GENETIC DIVERSITY OF RICE (*Oryza sativa* L.) LOCAL ACCESSIONS BASED ON MORPHOLOGICAL AND MOLECULAR CHARACTERISTICS

By
MUHAMMAD ISMAIL

Faculty of Biological Sciences
Quaid-i-Azam University
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IN THE NAME OF ALLAH,
THE MERCIFUL,
THE BENEFICIENT

It is **who** sends down water from the skies and brings out of it every thing that grows, the green foliage, the grain lying close, the date palm trees with clusters of dates and the gardens and olives and pomegranates, so similar yet so unlike.

(Al-Quran, sura Al-Aanam. 99)

Dedicated

To

My affectionate late  who always wished to see me at the highest peaks of humanitarian level and consumed energies of his life to bloom it. May Allah Pak set his soul in heavens under His warmth blessings for ever and ever (Aamin)

DECLARATION

This thesis submitted by **Muhammad Ismail** is accepted in its present form by the Faculty of Biological Sciences, Quaid-i-Azam University Islamabad as fulfilling the thesis requirement for the degree of Master of Philosophy in Biotechnology.

Supervisor:

(Prof. Dr. M. Fayyaz Chaudhary)

External Examiner:

(Prof. Dr. Ehsan Elahi)

Chairman:

(Prof. Dr. M. Fayyaz Chaudhary)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURS

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(Muhammad Ismail)

ABSTRACT

The present study was carried out on 72 accessions of rice (*Oryza sativa* L.) plotted in augmented design at Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agriculture Research Centre (NARC), Islamabad. All accessions were local, out of which 70 were wild and 2 commercial varieties. evaluated. The germplasm were provided by gene bank of IABGR, NARC, Islamabad. All of these accessions completed life cycle successfully and were analyzed for morphological and molecular (SDS-PAGE) level to ascertain the extent of genetic diversity among the accessions. At morphological level 19 different characteristics were studied, out of which 7 were qualitative and 12 were quantitative characteristics. For qualitative characters most significant level of diversity was observed in seed coat color (frequency 8, 11.11%). Significant level of variability was seen in flag leaf (Frequency 4, 5.55%) and panicle exertion (frequency 4, 5.55%). Data regarding quantitative traits were analyzed by descriptive statistics and association among various traits was estimated by correlation analysis. Descriptive statistics of quantitative traits revealed that considerable extent of diversity exists in six quantitative traits. Plant height (113.7 ± 33.72%), productive tillers per plant (7.5 ± 37.25%), panicle length (25.3cm ± 19.21%), spikelets per panicle (11.5 ± 17.7%), 100-seed weight, (1.3 ± 46.16%) and straw yield (95.2 ± 18.8%). Results based on correlation analysis indicated that these 12 characters are associated positively as well negatively. Seeds protein banding profiles were observed by using SDS-PAGE analysis, which showed considerable variation for glutelin and prolamin bands. Variability was seen in the whole germplasm on the basis of acidic glutelin (α3, α4) bands, basic glutelin (β3) band and prolamin (13a, b) bands. SDS-PAGE analysis showed stability of the characters under consideration i.e. free from environmental stresses, phylogenetic relationship among the accessions, diversity among accessions at gene level, sorting the accessions of the same locality, variation occurred in the accessions by out breeding, intimation for the selection of accessions with best performance (diversity level).

CHAPTER-1

INTRODUCTION

Rice:

Rice, a member of the grass family, along with wheat and corn, is one of the three crops on which the human species largely subsists. The distribution of people and the basic grain they consume is one of environmental determination, dating to the agricultural revolution of pre-history with redistributions in the last centuries.

Rice belongs to the genus *Oryza* and has two cultivated and 22 wild species. The cultivated species are *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* is grown all over the world while *Oryza glaberrima* has been cultivated in West Africa for the last ~3500 years (IRRI, 2001). Rice is grown under many different conditions and production systems, but submerged in water is the most common method used worldwide. Rice is the only cereal crop that can grow for long periods of time in standing water (Gramene, 2004). 57% of rice is grown on irrigated land, 25% on rainfed lowland, 10% on the uplands, 6% in deepwater, and 2% in tidal wetlands (Chopra, V.L. and S. Prakash, eds., 2002).

Historically the basic food source for mankind, rice along with wheat, has been selected and domesticated by the ancestors as a staple crop from it's wild progenitors. Now rice, wheat and maize are the three leading food crops in the world; together they directly supply more than 50% of all calories consumed by the entire human population, in which rice provides 20% of global human per capita energy and 15% of per capita protein (IRRI, 1993).

According to Chowdhury and Ghose (1953) excavation record shows the existence of rice by 1000BC in Northern India. Morinaga (1967, 1968) believed that the birth place of cultivated rice was southern foot of Himalaya mountains. There lies great possibility that, while it's cultivation spread in Indian subcontinent, it also reached in the areas of today's Pakistan.

Today, rice plants are grown to about 1,500 million hectares, more than 90% of which extends in East Asian countries, and feed more than one-third of the world's population. Rice is most closely associated with the South, Southeast, and

East Asian nations extending from Pakistan to Japan (IRRI, 1993). Rice plants grown in this region is *Oryza sativa* L. of family Poaceae. Genomes A, B, C, D, E and F have been found in genus *Oryza* while A is contained in the Sativa complex (Matsuo et al., 1997).

Kato (1930) found differentiation of *O. Sativa* into Japanese (*japonica*) and Indian (*indica*) subspecies. Chu (1967) also reported that in view of the peroxidase zymogram, Asian cultivars of *O. sativa* could be divided into two groups that largely correspond to *Indica* and *japonica* types.

According to report of Katsuta and Okuno (1992) the local varieties in northern Pakistan are typically classified into two groups, based on the shape of grain. According to them, varieties of the round shaped are adapted to higher locations, whereas, varieties of the slender shaped are distributed in the location below 1000m. Similar results were illustrated by Nagamine *et al.* (1992) for the distribution of Chinese germplasm from Yunnan province.

Rice is grown in widely diverse production environments in terms of topography, soil type, water regime and climatic factors. Aside from irrigated areas, there are four other types of environments, based mainly on the water regime- rainfed lowland including tidal wetland, deep water, upland and dry land areas (Kush, 1984). Rice is also characterized as an ideal plant material for genetic researches because of it's larger genetic diversity and smaller genome size, compared with other cereal crops.

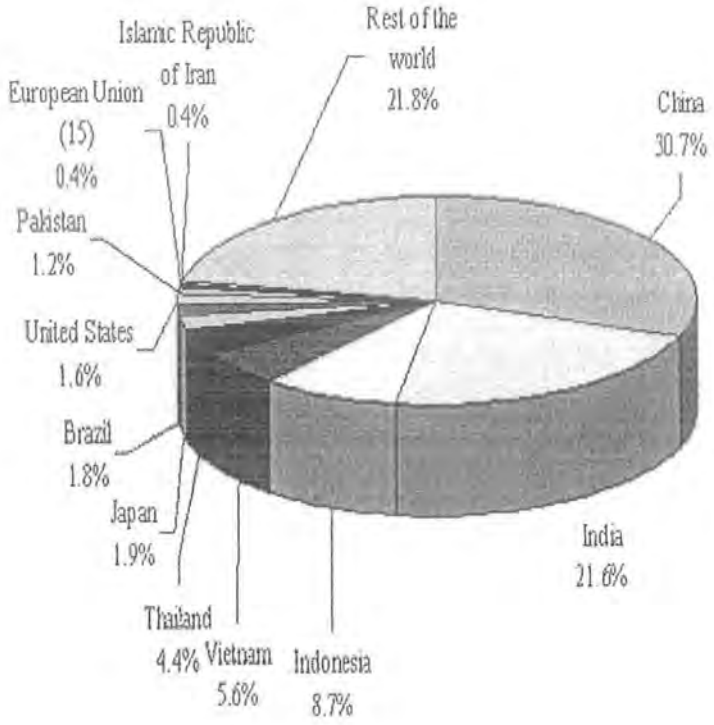
Rice is the staple food of the majority of the world's population and rice crop sales account for nearly 10% of the value of the global market. Rice is central to the lives of billions of people around the world. Possibly the oldest domesticated grain (~10,000 years), rice is the staple food for 2.5 billion people (International Year of Rice, 2004. "Gender and rice" factsheet) and growing rice is the largest single use of land for producing food, covering 9% of the earth's arable land. Rice provides 21% of global human per capita energy and 15% of per capital protein (IRRI, 2002). Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake (IRRI, 2001). As expected, Asia accounts for over 90% of the world's production of rice, with China, India, Thailand, Pakistan, Iran and Indonesia producing the most (IRRI, 2003). Only 6-7% of the world's rice crop is traded in the world market. Thailand,

Vietnam, China and the United States are the world's largest exporters (IRRI, 2004).

According to reports of Food & Agriculture Organization (FAO) of United Nations during the years 2004-2005 total rice production in the world is 608.50 million tons on an area of 153.25 million hectares. Production is geographically concentrated in Western and Eastern Asia with more than 90 percent of world output. China and India, which account for more than one-third of global population (52.3%), supply over half of the world's rice. Brazil is the most important non-Asian producer, followed by the United States. Italy ranks first in Europe.

World production has shown a significant and very steady growth, almost exclusively due to increasing production in Western and Eastern Asia.

Fig 1.1 Distribution of the world paddy rice production (FAO 2004-2005)



Pakistan

The future prosperity and economic stability of Pakistan mainly depends upon the quantum of material resources and their judicious exploitation and utilization. The population of Pakistan is increasing at an alarming rate of 2.0% per annum (Govt. of Pakistan, 2005). Therefore, there is dire need for advanced planning and research to increase food production and improve quality in order to meet the needs of ever increasing population.

Rice is a versatile crop, it can grow at the elevation of more than 3000 m in the Himalayas and at sea level in the deltas of great rivers of Asia. It is one of the most important cereal crops and is the staple food of majority of the people in the world. In Pakistan it ranks 2nd in consumption after wheat. During 2004-05 Pakistan exported 1.916 million tons rice and earned $ 540 million in the form of valuable foreign exchange (MINFAL, Govt. of Pakistan, 2005). Rice accounts for 5.7% value added in agriculture and 1.3% in GDP. In Pakistan, it is grown on an area of 2503 thousand hectares with the average yield of 1994 kg per hectare (MINFAL, Govt. of Pakistan, 2005).

