs D, Cantrell I, Harris N. 1996. The development, structure and composition of the barley grain in relation to its end use properties. In: Cereals 96. *Proceedings of the 46th Australian Cereal Chemistry Conference*, Sydney, September 1996, 158–162.
- Shewry PR, Tatham AS, Barro F, Barcelo P, Lazzeri P. 1995. Biotechnology of breadmaking: unravelling and manipulating the multi-protein gluten complex. *Biotechnology* 13, 1185–1190.
- Shewry PR, Tatham AS, Halford NG (1999). The prolamins of the Triticeae. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 35–78.
- Shewry PR, Tatham AS. (1990). The prolamin storage proteins of cereal seeds: structure and evolution. *Biochemical Journal* 267, 1–12.
- Shotwell MA (1999). Oat globulins. In: Shewry PR, Casey R, eds. Seed proteins. *Dordrecht: Kluwer Academic Publishers*, 389–400.
- Shu QY, Wu DX, Xia YW, Gao MW, Aryes NM, Larkin PD, Willam PD (1999). Microsatellites polymorphism on waxy gene locus and their relationship to amylose content in indica and japonica rice, *Oryza sativa* L. *Acta Genet Sin*, 26(4): 350-358.
- Shukla, V ., Singh , S. and Singh H. And Pradhan S.K. (2006). Multivariate analysis in tropical japonica “New Plant Type” rice (*Oryza sativa* L.). *Oryza*, 43 : 203-07.

- Siddiqui SU, Satoh H, Kumamaru T (2003). Variation in seed storage proteins of Pakistani rice germplasm. In: Khush G S, Brar D S, Hardy B. *Advance in Rice Genetics*. Manila, Philippines: IRRI. 198-200.
- Singh NK, Donovan GR, Carpenter HC, Skerritt JH, Langridge P (1993). Isolation and characterization of wheat triticin cDNA revealing a unique lysing-rich repetitive domain. *Plant Molecular Biology* 22, 227-237.
- Singh NK, Shepherd KW (1985). The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. *Theoretical and Applied Genetics* 71, 79-92.
- Singh NK, Shepherd KW, Langridge P, Gruen LC, Skerritt JH, Wrigley CW (1988). Identification of legumin-like proteins in wheat. *Plant Molecular Biology* 11, 633-639.
- Singh, L.; Singh, J.D. and Sachan, N.S. (2002). Inter character association and path analysis in Paddy. *Annals of Biology*, 18(2): 125-128.
- Singh. U.K., Mishra, S.B. and Jha, P.B. (2000). Variability and interrelationship studies on some quantitative traits in boro rice. *Oryza*. 37(3): 187-190.
- Sinha, S.K., (2004). New approaches and techniques of cost-saving potential in crop improvement. In: Souvenir, Golden Jubilee Celebration, College of Agriculture, Bhubaneswar, Orissa, pp.32-38.
- Sinha, P.K., Variar, M., Singh, C. V., Prasad, K. and Singh, R.K. (1991). A new upland rice variety "Vandana" for Bihar plateau. *Indian Farm*, 44 (33), 1-3.
- Smith DB, Lister PR (1983). Ge 1 forming proteins in barley grain and their relationship with malting quality. *Journal of Cereal Science* 1, 229-239.
- Sokal, R.R. and Michener, C.D. (1958). A statistic method for evaluating systematic relationships. *University Kansas Scientific Bulletin* 28 :1409-1438.
- Song TM, Zheng DH, Liu Y (1996). Identification of corn varieties using lactate polyacrylamide gel electrophoresis of seed albumins and globulins. *Acta Bot Sin*, 38(8): 599-604.
- Sørensen MB, Cameron-Mills V, Brandt A (1989). Transcriptional and post-transcriptional regulation of gene expression in developing barley endosperm. *Molecular and General Genetics* 217, 195-201.
- Sun YQ, Nakayama H, Katsutoshi O, Kazuo I (2000). Variation in endosperm protein of rice germplasm in Yunnan Province. *Acta Agron Sin*, 26(4): 438-443.