A considerable yield gap exists between average and potential yield of rice in Pakistan. This gap is attributed to many factors such as high weed infestation, imbalanced use of fertilizers, inadequate supply of irrigation water and improper plant protection measures but sub-optimum plant population and uneven crop stand resulting from poor nursery seedlings is one of the most important yield limiting factors in rice (Sharma, 1998). Our farmers have been adapting traditional seed soaking for rice nursery sowing since decades. This results in poor germination and uneven nursery stand (Ahmad, 1998). Improved seed invigoration techniques are being used in many parts of the world to reduce the germination time, synchronize germination, improved germination rate and better seedling stand (Khan, 1992; Lee and Kim, 2000).

Pakistan is located in between 24° and 36° N latitude and 62° and 76° E longitude. The wedge shaped country is bounded on the south by the Arabian sea, on the southwest by Iran, on the northwest by Afghanistan, on the northeast by Peoples Republic of China and on the east by India. Elevation ranges from sea level on the Arabian sea to over 7600 m in the north. The country is roughly divided into three geographical regions: the highlands, where the western extension

of Himalayan mountains meet that of the Hindu Kush; the vast southwestern plateau and mountains on the northwestern flank of the country; upper and lower plains of the Indus River. Pakistan lies in AEZ5, characterized as warm arid and semiarid subtropics with summer rainfall (IRRI 1993).

Pakistan is fortunate to be one of the centers of diversity for many plant species. It is located in the ancient passage for travelers; so many crops were introduced by early migrants; while many domesticated thousands of years ago in the region. However, this germplasm is faced with the threat of genetic erosion due to replacement by newly introduced varieties and change in land utilization. From all over Pakistan germplasm of different crops has been collected. The collected cultivars belong to different agro climatic conditions and distributed in different locations in altitude. In the National gene bank at Institute of Agricultural Biotechnology & Genetic Resources (IABGR), National Agriculture Research Center (NARC), Islamabad, Pakistan thousands of accessions of different crops are preserved out of which 2500 are rice accessions which include 655 base samples, many of which are mixed populations.

Rice is the 2nd most important food crop in Pakistan, not only in respect of local consumption but also in view of its large exports. About 1/3 of total rice production is exported, which contributes about 20% of the foreign exchange earnings (MINFAL, 2005). Pakistan rice export was about 10% of the world rice trade, in which the international market price for basmati rice has been three times more than that of the modern varieties.

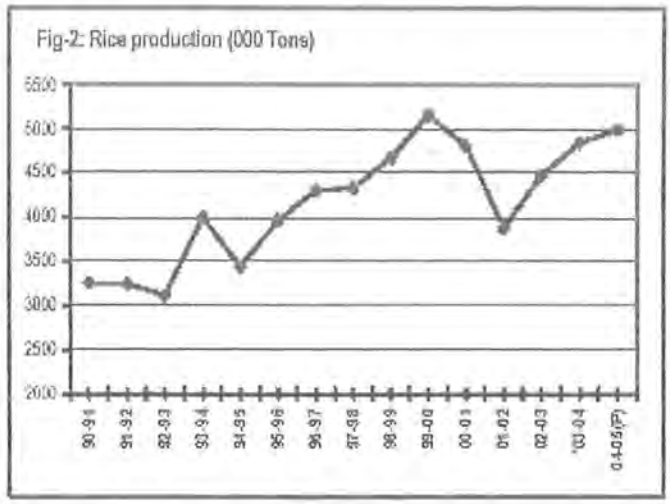
Table 1.1 Statistics of rice production in Pakistan during 2000-2005

(MINFAL 2005)

Year	*Area (Hectares*	*Production (000 tons)*	*Yield (Kgs/Hec)*
2000-01	2377	4803	2021
2001-02	2114	3882	1836
2002-03	2225	4477	2013
2003-04	2461	4848	1970
2004-05	2503	4991	1994

According to MINFAL (2005) reports annual production of rice has increased in Pakistan from year 1990-91 to 2004-05, however there was fluctuation in this productivity during years 1995-96 and 2001-02 due to water shortage in the country.

Fig: 1.2 Annual rice production data from 1990-2005.



Takahashi (1997) stated that Oryza sativa L. the cultivated rice crop native in Asia, has a genetic diversity of ecological conditions, including light, temperature, moisture and soil. Though rice is grown all over Pakistan from sea level up to 2250 m in the mountain region. Rice production in Pakistan is concentrated in four, more or less distinct agro-ecological zones. Each zone represents a diverse climatic, hydrological and edaphic condition.

Zone-I: It consists of northern mountainous areas of the country. Irrigated rice is grown either in flat valleys or terraced valley's side. The climate is sub-humid monsoon with 750-1000 mm average rain fall, mostly concentrated in summer.

Zone-II: This area lies in the broad strip of land between river Ravi and Chenab, where both canal and sub-soil water are used for irrigation. The climate is sub-humid, sub-tropical type with 400-700 mm rainfall, most of which precipitates in July-August.

Zone-III: It consists of the large tract of land on the west bank of river Indus. It has an arid sub-tropical climate with 100 mm of average rainfall and maximum temperature higher than zones I and II.

Zone-IV: It is Indus delta that consists of vast spill flats and basins; the later mostly irrigated. The climate is arid tropical marine, with no marked season.

Fig 1.3 Rice Cultivation Zones in Pakistan.

Diversity in Rice Germplasm

Studies on rice germplasm conserved in National Genebank for agronomic traits and phenotypic characters has revealed the germplasm to possess wide variation both in qualitative and quantitative characters (PGRI, 1995a).

In Pakistan research activities on rice are targeted for increase in yield and resistance to disease and pest. In this regard mechanization of rice cultivation, adaptation of improved varieties and more recently, use of biotechnology for the incorporation of gene for disease resistance have come up (PARC, 2000b) and salt tolerance studies are also in progress, but no studies have been marked for grain quality. However, recently it was realized at national level in Pakistan that rice with better grain quality should be produced and research to some extent have also been started (PARC, 2000c).

According to Harrison and Schwarzacher (2000) the knowledge of the crops and their relatives, combined with assistance given by breeders using advanced technologies can have rapid results in increasing the productivity of such crops and correcting the minor agronomic weakness. In their view a final challenge for breeders is the understanding, manipulation and utilization of the genomic responses of plants to a changing and apparently more variable climate in many of the most productive crop areas.

Land races are crop populations in balance with their environment and remain relatively stable over long periods of time. Yet their population structure has the effect of retaining a potential for adaptive change, especially where there are opportunities for gene exchange and introgression. The transition from primitive to advanced cultivars has the effect of narrowing the genetic base, mainly due to selection for uniformity. Thus man and plant in interaction with each other and with the range of environment produced a range of population from which our gene centers are derived (Frankel and Bannett, 1970). Sano (2000) has stated that genetic diversity at the molecular level generally decrease due to genetic bottlenecks during domestication in crop plants, however, phenotypic diversity increases during domestication as a general trend in crop plants. Even the lately developed cultivars and advanced lines need detailed evaluation as Hutchinson and Hawkes (1987) stated that in three of their major crops, cotton, wheat and rice, the greatest advances have come from the exploitation of diversity within the advanced cultivars.

According to Harrison and Schwarzacher (2000) since most major resistance genes act in a race specific way and will be overcome by time, there is a permanent demand for identification of novel genes/alleles. While Gill *et al.* (2000) said that conservation of the biodiversity of the wild relatives of crop plants and it's efficient management and utilization in crop improvement is critical for food security of the world. Tsujimoto *et al.* (2000) realized that although many landraces and wild species are maintained in the world as genetic resources for crop improvement, their use for breeding is still limited, and we are challenged as to how to use this biodiversity for practical crop breeding. Wilson (2000) stated that, it is estimated that merely less than 1% of species out of 10 millions of species in this planet are studied on the biodiversity aspect.

Genetic diversity is a prerequisite for increasing yields and for stabilizing production in the face of disease epidemic and fluctuating environmental conditions. Vast reservoirs of genetic diversity have been collected and are maintained in germplasm collections around the world. However, there has been relatively little utilization of these genetic resources to enhance the performance of elite rice cultivars, other than a source of single genes for disease and insect resistance (Moeljopawiro *et al.*, 2000). New strategies that simultaneously target variety development and diversification of the gene pool are urgently needed. According to them, Asian and American rice breeders have made virtually no use of *Oryza rufipogon* in commercial inbred or hybrid development. This can be attributed both to the many agronomically undesirable traits apparent in progenies of these materials, and to breeders inability to identify useful genetic variation based on field evaluation of such progenies. Takeda (2000) is also of the view that, efficiency of plant breeding or selecting super genotype is dependant upon the efficiency of selection and magnitude of genetic variation existing in the breeding population. Thus the diversity of genetic resources is vitally important for the future plant breeding. According to him, improving stress tolerance is important for low input sustainable agriculture. They have collected barley germplasm from all over the world and evaluated their genetic traits from the view point of phylogeny and plant breeding.

Watanabe (2000) said that plant genetic resources have provided life to human being and to the earth. Wolfe (2000) stated that, intra-specific genetic variation, which creates genetic diversity, is fundamental material for plant

breeding. Increase in intra-locus variation by the enhancement of genetic diversity in the available genetic resources, had been demonstrated drastic gain in the agronomic traits. Adequate use of such genetic diversity by participatory plant breeding gained success in combating major constrains such as rice crop against rice blast.

When plant material is introduced from elsewhere, there is risk of introducing disease, pest or weed, hence plant quarantine procedures must be applied (Frankel and Bennett, 1970). Therefore local plant genetic resources are of greater choice for evaluation as they are in harmony with the environmental conditions and free from introduction of alien pest and disease.

Satoh *et al.* (2000) have stated that mainly the storage proteins in a grain determine nutritional values of rice. Therefore, the development of improved germplasm with superior nutritional and cooking quality is one of the most important subjects in rice breeding. According to them, although, the artificial nutrition technique is one of the useful methods to develop the to develop the novel genetic resources, spontaneous mutations preserved in the local rice germplasm are the most important genetic resources. They also reported a wide variation for grain morphology(Satoh *et al.* 1990c, d, e, f) and for the storage starch as well as storage proteins among the local rice collected from different countries(Satoh et al. 1990a, b, g, h).

Plant storage proteins generally consist of several groups, each of which contains similar polypeptides possessing only a small difference from each other in chemical structures (Nakamura and Hattori, 1989).

For the classification of plant storage proteins, Osborn's method is still used, where it depends on relative solubility of the target proteins in standard solvents (Osborn, 1924). This classification has been very valuable in grain protein research for the improvement of their nutritional value. In this classification, proteins which are soluble in water, 0.5 M sodium chloride and 70% ethyl alcohol are called albumin, globulin and prolamin, respectively. While the proteins which are soluble in 0.1 M acid or alkaline solution are glutaline.

Juliano (1972) also found rice protein to be mainly composed of four types of protein fractions viz. albumins, globulins, prolamins and glutelin. However, gluteline and prolamin are the major storage proteins in rice. Moreover, these

proteins are stored in different sub-cellular components (Krishnan *et al.*, 1986; Yamagata and Tanaka, 1986; Muench *et al.*, 1998).

The major seed storage protein in most of the cereals is the alcohol soluble protein. In contrast rice stores a high content (about 75% of total proteins on the weight basis) of glutelins in starchy endosperm making itself unique in cereal crops, except for oat. Comparing to Prolamins, glutelins are more easily digested (Tanaka *et al.*, 1975; Ogawa et al. 1987; Resurrection *et al.*, 1993) illustrating the reason of why glutelin is much better than prolamin in nutritional value.

Tanaka and Ogawa (1986) stated that in molecular and biological terms, we must try to breed rice seeds with an enhanced expression of glutelin genes. However, Iida *et al.* (1997) feel the need of low protein content as well, and stated that protein content of rice endosperm correlated negatively with taste.

Yamagata *et al.* (1982) has also stated that rice prolamin is accumulated in protein body type-I (PB-I) and rice glutelin is accumulated in protein body type-II (PB-II). Whereas, Ogawa *et al.* (1987) has found that PB-I is nutritionally inferior to PB-II, and that PB-I is 20 to 25% of the total rice endoplasm protein. Bhowmik *et al.* (1990) have stated that for the quality improvement of protein, PB-I has to be decreased and PB-II has to be increased.

For improving the storage protein qualities, research and breeding efforts have been devoted to increase total protein content or lysine via use of mutants for seed storage protein (Beachell *et al.*, 1972, Kambayshi *et al.*, 1984). Shin *et al.* (1977) reported high lysine in rice variety "Hiyamizu".

The mutants in maize e.g. waxy, sugary, etc. have greatly contributed to the improvement of the grain quality of maize. These also expanded the use of maize not only as the food for men and domestic animals but also for the important industrial materials of food chemistry. In addition these mutants have offered much valuable information concerning gene action in the biological processes of metabolic regulation in the high plants (Satoh 1985). In wheat (Paynep *et al.*, 1981), oat (Robert *et al.*, 1983) and soyabean (Kitamura *et al.*, 1981) etc. similar mutants have been reported and used for genetic and breeding studies.

Similarly, successful improvement of rice storage proteins relies on a thorough understanding of its inheritance, characterization, biological and genetic regulation mechanism of biosynthesis and deposition. These depend on the availability of the mutants to use as material to study these mechanisms and as

material to incorporate by breeding or other techniques. Investigation on the genetics and the effect of specific gene on qualitative and quantitative changes in carbohydrates, proteins, or lipids in the rice endosperm started with the production of enormous amount of induced mutants by Satoh and Omura (1979). Later, mutants with high lysine content were reported for rice (Kumamaru *et al.*, 1997). Similarly, mutants with increased protein were also reported (Kambayshi *et al.*, 1984). Due to the importance of mutants in genetic studies and being material source for improvement in existing varieties, search for mutants was carried out by Bhowmik *et al.*, (1990) who screened 118 rice varieties/lines from Bangladesh and reported breeding material containing comparatively higher glutalin content; Satoh *et al.* (1990a, b; 1995) reported variation in seed storage proteins in rice collected from Tanzania, Madagascar and North Asian countries.

Molecular Diversity

Electrophoresis is a technique for separating and characterizing proteins and nucleic acids. For protein studies, one can use it to show in a semi-quantitative manner that how many proteins are there in sample, whether two samples differ qualitatively or quantitatively in their protein composition, and whether a specific protein is present in a sample. One might, for instance, use electrophoresis to tentatively document changes in protein composition that occur as an organism progresses from one developmental stage to another, changes in protein composition that occur upon exposure to a given treatment, and differences in the protein composition of two tissues. Comparison of "protein profiles" obtained from tissue under different conditions can indicate similarities (e.g. protein present in all), differences (e.g. proteins unique to one condition), and quantitative changes in the level of a given protein. Proteins unique to a particular condition are potentially useful in ultimately understanding the cellular events that are occurring at that time. Similarly, a comparison of protein profiles from unstressed and stressed organisms could lead to the identification of proteins associated directly of indirectly with the stress response. It is important to realize, however, that electrophoresis indicates potentially interesting differences. It does not, by itself, give the identity of a protein(s).

Electrophoresis depends on the observation that different proteins are different sized and have different overall surface charges at a fixed pH. Differences in size are due to differences in the number of amino acids that make each protein.

Different proteins of similar size, however, will likely vary in overall charge due to the specific amino acid sequence that each different protein has. Thus, different proteins can be separated from one another by taking advantage of differences in size and/or differences in overall surface charges. Electrophoresis in general, consists of placing a mixture of charged molecules in solid supporting medium (e.g. gel slab), immersing the gel in a buffered salt solution and establishing an electric field between two electrodes positioned at both ends of the gel. The charged molecules will migrate towards the positive or negative electrode, the direction depends upon their overall charge (e.g. molecules with an overall negative surface charge will migrate towards the positive electrode). Several variations of electrophoresis are available for the separation of nucleic acids and proteins. Descriptions of the different kinds of electrophoresis that can be used to separate proteins are described below.

One kind of electrophoresis uses a starch or agars gel to separate proteins that are identical in size (may even have the same activity), but differ in their overall surface charge because of amino acid substitutions.

Another type of electrophoresis is PAGE (Poly-Acrylamide Gel Electrophoresis). Polyacrylamide is a polymer that forms a gel with pores that are relatively small, approximately the size of proteins. "Native" PAGE is performed under conditions of high pH, a condition which causes almost all proteins to have overall negative charges.

The system separates proteins both by their surface charge density and their size (larger proteins move more slowly because their ability to move through the pores of the gel is restricted). The addition f Sodium Dodecylsulfate (SDS), a detergent, to the PAGE system led to one of the most widely used techniques in the study of proteins-SDS-PAGE. SDS coats individual proteins, negating the charge differences due to amino acid composition. Samples are first heated (100C) in a buffer containing SDS and reducing agents (e.g. mercaptoethanol or dithiothreitol). These conditions denature polypeptide chains as well as separate protein subunits in oligomers. As previously noted, the SDS then surrounds individual polypeptide chains, giving each chain the same overall surface charge (all proteins will have the same net negative charge). Under these conditions, electrophoresis separates proteins by size. The addition of protein molecular weight standards (a series of

proteins of known molecular size) can be used to determine the relative size of an unknown protein (Koranyi, 1989).

SDS-PAGE is considered to be reliable method because seed storage proteins are largely independent of environmental fluctuations (Gept, 1989; Murphy *et al.*, 1990). Although seed proteins can be fractionated by high-performance liquid chromatography (Smith and Smith, 1989) and some other techniques but SDS-PAGE is currently the favored technique for rapid analysis (Cooke 1984). Protein fractionation by SDS-PAGE is relatively rapid and inexpensive as compared to isozyme and DNA analysis (Higginbotham *et al.*, 1991).

Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky and Hymowitz, 1979; Murphy *et al.*, 1990; Khan, 1992; Das and Mukarjee, 1995). Varieties discrimination and identification have been achieved in a range of agriculture crops by means of electrophoretic techniques (Moller and Spoor, 1993), according to them, means of electrophoresis are independent from the growing season, no need for plant cultivation, availability of material year round, ease of storing material, the relative speed of examination, small size of sample needed etc. They also reported that the advantages of examining denatured protein are independent from seed vigor and physiological seed activity. More over, because proteins are the primary gene products, they provide valuable means of making genetic system; this variation in protein composition is a reflection of genotypic variation.

Electrophoretic procedures able to detect qualitative and quantitative chemical deference among cultivars have been applied successfully for cultivar analysis in various crops (Cooke, 1984; Ferguson and Grabe, 1986; Gardiner and Forde, 1988. Gadgil, *et al.*, 1989; Koranyi, 1989; Mooler and Spoor, 1993; Ahmad *et al.*, 1992 and Jha and Ohri, 1996). However, few studies indicate that cultivar identification was not possible with the SDS-PAGE method, as electrophoretic patterns of proteins were similar among the cultivars (Ladzinsky and Hymowitz, 1979; Raymond *et al.*, 1991; Ahmad and Slinkard, 1992; de Vries, 1996).

For plant germplasm management seed protein polymorphism may serve as genetic markers because they can be quite polymorphic, generally substantially

more so than are isomers (Gepts, 1990), and the variability is generally high heritable (Smith and Smith, 1986).

Moller and Spoor, (1993) used SDS-PAGE for discrimination and identification of Lolium species and reported difference in the resulting seed protein banding pattern for identification. Das and Mukarjee, (1995) while working on seed protein for species homology and genetic relationship, reported three major groups on the bases of cluster analysis. Przybylska and Przybylska, (1995) reported marked difference in seed texture (rough and smooth seeded) based on SDS-PAGE analysis.

SDS-PAGE protein analysis has been used widely in biosynthetic study of several plant species (Sanches Yelamo *et al.*, 1995; Sheidai *et al.*, 1999) as identification of seed protein by electrophoresis has indicated that seed protein profile is highly stable and species specific. Moreover, seed protein profile is hardly affected by experimental conditions (Gray *et al.*, 1973; Ladizinsky, 1979).

de Vries, (1996) reported pattern of proteins of *Lctuca sativa* cultivars, mutually compared with wild relatives *Lactuca saligna, L. serriola* and *L. virosa* on the basis of SDS-PAGE. *L.virosa* and *L. saligna* were easily identified and characterized by typical banding pattern. They further reported that cultivar identification was not possible with the help of SDS-PAGE analysis.

Singh *et al.*, (1996) reported little variation for protein band in groundnut, which indicated that most of the accessions were the members of same conservative species.