- Tahir, M., Wadan, D. and Zada A. (2002). Genetic variability of different plant and yield characters in rice. *Sarhad journal of Agriculture.*, 18(2):207-210.
- Takaiwa F, Ogawa M, Okita TW. (1999). Rich glutelins. In: Shewry PR, Casey R, eds. Seed proteins. Dordrecht: *Kluwer Academic Publishers*, 401– 425.
- Takeda M, Namba Y, Nunokawa Y (1970). Heterogeneity of rice glutelin. *Agric Biol Chem* 34: 473-475.
- Talukdar, A. and Takukdar, P. (1998). Inheritance of photoperiod insensitivity and genetic relationship of grain yield with some attributes in scented rice. *Indian Journal of Hill Farming*, **11**(1-2): 39-45.
- Tanaka K, Sugimoto T, Ogama M, Kasai Z (1980). Isolation and characterization of two types of protein bodies in the rice endosperm. *Agric Biol Chem* 44: 1633-1639.
- Tecsi L, Darlington HF, Harris N, Shewry PR (2000). Patterns of protein deposition and distribution in developing and mature barley grain. In: Barley genetics, VIII. *Proceedings of the 8th International Barley Genetics Symposium*, Adelaide, Australia, Vol. 2, 266–268.
- Tecson EMS, Esmama BV, Lontok LP, Juliano BO (1971). Studies on the extraction and composition of rice endosperm glutelin and prolamin. *Cereal Chem* 48: 168-181.
- Thomas MS, Flavell RB. (1990). Identification of an enhancer element for the endosperm-specific expression of high molecular weight glutenin. *The Plant Cell* 2, 1171–1180.
- Torrent M, Geli MI, Ruiz-Avila L, Canals J, Puigdomenech P, Ludevid MD. (1994). Role of structural domains for maize c-zein retention in *Xenopus* oocytes. *Planta* 192, 512–518.
- Vanaja, T., Babu, L.C., Radhakrishnan, V.V. and Unnithan, V.K.G. (2003). Genetic divergence in high yielding rice genotypes. *Oryza*, 40(1&2):40-42.
- Vivekanandan and Subramanian, S. (1993). Genetic diversity in rice. *Oryza*, 30:60-62.
- Vivekanandan, P. and Giridharan, S. (1998). Genetic variability and character association for kernel and cooking quality in rice. *Oryza*, **35**(3): 242-245.
- Wallace NH, Kriz AL (1991). Nucleotide sequence of a cDNA clone corresponding to the maize Globulin-2 gene. *Plant Physiology* 95, 973–975.

- Wei-dong Jin , Na Li and De-lin HONG (2006). Genetic Diversity of Seed Storage Proteins in Different Ecotype Varieties of japonica Rice and Its Application. *Rice Science*,13(2): 85-92.
- Wu H and Chen J (1978). Protein bodies in developing rice grain. *Proc Natl Sci Counc. Repub China* 2: 281-292.
- Xu JH, Messing J (2009). Amplification of prolamin storage protein genes in different subfamilies of the Poaceae. *Theor Appl Genet* 119: 1397–1412
- Yadav, R. K.(2000). Studies on genetic variability for some quantitative characters in rice (*Oryza sativa* L.). *Advances in Agricultural Research in India*.13: 205-207.
- Yadav, R. K., Suresh, B. G., Pandey , P. and Binod K. (2001) . Assesment of genetic variability , correlation and path association in rice (*Oryza sativa* L.).*J. Bio.Sci.* 18:1-8.
- Yamagata H, Sugimoto T, Tanaka K, AND Kasai Z(1982). Biosynthesis of storage proteins in developing rice seeds. *Plant Physiol.* (1982) 70, 1094-1100.
- Yamagata H, Tanaka K (1986). The site of synthesis and accumulation of rice storage proteins. *Plant and Cell Physiology* 27, 135–145.
- Yang TY, Shen YH, Huang XG, He JH, Wu GZ(2005). Identification of genetic diversity in the foxtail millet (*Setaria italic* L. Beauv.) by A-PAGE. *Acta Agron Sin*, 131(1): 131-133.
- Yu C M, Chen P D. Study on common wheat (*T. aestivum* L.) germplasm for waxy protein. *J. Nanjing Agric Univ*, 2003, 26 (3): 1-6.
- Yupsanis T, Burgess SR, Jackson PJ, Shewry PR (1990). Characterization of the major protein component from aleurone cells of barley (*Hordeum vulgare*) L. *Journal of Experimental Botany* 41, 385–392.
- Zhan XY and Lin RH (1991). Electrophoresis analysis on glutelin of cultivar rice and wild rice. *Chinese J Rice Sci*, 5(3): 109-113.
- Zhang CQ, Yin YP, Gao RQ, Jia JZ (1998). Polymorphism of seed protein and identification of cotton cultivar. *Sci Agric Sin*, 31(4): 16-19.
- Zuo Qingfan, Liu Yibai, Pan Xiao Yun, Zhu Yun and Xie Ping (2001). Genetic correlation analysis of yield and quality characters of rice (*Oryza sativa* L.) multiple environments. *Acta Agriculturae Universitatis Jiangxiensis*, 23 (1):16-23.