Genetic diversity of seed storage proteins have been reported for many crops; Sunflower(Raymond *et al.* 1991), *Ipomoea* (Das and Mukhergee, 1995), Groundnut (Oldeigah and Osanyinpeju 1998), *Lathyrus* species (Przybylska et al., 1998), *Capsicum* species (Odeigah et al., 1999), *lima* bean (Lioi *et al.*, 1999), *Triticale* (Igrejas *et al.*, 1999) and *Phaseolus vulgaris* (Ferreira *et al.*, 2000) etc. these studies on seed storage proteins, not only helped in the identification and characterization of diversity in crop varieties, cultivars and their wild varieties but also in determining the out crossing rate, phylogenetic relationships, etc.

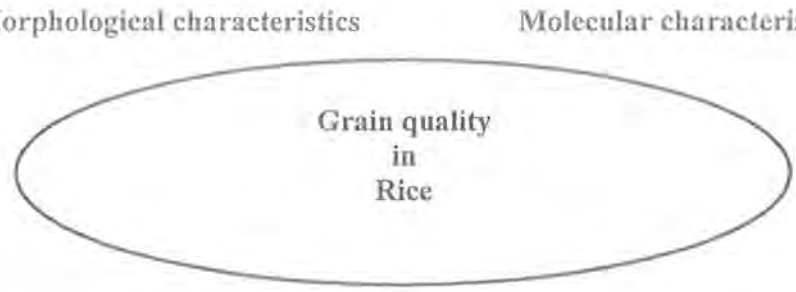
Rice storage proteins have also been studied for their variation in composition and electrophoretic pattern (Satoh 1985, Hibino *et al.*, 1989; Ogawa *et al.*, 1989; Uemera *et al.*, 1996). Inter and intra specific varietals difference in electrophoretic, isoelectric focusing and chromatographic patterns of rice storage

proteins have also been reported extensively with great detail (Bhowmik *et al.*, 1980; Kagwa *et al.*, 1988; Kumamaru et al. 1988; Satoh *et al.*, 1990a,b; Uemura *et al.*, 1996)

According to Glasmann (1986) a main part of the genetic improvement of a crop resides in the creation of new genetic combination from the available germplasm. In this respect, the first concern of breeders is to know the genetic structure of the existing germplasm. Jiang (1993) has also emphasized on the genetic resource utilization for breeding and the need for collection and evaluation of the germplasm. Therefore, it is imperative to evaluate local rice genetic resources for useful variation/mutation for their utilization in the improvement of seed storage proteins.

Grain quality is a very wide subject encompassing diverse characters that are directly or indirectly related to exhibit one quality type. Fig 1.2 describes some of the important grain characteristics that constitute the grain quality of rice grain. Variation in any one character or character combination results in the changed quality of rice grain. If research on grain quality improvement of rice is to be done, the local rice genetic resources in Pakistan must be evaluated to outline the variation.

Fig 1.4 Some important factors contributing to grain quality in rice.



Grain morphology is among the first to be a visible character for selection and quality marking. In the southern US, long grain rice constitute 80-90% acreage, while in California, medium grain rice constitutes a majority of rice acreage (Gravios and Webb 1997). They stated that each grain type has been bred to be associated with specific cooking quality characteristics. They also noticed a particular trend of grain cooking quality characters related to grain shape.

Nutritional value of rice is determined by its storage proteins in a grain, therefore the variation in seed storage proteins signifies the diversity in the nutritional quality, which may be used in manipulating the nutritional component of grain quality. Hence, grain morphology and storage proteins were studied in this research evaluation.

The objectives of this research work are as follows;

- Germplasm collection, evaluation and availability for breeding purposes.
- Protein profile studies so as to select the promising lines for further utilization.
- Identification of genetic diversity occurring in qualitative, quantitative and agronomic traits of rice (*Oryza sativa* L.).

CHAPTER-2

Materials and Methods

The research work was carried out during Feb, 2005 to end of September, 2005 under field conditions at Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agricultural Research Center (NARC) Islamabad. Experimental plant was cultivated rice (*Oryza sativa* L.) consisting of 70 accessions provided by gene bank of IABGR, NARC, Islamabad, which were collected from various parts of the country that represent a wide ecological variation from dry mountains to irrigated plains. The research work comprised of two phases conducted under field and laboratory conditions.

Field Phase:

All the 70 accessions and two control varieties of *Oryza sativa* were first planted in a small field for nursery growing. When seedlings were 20 days old they were transferred to the field and plotted in augmented design. The row length was 5m with 75cm row spacing and intra row distance was kept at 15cm. Recommended cultural practices were followed throughout the crop season to get healthy and vigorous crop. Proper water treatment was applied to avoid water stress, flooded irrigation was continued after every 15 days till maturity of crop. Experimental field received two hoeings, one during nursery transplantation and other after one month. Fungicide Capton was sprayed twice to save the crop from fungal infections.

Laboratory Phase:

Healthy and mature seed of 70 accessions and two control varieties was used for molecular analysis of total seed protein. SDS-PAGE technique was used to identify molecular diversity of rice accessions available. Different molecular level characteristics were studied.

Table 2.1: Summery of Experiments

Experiment	*Accessions*	*Experimental Condition*	*Characters Studied*
Morphological Characters	70 accessions 2 controls	Field Pots	Qualitative traits 7 Quantitative traits 12
Molecular Characters (SDS-PAGE)	70 accessions 2 Control Varieties	Laboratory	Presence/Absence High intensity/Low intensity

Morphological Characteristics:

For morphological characteristics, five competitive plants (accessions/genotypes) were sampled at random for data collection as reported by Satoh *et al.* (1990c,d,e,f). The mean values of each character for each entry were used for statistical analysis according to Adair *et al.* (1973). Descriptive statistical analyses were performed using the statistical programs STATISTICA, MINITAB and Microsoft Office EXCEL. Morphological characters are divided into two parts, Qualitative and Quantitative.

Qualitative Characteristics:

Total number of qualitative traits studied was 7, which were considered most suitable characters according to breeding point of view.

Flag Leaf:

The first leaf which appears on the stem and does not take part in photosynthesis is called flag leaf. It is one of the characteristics of cereal crops. Four different types of flag leaves were studied in rice.

1) Erect
2) Intermediate
3) Horizontal
4) Descending

Lodging:

The flag leaf may be in lodged condition or in erect form so it has only two phenotypes presence or absence which are numbered as follows;

1) Present
2) Absent

Panicle Type:

Panicle is a type of inflorescence which represents flower position on the stem. Three forms of panicle were seen in rice which are numbered as follows;

1) Compact
2) Intermediate
3) Open

Panicle Exertion:

On the basis of exertion panicles are of three different types, which have following numberings;

1) Well exerted
2) Moderately exerted
3) Enclosed

Awning:

The extension of glume is called awn. Three types of awnings were seen in rice which is numbered as follows;

1) Awned
2) Awnletted
3) Awnless

Awn Color:

Following three main colors were observed in rice awns;

1) No awn
2) White
3) Red black

Seed Coat Color:

Eight different colors of seed coat were observed;

1) White
2) Light brown
3) Speckled brown
4) Brown
5) Variable purple
6) Purple
7) Redish brown
8) Red

Quantitative Characteristics:

Total number of quantitative traits studied was 12, which were considered most suitable characters according to breeding point of view.

Day of Flowering:

The first quantitative trait studied was day of flowering after sowing. Date was carefully noted when flowering initiated in each accession.

Day of Maturity:

When flowers were fully gloomed and plant was fully mature for fertilization, day of maturity was also noted carefully.

Plant Height:

After maturity plants reach to their maximum size. Plant height of each accession was measured in centi-meters (cm).

Productive Tillers/Plant:

Tillers having mature and healthy seeds per plant were counted in five samples from each accession and then their average was taken for further analysis.

Panicle Length:

Panicle length was measured in centi-meters (cm) in five samples of each accession and then average was taken for further study.

Spiklets/Panicle:

Number of spiklets per panicle in each accession were counted in the same way as mentioned above (5 samples from each accessions).

Grain Yield/Plant:

Total grains of a plant were taken from each accession and their yield was calculated in grams (g). Experiment was repeated five times to avoid experimental error.

Straw Yield/Plant:

Straw yield was also measured in grams (g) in the same way as grain yield of each accession.

Grain Length:

Five samples of healthy and mature grains were taken from bulk of each accession, their length was measured in milli-meters (mm) with the help of digital Vernier Caliper and average length was taken as consideration.

Grain Width:

Grain width was measured in the units of milli-meters (mm) in the same way as grain length.

Grain Length/Width ratio:

Ratio of grain length and width was taken for each accession to determine average size of grain.

100 Seed-Weights:

Five samples of healthy and mature 100 seeds were taken from lot of each accession and weighed in units of grams (g). Average of 100 seed-weights of each accession was taken as data.

Molecular Characteristics:

Molecular evaluation involves the use of molecular techniques for assessing genetic diversity of plant germplasm and identification of molecular markers for crop improvements. Following molecular techniques are involved in plant genetic resources;

- Total seed protein analysis: SDS-PAGE electrophoresis.
- Isoelectric focusing of proteins.
- Isozyme analysis.
- DNA polymorphism analysis.

Diversity of total seed protein of all 72 accessions/varieties was checked in laboratory phase. Electrophoresis was carried out in the discontinuous Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) system of Leammli (1970) using 11.25% (w/v) separating gel and 4.5% (w/v) stacking gel.

Total Seed Protein Analysis: SDS-PAGE Electrophoresis:

In Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) separations, migration is determined by molecular weight. Sodium Dodecylsulphate (SDS) is an anionic detergent that denatures proteins by wrapping the hydrophobic tail around the polypeptide backbone. For almost all proteins, SDS binds at a ratio of approximately 1.4g SDS per gram of protein, thus conferring a net negative charge to the polypeptide in proportion to its length. The SDS also disrupts hydrogen bonds, blocks hydrophobic interactions, and substantially unfolds the protein molecules, minimizing differences in molecular form by eliminating the tertiary and secondary structures. The proteins can be totally unfolded when a reducing agent is employed. The SDS denatured and reduced polypeptides are flexible rods with uniform negative charge per unit length. Thus, because molecular weight is essentially a linear function of peptide chain length, in sieving gels the proteins separate by

molecular weight. There are two types of buffer systems used in protein gel electrophoresis: Continuous and discontinuous.

In a discontinuous system, a nonrestrictive large-pore gel called a stacking gel is layered on top of a separating (resolving) gel. The two gel layers are each made with a different buffer, and the tank buffers differ from the gel buffers. In this system the protein mobility, a quantitative measure of the migration rate of a charged species in an electric field, is intermediate between the mobility of the buffer ion of the same charge (usually negative) in the stacking gel (leading ion) and the mobility of buffer ion in the upper tank (trailing ion). When electrophoresis is started, the ions and the proteins begin migrating into the stacking gel. The proteins concentrate in a very thin zone, called the stack, between the leading ion and trailing ion. The proteins continue to migrate in the stack until they reach the separating gel. At that point, due to a pH or an ion change, proteins become the trailing ion and "unstuck" as they separate on the gel. Denaturing gel electrophoresis can resolve complex protein mixtures into hundreds of bands on a gel.

Preparation of Buffers

Protein Extraction Buffer

(0.05 M Tris-HCl pH 8.0, 0.2% SDS, 5M Urea, 1% ß-mecaptoethanol)

Tris	0.6057g
Sodium Dodecylsulphate (SDS)*	0.2g
Urea*	30.3g
Distilled water	About 70ml
HCl (conc.)	Adjust to pH 8.0
2-Mercaptoethanol	1ml
	Ttotal volume of 100ml

A little bit Bromophenol blue (BPB) was added. Buffer solution was stored in a refrigerator.

Tris; Tris (hydroxymethyl) aminomethane

*SDS and urea solubilize and denature proteins.

Solutions for Electrophoresis

Solution A

(3.0 M Tris-HCl pH 9.0, 0.4% SDS)

Tris	36.3g
SDS	0.4g
Distilled water	About 70 ml
HCl (conc.)	Adjusted to pH 8.8
	Total volume of 100ml

Stored in a refrigerator

Solution B

(0.493 M Tris-HCl pH 7.0, 0.4% SDS)

Tris	5.98 g
SDS	0.4 g
Distilled water	About 80 ml
HCl (conc.)	Adjusted to pH 7.0

Stored in a refrigerator

Solution C

(30% Acrylamide, Acrylamide/Bis = 30: 0.8)

Acrylamide*	30g
Bis-acrylamide (Bis)*	0.8g
Distilled water	Total volume of 100 ml

Stored in refrigerator

*Acrylamide and Bis-acrylamide are highly toxic and carcinogenic. Gloves were used while preparing solution using these reagents.

10% APS

Ammonium Persulfate (APS)	0.1g
Distilled water	Total volume 1 ml

Can be stored in a refrigerator for several days but it was prepared fresh all the times for better performance.

Electrode Buffer Solution

(0.025 M Tris, 0.129 M Glycine, 0.125% SDS)

Tris	3.0g
Glycine	14.4g
SDS	1.25g
Distilled water	Total volume of 1000 ml

Stored at room temperature

Staining Solution

Methanol	440 ml
Acetic Acid	60 ml
Distilled water	500 ml
Coomassie Brilliant Blue (CBB)* R250	2.25g
	Total volume of 1litre

Solution was stirred for 30 minutes and then filtered, stored at room temperature. *CBB is a protein staining dye.

Destaining Solution

Methanol	200 ml
Acetic Acid	50 ml
Distilled water	750 ml
	Total volume 1 litre

Stored at room temperature

Preparation of Seed Samples

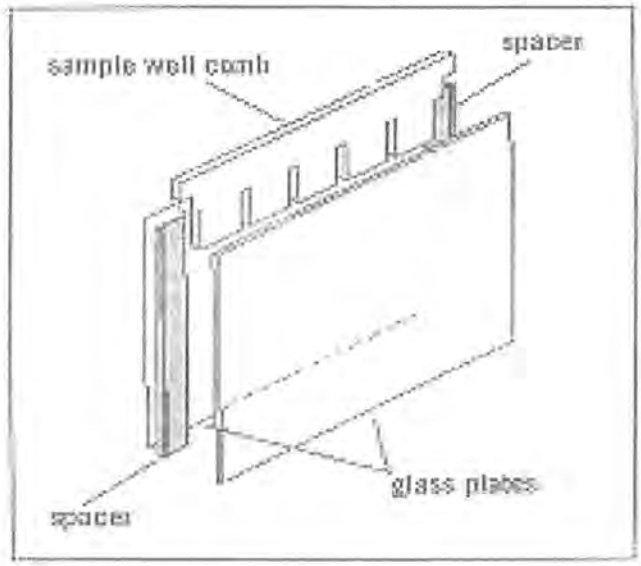
Single seed of each accession was taken, crushed and grinded in mortar and pestle. 10mg (0.01g) seed flour was weighed by an electronic balance and put into 1.5 ml micro tube. After each sample weighing mortar and pestle were cleaned with great care so that their should not be even a single particle of last seed flour. To extract

proteins from flour, 400µl of the protein extraction buffer was put into the microtube and mixed well by the test tube mixer (vortex). This sample was preserved in a freezer (- 20°C).

Preparation of Electrophoretic Gel

Glass plates used for electrophoresis were cleaned up from internal side with 80% Ethanol and Kimwipe. Gaskets were sealed on glass plates with spacer, it was kept in mind that gaskets should not overlap with spacer of plates. Sets of glass plates were fixed with double clips and marked 2cm from the top. To make sure that there is no leakage; glass plate set ups were filled wit water and placed for some time.

Fig 2.1: Electrophoretic Gel Assembly



Following separation gel solution was prepared after setting up the apparatus;

Separation Gel with 1mm thickness (For two mini gels)

Separation gel	**11.25%**
Solution A	5ml

Solution C	7.5ml
10% APS	200µl
Distilled water	7.5ml
TEMED	15µl

TEMED (N-N-N-N-Tetramethylethylenediamine) was added at the end and shaken well.

Separation gel was put into the space between a set of glass plates (up to 2cm from the top). Small amount of distilled water (120µl) was added on separation gel gently to prevent gel surface from air and promote fixation. The set up was left for 30 minutes so that gel was fixed. However, it depends upon the room temperature. Some times it was seen that gel was fixed before 30 minutes when their were cool days. During the fixation time of separation gel, stacking gel was prepared.

Stacking Gel (For two mini gels)

Stacking gel	**4.5%**
Solution B	2.5ml
Solution C	1.5ml
10% APS	70µl
Distilled water	6.0ml
TEMED	17µl

TEMED was added at the end and shaken well.

When separation gel was fixed, distilled water was removed from it's top and stacking gel solution poured on it. Combs were fixed into the stacking gel. Combs were put with special care and it was confirmed that their was no any air bubble at the bottom of the combs. The set up was left for 15 minutes so that the stacking solution became gel. Combs, clips and gaskets were removed from glass plates carefully and confirmed there was no any air bubble at this stage. Gel plates were freshly used for electrophoresis but is was also possible that these would be wrapped in aluminum foil and could be used even for one week.

Electrophoresis

Electrophoresis procedure was carried out using slab type SDS-PAGE model: AE-6530M, ATTA Japan, with 11.25% polyacrylamide gel. The molecular weight of

dissociated proteins was estimated by using molecular weight standard proteins "MW-SDS-70 Kit".

Electrode buffer solution was put into the bottom pool of the apparatus. Gel plates were placed in the apparatus, here again air bubble formation was avoided. Electrode buffer solution was also put into the top pool of the apparatus, wells formed by combs were washed by syringe. Seed samples were centrifuged at 15,000 rpm for 10 minutes, 15 µl of supernatant was put into wells with the help of micropipette. Protein molecular weight marker marker was put in first well of each glass plate. The numbering of seed samples and wells were noted to avoid repetition. The apparatus was connected with + (red) and – (black) electrodes of power supply. The voltage of apparatus was kept constant at 100V and apparatus was left until a blue line of BPB came at the bottom of the gel plates.

Detection of Proteins

(Staining and Destaining of Separation gel)

When blue line reached at the bottom of the gel plates, electric supply was disconnected. Gel plates were taken out from the apparatus and separated by spatula. Stacking gel was removed with the help of same spatula. Separation gel was put in the box which contained staining solution. Box was put on the shaker for two hours. Staining solution was exchanged by destaining solution and the box was shaked gently almost overnight until the background of the gel disappeared to absorb excess CBB, a piece of Kimwipe was put in the destaining solution to check absorbance.

Drying of separation Gel

Wet filter paper was placed on the plate of gel dryer. Separation gel was carefully placed on the paper and covered with a wrap. It was dried in a drier for about 1.5 hours at 60°C. When gel sheet was completely dried it was taken out while the pump was still running. All gels were dried with the same manner.

CHAPTER-3

RESULTS

To assess the degree of genetic diversity in the rice germplasm 72 accessions/genotypes were used as experimental material, which were studied for 7 qualitative and 12 quantitative traits. Qualitative traits comprise flag leaf, lodging, panicle type, panicle exertion, awning, awn color, seed coat color. Quantitative traits include day of flowering, day of maturity, plant height, productive tillers/plant, panicle length, number of spiklets per panicle, grain yield per plant, straw yield per plant, grain length, grain width, grain length/width ratio, 100 seed weight. SDS-PAGE technique was used as molecular marker to estimate the extent of genetic diversity (Phylogenetic relationship among these accessions) existing in the germplasm.

Genetic diversity based on Morphological Traits

Morphological traits were observed under field conditions. Experiments were recorded watchfully and observantly. Morphological traits were divided into qualitative and quantitative.

Qualitative Traits

Qualitative traits studied in this research work were flag leaf position, lodging, panicle type, panicle exertion, awning, awn color, seed coat color. The data of all 72 accessions was taken carefully and results were prepared with keen interest. Out of these 72 accessions 2 were cultivated varieties; BAS-385 and DR-92. The results of 7 qualitative characteristics are mentioned as follows.

Flag Leaf

Flag leaf trait was categorized into four groups; erect with abbreviation code 1, intermediate with code 2, horizontal with code 3, descending with code 4. Most of the plants have erect flag leaf with frequency 55 and frequency percentage 76.38%. Plants having horizontal flag leaf have frequency 11 and frequency percentage 15.27%. Intermediate flag leaf plants have frequency 6 and frequency percentage 8.33%. There was no any plant out of 72 accessions which would have descending flag leaf. Both

cultivated varieties BAS-385 and DR-92 have erect flag leaf. Results for frequency distribution for flag leaf are summarized in table 3.1.

Table 3.1 Frequency distribution for Flag Leaf

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %age*
Flag Leaf	Erect	1	55	76.38
	Intermediate	2	6	8.33
	Horizontal	3	11	15.27
	Descending	4	0	0

Lodging

For the lodging trait all 72 accessions were distributed into two main groups, one having lodging and other without it. Abbreviations Present with code 1 and Absent with code 2 were given to these categories. The plants in which lodging was present have frequency 13 and frequency percentage 18.05%. The plants in which lodging was absent have frequency 59 and frequency percentage 81.94%. In both of the cultivated varieties BAS-385 and DR-92 lodging was absent. Frequency distribution for lodging is shown in table 3.2.

Table 3.2 Frequency Distribution for Lodging

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Lodging	Present	1	13	18.05
	Absent	2	59	81.94

Panicle Type

The trait of Panicle type is divided into three groups in rice plant. Abbreviations for this trait are Compact with code1, Intermediate with code 2 and Open with code 3. Out of 72 accessions 21 are compact with frequency percentage 29.16%, 37 are intermediate with frequency percentage 51.38% and 14 are open type with frequency percentage 19.44%. Most of the plants have intermediate panicle types. The cultivated varieties BAS-385 and DR-92 have also intermediate panicle type. Frequency distribution for panicle type is categorized in table 3.3.

Table 3.3 Frequency Distribution for Panicle Type

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Panicle Type	Compact	1	21	29.16
	Intermediate	2	37	51.38
	Open	3	14	19.44

Panicle Exertion

Panicle exertion was fourth trait studied in rice plants. It was categorized into three groups; well exerted with abbreviation code 1, moderately exerted with code 2 and Enclosed with code 3. The highest frequency was shown by moderately exerted group with frequency 35 and frequency percentage 48.61 %. The plants having enclosed panicle exertion have frequency 20 and frequency percentage 27.77%. The plants having well exerted panicle exertion have frequency 17 and frequency percentage 23.61%. Out of two cultivated varieties BAS-385 has enclosed while DR-92 has moderately exerted panicle exertion. Frequency distribution for panicle exertion is given in table 3.4

Table 3.4 Frequency Distribution for Panicle Exertion

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Panicle Exertion	Well exerted	1	17	23.61
	Moderately exerted	2	35	48.61
	Enclosed	3	20	27.77

Awning

According to the appearance and position of awn rice plants were divided into three groups. Awned plants with abbreviation code 1, awnletted with code 2, and awnless with code 3. Frequency of awnless plants was highest of all. These have frequency 40 with frequency percentage 55.55%. Awnletted plants showed frequency 21 with frequency percentage 29.16%. The plants having awns fall in frequency 11 with frequency percentage 15.27%. The plants of cultivated variety BAS-385 were awnletted while DR-92 showed awnless character. Frequency distribution for awning trait is shown in table 3.5.

Table 3.5 Frequency distribution for awning

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Awning	Awned	1	11	15.27
	Awnletted	2	21	29.16
	Awnless	3	40	55.55

Awn Color

The trait of awn color is categorized into three groups. Plants with no awn were given code 1, plants with awn color white were given code 2 and those with awn color red black were given code 3. The plants with no awn have same frequency as that in awning trait that is 40 with frequency percentage 55.55%. The plants having white awn color have frequency 18 with percentage 25%. The plants with red black awn color have frequency 14 and frequency percentage 19.44%. Both the cultivated varieties BAS-385 and DR-92 fall in the category of no awn color. Frequency distribution for awn color is summarized in table 3.6.

Table 3.6 Frequency distribution for Awn color

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Awn color	No Awn	1	40	55.55
	White	2	18	25
	Red black	3	14	19.44

Seed Coat Color

Seed coat color is very important trait of rice plant. According to reports of International Rice Research Institute (IRRI, 2000) there are eight traits for seed coat color. These are abbreviated as white with code 1, light brown with code 2, speckled brown with code 3, brown with code 4, reddish brown with code 5, red with code 6, variable purple with code 7 and purple with code 8. The data used in this research work comprised of 72 accessions out of which most of the plants have seed coat color brown having frequency 25 and frequency percentage 34.72%. The second most abundant seed coat color was light brown with frequency 18 and frequency percentage 25%. Speckled brown color was observed in 12 accessions with frequency percentage 16.66%. Reddish

brown showed frequency 7 with frequency percentage 9.72%. Pure white color of seed was seen in 6 accessions with frequency percentage 8.33%. Red color have frequency 4 with percentage 5.55%. Non of the accessions were observed as variable purple or full purple in coloration. Out of two cultivated varieties BAS-385 has light brown while DR-92 has speckled brown color. Frequency distribution for seed coat color is shown in table 3.7

Table 3.7 Frequency distribution for Seed Coat Color

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Seed Coat Color	White	1	6	8.33
	Light brown	2	18	25
	Speckled brown	3	12	16.66
	Brown	4	25	34.72
	Reddish brown	5	7	9.72
	Red	6	4	5.55
	Variable purple	7	0	0
	Purple	8	0	0

The most abundant or maximum values for each qualitative trait were observed in rice plant to check degree of abundance for different traits. The trait of flag leaf showed maximum value as erect leaf with frequency 55 and percentage 76.38%. Most of the plants have no lodging; their number was 59 with percentage 81.94. Panicle type was observed intermediate in most of plants with frequency 35 and percentage 48.61%. Panicle exertion was moderately exerted in abundance with frequency 35 and frequency percentage 48.61%. Most of the plants were seen awnless during observation of awning trait with frequency 40 and percentage 55.55%. The character of awn color was also dominated by no awns with frequency 40 and frequency percentage 55.55%. Brown seed coat color was most abundant of all with frequency 25 and frequency percentage 34.72%. Degree of abundance for qualitative traits is shown in table 3.8

Table: 3.8 Degree of abundance for qualitative traits

Trait	*Max. value*	*Code*	*Frequency*	*Frequency %*
Flag Leaf	Erect	1	55	76.38
Lodging	Absent	2	59	81.94
Panicle Type	Intermediate	2	37	51.38
Panicle Exertion	Moderately exerted	2	35	48.61
Awning	Awnless	3	40	55.55
Awn color	No Awn	1	40	55.55
Seed Coat Color	Brown	4	25	34.72

Results for all qualitative characteristics are summarized in table 3.8

Table 3.9 Summery of Results for Qualitative Traits

Trait	*Abbreviation*	*Code*	*Frequency*	*Frequency %*
Flag Leaf	Erect	1	55	76.38
	Intermediate	2	6	8.33
	Horizontal	3	11	15.27
	Descending	4	0	0
Lodging	Present	1	13	18.05
	Absent	2	59	81.94
Panicle Type	Compact	1	21	29.16
	Intermediate	2	37	51.38
	Open	3	14	19.44
Panicle Exertion	Well exerted	1	17	23.61
	Moderately exerted	2	35	48.61
	Enclosed	3	20	27.77
Awning	Awned	1	11	15.27
	Awnletted	2	21	29.16
	Awnless	3	40	55.55
Awn color	No Awn	1	40	55.55
	White	2	18	25

	Red black	3	14	19.44
Seed Coat Color	White	1	6	8.33
	Light brown	2	18	25
	Speckled brown	3	12	16.66
	Brown	4	25	34.72
	Reddish brown	5	7	9.72
	Red	6	4	5.55
	Variable purple	7	0	0
	Purple	8	0	0

Quantitative Traits

Quantitative data on characters such as Days of flowering, Days of maturity, Plant Height (cm), Productive tillers per plant, Panicle length (cm), Spiklets per panicle, Grain Yield per plant (gm), Straw Yield per plant (gm), Grain length (mm), Grain width (mm), 100-seed weight were recorded and tabulated for frequency and percentage distribution, descriptive statistics and correlation matrix.

Day of Flowering

Day of flower initiation ranged from 60 to 131 with mean 86.9, standard deviation 11.43 and co-efficient of variance 13.15. Maximum value 131 was shown by accession No. PAK 007916 while minimum value 54 was exhibited by two accessions (PAK 007961, PAK007962) as shown table 3.10

On the basis of class interval this character was divided into five classes. It was cleared from the results that 12 accessions which were 16.66% of 72 accessions, ranged in days of flower initiation from ≤ 74 which was minimum range for day of flowering. This group was followed by the group with 23 accessions, which comprised of 31.94% of the total germplasm ranged in days to flowering from >74 to ≤ 88 and accessions exist in this range possessed medium value of days to flowering. In the remaining three groups, the group ranged from >88 to ≤ 103 exist 34 accessions with 47.22 % of total germplasm and it was the largest group having maximum number of accessions from the grermplasm. The next group having range from >103 to ≤ 117 contained only 2

accessions with 2.77 % and only one accession was observed in last group having range from >117 to ≤ 131 with 1.38 frequency percentage, given in table 3.11 and Fig3.1

Table 3.10 Descriptive Statistics for Day of Flowering.

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Day of Flowering	86.9	131	54	11.43	13.15

Table 3.11 Range, Frequency Distribution for Day of Flowering.

Trait	*Range*	*Frequency*	*Frequency %*
Day of Flowering	< 74	12	16.66
	< 88	23	31.94
	< 103	34	47.22
	< 117	2	2.77
	< 131	1	1.38

Day of Maturity

Day of maturity ranged from 60 to 133 with mean value 110.1, standard deviation 14.99 and co-efficient of variance percentage 13.61%. Analysis of sample variance and Standard deviation for this trait showed that variation was highly significant so it was concluded that a lot of variation exists among accessions. Maximum value (133 days) for day of maturity was shown by accession No. PAK 007998 and PAK007916 while minimum value (60 days) was shown by two accessions PAK 007961 and PAK 007962, as shown in table 3.12

Day of maturity on the basis of class interval was divided into 5 groups. It was clear from the results that two accessions PAK 007961, PAK 007962 existed in first group with 2.77 % of total germplasm having range ≤ 70. Second group having range >70 to ≤ 86 comprised no accession. This group was followed by next group having range >86 to ≤ 101 which existed 22 accessions with 30.55 % of total germplasm. Fourth group with range >101 to ≤ 117 had 24 accessions having 33.33% frequency percentage. Last group the members of which took maximum time for maturity comprised 24

accessions with 33.33% of total germplasm having range >117 to ≤ 133 (table 3.13, Fig 3.2)

Table 3.12 Descriptive statistics for Day of Maturity.

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Day of Maturity	110.1	133	60	14.99	13.61

Table 3.13 Range, Frequency distribution for day of maturity.

Trait	*Range*	*Frequency*	*Frequency %*
Day of Maturity	<70	2	2.77
	<86	0	0
	<101	22	30.55
	<117	24	33.33
	<133	24	33.33

Plant Height

Plant height in the germplasm varied from 58 to 191.7 cm with mean value being 113.7, standard deviation 38.35 and co-efficient of variance percentage 33.72 %. The maximum plant height was observed in accession No. PAK 007060 which showed 191.7cm height and minimum value for plant height was 58.0cm exhibited by accession No. PAK 007983 (table 3.14).

On the basis of class interval 5 groups were in order to show frequency distribution for plant height. Results revealed that 26 accessions which were 36.11% of total population ranged in plant height from 58 to ≤ 84.74cm, this group was followed by other group that ranged from > 84.74 to ≤ 111.48 which comprised 14 accessions with 19.44% of total germplasm. Next group having range from >111.48 to ≤ 138.22cm existed 10 accessions with 13.88% of all 72 accessions; this group was considered having plants of intermediate height. Fourth group of this category was established having range from > 138.22 to ≤ 164.96cm which contained 14 accessions with frequency percentage 19.44%. Last group having the highest plants was ranged from >164.96 to ≤ 191.70cm which had 8 accessions with 11.11% of total germplasm. Results are mentioned in table 3.15 and Fig 3.3

Table 3.14 Descriptive statistics for Plant Height

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Plant Height	113.7	191.7	58.0	38.35	33.72

Table 3.15 Range, Frequency distribution for Plant Height

Trait	*Range*	*Frequency*	*Frequency %*
Plant Height	<84.74	26	36.11
	<111.48	14	19.44
	<138.22	10	13.88
	<164.96	14	19.44
	<191.70	8	11.11

Productive Tillers per Plant

The trait of productive tillers per plant ranged from 2.2 to 24.0 with mean value 7.5, standard deviation 2.81 and co-efficient of variance 37.25%. The maximum tillers per plant were 24.0 exhibited by accession No. PAK 007060 while the minimum tillers per plant 2.2 were shown by accession No. PAK 007916, given in table 3.16

On the basis of frequency distribution whole germplasm was distributed into 5 groups. Among these groups 32 accessions were having the percentage 44.44% ranged from 2.2 to ≤ 6.56 tillers per plant. 35 accessions were having percentage 48.61% ranged in >6.56 to ≤ 10.92 tillers. 4 accessions having percentage 5.55% ranged from >10.92 to ≤ 15.28. Next group having range >15.28 to ≤ 19.64 comprised no accessions. Finally the group having maximum number of tillers per plant ranged >19.64 to ≤ 24.00 contained only one accession PAK 007060 with frequency percentage 1.38%. Descriptive results for frequency distribution of productive tillers per plant are shown in table 3.17, Fig 3.4

Table 3.16 Descriptive Statistics for Productive Tillers per Plant

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Tillers per Plant	7.5	24.0	2.2	2.81	37.25

Table 3.17 Range, Frequency Distribution for Productive Tillers per Plant

Trait	*Range*	*Frequency*	*Frequency %*
Tillers per Plant	<6.56	32	44.44
	<10.92	35	48.61
	<15.28	4	5.55
	<19.64	0	0
	<24.00	1	1.38

Panicle Length

Panicle length ranged from 44.0 to 17.8cm with the mean value 25.3, standard deviation 2.81 and co-efficient of variance percentage 37.25%, which showed that diversity was present in the accessions. Minimum value 17.8cm of panicle length was observed in accession No. PAK 007060 while maximum value 44.0cm in accession No. 007896, shown in table 3.18

On the basis of frequency distribution the whole germplasm was distributed into five groups. Among these groups 33 accessions were having the percentage 45.83% of total population ranged from 17.8 to ≤ 23.04 have the minimum panicle length. 24 accessions were having the percentage 33.33% ranged from > 23.04 to ≤ 28.28, 10 accessions having percentage 13.88% ranged from >28.28 to ≤ 33.52, 4 accessions having percentage 5.55% ranged from >33.52 to ≤ 38.76 and last group with maximum panicle length comprised only one accession (PAK 007896) having percentage 1.38 was of the length 44.00 (table 3.19, Fig3.5).

Table 3.18 Descriptive Statistics for Panicle Length

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Panicle Length	25.3	44.0	17.8	2.81	37.25

Table 3.19 Range, Frequency Distribution for Panicle Length

Trait	*Range*	*Frequency*	*Frequency %*
Panicle Length	<23.04	33	45.83
	<28.28	24	33.33

	<33.52	10	13.88
	<38.76	4	5.55
	<44.00	1	1.38

Spikelets per Panicle

Spikelets per panicle ranged from 5.8 to 16.8 with mean value 11.5, standard deviation 2.04 and co-efficient of variance 17.74 which revealed that diversity was present in the accessions. Maximum value 16.8 was observed in accession No. PAK 007959 while minimum value in accession No. PAK 007923, given in table 3.20

On the basis of class interval germplasm was divided into five groups. It was obvious from the results that 6 accessions which were 8.33% of total germplasm ranged from 5.8 to $\leq$ 8.0 in spikelets per panicle, this group was followed by the group which ranged from >8.0 $\leq$ 10.2 having 20 accessions with percentage 27.77%. Next group with respect to frequency 31 and percentage 43.05% ranged from >10.2 to $\leq$ 12.4, followed by the group having range >12.4 to $\leq$ 14.6 with 12 accessions and 16.66% of total germplasm. Maximum number of spikelets per plant was observed in the group having range > 14.6 to $\leq$ 16.8 which consist only 3 accessions with percentage 4.16% (table 3.21, Fig 3.6).

Table 3.20 Descriptive Statistics for Spikelets per Panicle

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Spikelets/Panicle	11.5	16.8	5.8	2.04	17.74

Table 3.21 Range, Frequency Distribution for Spikelets per Panicle

Trait	*Range*	*Frequency*	*Frequency %*
Spikelets/Panicle	<8.0	6	8.33
	<10.2	20	27.77
	<12.4	31	43.05
	<14.6	12	16.66
	<16.8	3	4.16

Straw yield per Plant

Straw yield per plant ranged from 62.2 to 143.7 with mean value 95.20, standard deviation 22.2 and co-efficient of variance 18.81 showing diversity in straw yield. Maximum value 143.7g was observed in accession No. PAK 008015 while minimum value 62.2g was seen in accession No. PAK 007940 as shown in table 3.22

Straw yield per plant on the basis of frequency distribution was divided into five groups (given in table 3.23, Fig 3.7).

25 accessions were having straw yield range from 62.2 to ≤ 78.5g with frequency 25 and percentage 34.72% of total germplasm, 17 accessions were having range >78.5 to ≤ 94.8g with percentage 23.61%, 14 accessions were having range from > 94.8 to ≤ 111.1g with percentage 19.44%, 10 accessions were having range > 111.1 to ≤ 127.4g with percentage 13.88% and 6 accessions have maximum straw yield per plant with percentage 8.33% ranging from >127.4 to ≤ 143.7g.

Table 3.22 Descriptive Statistics for Straw yield per plant

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Straw yield/plant	95.20	143.7	62.2	22.2	18.81

Table 3.23 Range, Frequency Distribution for Straw yield per Plant.

Trait	*Range*	*Frequency*	*Frequency %*
Straw yield/plant	<78.5	25	34.72
	<94.8	17	23.61
	<111.1	14	19.44
	<127.4	10	13.88
	<143.7	6	8.33

Grain Yield per Plant

Range for grain yield per plant was observed from 5.2g to 16.8g with mean value 8.98, standard deviation 2.83 and co-efficient of variance percentage 2.14%. It is obvious from the results that diversity exists in this character. Maximum value for grain 16.8g

was observed in accession No. PAK 008015 and minimum value 5.2g was observed in accession No. PAK 007999 as mentioned in table 3.24

On the basis of class interval total population of 72 accessions was divided into five groups for grain yield trait. Among these groups minimum grain yield was observed in group having range 5.2 to ≤ 7.5g comprising 28 accessions with frequency percentage 38.88%, this was followed by the next group having range >7.5 to ≤ 9.8g with 27 accessions containing 37.50% of total germplasm. Another group having range >9.8 to ≤ 12.2g comprised 6 accessions with frequency percentage 8.33%, 7 accessions of next group have percentage 9.72% with >12.2 to≤ 14.5g range. Last group having maximum grain yield ranged from >14.5 to ≤ 16.8g comprised 4 accessions with percentage 5.55%, results are shown in table 3.25 and Fig 3.8

Table 3.24 Descriptive Statistics for Grain Yield per Plant

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Grain Yield/plant	8.98	16.8	5.20	2.83	2.14

Table 3.25 Range, Frequency Distribution for Grain Yield per Plant

Trait	*Range*	*Frequency*	*Frequency %*
Grain Yield/plant	<7.5	28	38.88
	<9.8	27	37.50
	<12.2	6	8.33
	<14.5	7	9.72
	<16.8	4	5.55

Grain Length

Grain length varied from 5.7mm to 13.4mm with mean value 8.8, standard deviation 0.96 and co-efficient of variance percentage 10.93% which shows least diversity in this character.

Maximum grain length 13.4mm was observed in accession No. PAK 007567 while minimum grain length 5.7mm was seen in accession No. PAK 007060 (shown in table 3.26).

On the basis of frequency distribution whole germplasm was divided into five groups. First group having minimum grain length ranged from 5.70 ≤ 7.24mm with 9 accessions having frequency percentage 12.50%. Next group consist 32 accessions with percentage 44.44% having range >7.24 to ≤ 8.78mm, followed by the group which comprise 30 accessions with percentage 41.66% ranged > 8.78 to ≤ 10.32mm in grain length. Other group was ranged > 10.32 to ≤ 11.86mm comprised no accessions. The group having maximum grain length ranged > 11.86 to ≤ 13.40mm comprised only one accession (PAK 007567) with frequency percentage 1.38% (shown in table 3.27, Fig 3.9).

Table 3.26 Descriptive Statistics for Grain Length

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Grain Length	8.8	13.4	5.7	0.96	10.93

Table 3.27 Range, Frequency Distribution for Grain Length

Trait	*Range*	*Frequency*	*Frequency %*
Grain Length	<7.24	9	12.50
	<8.78	32	44.44
	<10.32	30	41.66
	<11.86	0	0
	<13.4	1	1.38

Grain Width

Grain width ranged from 1.6mm to 4.5mm with mean value 2.6, standard deviation 0.34 and co-efficient of variance 13.13% which revealed that least diversity is present in grain width trait of accessions. Maximum value 4.5mm of grain width was observed in accession No. PAK 007962 while minimum value 1.6mm was observed in accession No. PAK 007060, shown in table 3.28

On the basis of class interval the whole population was divided into five groups for grain width trait. The group having minimum grain width ranged from 1.6 to ≤ 2.18mm with 33 accessions and frequency percentage 45.83%, followed by the group

with 37 accessions percentage 51.38% ranging >2.18 to ≤ 2.80mm in grain width. Next group ranged >2.80 to ≤ 3.34mm comprised only one accession (PAK 007978). The group having range >3.34 to ≤ 3.92mm comprised no accession and the group of maximum grain width having range >3.92 to ≤ 4.50mm also contain only one accession (PAK007962), results are mentioned in table 3.29 and Fig 3.10

Table 3.28 Descriptive Statistics for Grain Width

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
Grain Width	2.6	4.5	1.6	0.34	13.13

Table 3.29 Range, Frequency Distribution for Grain Width

Trait	*Range*	*Frequency*	*Frequency %*
Grain Width	<2.18	33	45.83
	<2.80	37	51.38
	<3.34	1	1.38
	<3.92	0	0
	<4.5	1	1.38

Grain Length to Width Ratio

Grain length to width ratio ranged from 2.0 to 4.9 with mean value 3.5, standard deviation 0.46 and co-efficient of variance 13.38% which showed least amount of variability in this character. Maximum value of grain length to width ratio 4.9 was observed in accession No. PAK 008010 while minimum value 2.0 was seen in accession No. PAK 007962 (results given in table 3.30).

On the basis of frequency distribution for this trait whole germplasm was divided into five groups. The group ranging from 2.0 to ≤ 2.5 for grain length to width ratio consisted only two accessions which were 2.77% of total population of 72 accessions. The group having range >2.5 to ≤ 3.1 contained 11 accessions with frequency percentage 15.27% followed by the group of range >3.1 to ≤ 3.7 existed with maximum number of accessions which were 45 with percentage 62.5%. 12 accessions with 16.66% frequency percentage were having the range >3.7 to ≤ 4.3 and 2 accessions with percentage 2.77%

having range >4.3 to ≤ 4.9 comprised last group. Results for class interval are shown in table 3.31 and Fig 3.11

Table 3.30 Descriptive Statistics for Grain Length to Width Ratio

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
GL/GW	3.5	4.9	2.0	0.46	13.38

Table 3.31 Range, Frequency Distribution for Grain Length to Width Ratio

Trait	*Range*	*Frequency*	*Frequency %*
GL/GW	<2.5	2	2.77
	<3.1	11	15.27
	<3.7	45	62.5
	<4.3	12	16.66
	<4.9	2	2.77

100 Seed Weight

The 100 seed weight value varied from 0.3g to 3.1g with mean value 1.3, standard deviation 0.62 and co-efficient of variance percentage 46.16% which revealed little variation in this trait. Maximum value 3.1g was seen in accession No. PAK 007998 while minimum value 0.3g was observed in accession No. PAK 007975 (shown in table 3.32).

On the basis of class interval the whole population of 72 accessions was divided into five groups. The group having range from 0.3 to ≤ 0.9g comprised 23 accessions with frequency percentage 31.94%. Other group containing 24 accessions which were 33.33% of total germplasm ranged >0.9 to ≤ 1.4g. 14 accessions having percentage 19.44% ranged >1.4 to ≤ 1.9g made another group, 9 accessions with percentage 12.50% ranged >1.9 to ≤ 2.5g were also placed in separate group. The group having maximum 100 seed weight ranged >2.5 to ≤ 3.1g comprised only two accessions with frequency percentage 2.77%. Results for frequency distribution are given in table 3.33 and Fig 3.12

Table 3.32 Descriptive Statistics for 100 Seed weight

Trait	*Mean*	*Max.*	*Min.*	*SD*	*CV%*
100 Seed Weight	1.3	3.1	0.3	0.62	46.16

Table 3.33 Range, Frequency Distribution for 100 Seed Weight

Trait	*Range*	*Frequency*	*Frequency %*
100 Seed Weight	<0.9	23	31.94
	<1.4	24	33.33
	<1.9	14	19.44
	<2.5	9	12.50
	<3.1	2	2.77

Correlation

The correlation co-efficient was computed among all the quantitative traits such as; day of flowering, day of maturity, plant height, productive tillers per plant, panicle length, spikelets per panicle, grain yield, straw yield, 100 seed weight, seed length, seed weight, seed length to width ratio. The results regarding correlation matrix (Table 3.37) revealed that all the characters were correlated both positively as well as negatively which were father categorized into highly significant positive (≤0.5), significant positive (≤0.1), significant negative (≤-0.1) and highly significant negatively correlated (≤-0.5).

A critical review of the results showed that correlation between day of flowering and day of maturity was highly significant (0.657) positively correlated while day of flowering with seed weight was significant positive (0.0538). Day of flowering was negatively significant correlated with plant height (-0.007), panicle length (-0.194), productive tillers per plant (-0.050) and seed length (-0.0126) while negatively highly significant for spikelets per panicle (-0.217), grain yield (-0.336), straw yield (-0.261), 100-seed weight (-0.234), seed length to width ratio (-0.206).

Day of maturity is positively correlated with seed weight (0.164) and day of flowering (0.657) while it showed negative correlation with plant height (-0.0127),

productive tillers per plant (-0.0016), panicle length (-0.0789) and seed length (-0.089) which was negatively significant. Day of maturity also showed negative correlation with spikelets per panicle (-0.269), grain yield (-0.441), straw yield (-0.339), 100-seed weight (-0.260) and seed length to width ratio (-0.288) which was highly significant.

Plant height was positively correlated with Productive tillers per plant (0.227), panicle length (0.468), straw yield (0.398) and 100-seed weight which were highly significant for this trait. Plant height was also positively correlated with spiklets per panicle (0.0103) and grain yield (0.170) which showed significant positive correlation while it was significantly negative for day of flowering (-0.0072), day of maturity (-0.00166), seed length (-0.0702), seed width (-0.0569) and seed length to width ratio (-0.0088).

The trait of productive tillers per plant showed positive correlation with grain yield (0.257), plant height (0.227), 100-seed weight (0.213) which were highly significant and with panicle length (0.0361), spikelets per panicle (0.167), straw yield (0.195) which were significantly correlated to this trait. It showed negative correlation with day of flowering (-0.0502), day of maturity (-0.0016), seed weight (0.0093) which were significantly correlated and seed length (-0.284), seed length to width ratio (-0.277) which were highly significant negative for this trait.

Panicle length also showed both positive and negative correlations with other traits. It was positively correlated with plant height (0.468), straw yield (0.318), 100-seed weight (0.292) which were highly significant while seed length (0.0265), seed width (0.0077), seed length to width ratio (0.0959), grain yield (0.0656) were positively significant. Panicle length showed negatively significant correlation with day of flowering (-0.194), day of maturity (-0.0789), spikelets per panicle (-0.0073).

The trait of spikelets per panicle was positively correlated with grain yield (0.397), straw yield (0.355), 100 seed weight (0.300) which were highly significant for this trait while seed length (0.192), plant height (0.0103), productive tillers per plant (0.167) were significant. Negative correlation of this trait was seen with seed width (-0.197), seed length to width ratio (-0.0597) and panicle length (-0.0733) which were significant while day of flowering (-0.217) and day of maturity (-0.269) showed highly significant negative correlation.

Grain yield was positively correlated with productive tillers per plant (0.257), spikelets per panicle (0.397), straw yield (0.866) which were highly significant for this trait and plant height (0.170), panicle length (0.0656), 100 seed weight (0.158), seed length (0.0527) were significant. Negative correlation of grain yield was seen with seed width (-0.181), seed length to width ratio (-0.123), day of flowering (-0.336), day of maturity (-0.441) which were both significant and highly significant.

The trait of straw yield was also correlated with other traits both positively and negatively. Positive correlation of straw yield was observed with plant height (0.398), panicle length (0.318), spikelets per panicle (0.355), grain yield (0.866), 100 seed weight (0.221) which were highly significant and seed length (0.128), productive tillers per plant (0.195) which were significant. It showed negative correlation with seed width (-0.190), seed length to width ratio (-0.075) which were significant and day of flowering (-0.261), day of maturity (-0.339) which were highly significant for negative correlation.

100 seed weight was positively correlated with plant height (0.335), productive tillers per plant (0.213), panicle length (0.292), spikelets per panicle (0.300), straw yield (0.221) which were highly significant and grain yield (0.158) which was significantly correlated with this trait while it revealed significantly negative correlation with day of flowering (-0.234), day of maturity (-0.260), seed width (-0.0619) and seed length to width ratio (-0.0560).

Seed length also revealed positive and negative correlations with other traits. It was positively correlated with seed width (0.428), seed length to width ratio (0.258) which were highly significant and panicle length (0.0265), spikelets per panicle (0.192), grain yield (0.0527), straw yield (0.128), 100 seed weight (0.0645) which were significantly positive while negative correlation was seen with day of flowering (-0.0126), day of maturity (-0.0896), plant height (-0.0702) and productive tillers per plant (-0.284) which were significantly negative correlated.

The trait of seed width was positively correlated with seed length (0.0128), day of flowering (0.0538), day of maturity (0.164) and panicle length (0.0077) which showed significantly positive correlation with this trait. It was negatively correlated with plant height (-0.0569), productive tillers per plant (-0.0092), spikelets per panicle (-0.197),

grain yield (-0.181), straw yield (-0.190), 100 seed weight (-0.0619) and seed length to width ratio (-0.0551) which showed significantly negative correlation with this trait.

It was observed that seed length to width ratio was positively correlated with panicle length (0.0959) and seed length (0.258) which showed significant and highly significant positive correlation respectively. All other traits were negatively correlated with seed length to width ratio out of which day of flowering (-0.206), day of maturity (-0.288), productive tillers per plant (-0.277) were highly significant and plant height (-0.0088), spikelets per panicle (-0.0597), grain yield (-0.0123), straw yield (-0.753), 100 seed weight (-0.0560), seed width (-0.0551) were significantly negative.

Figure 3.1 Frequency Distribution for Day of Flowering

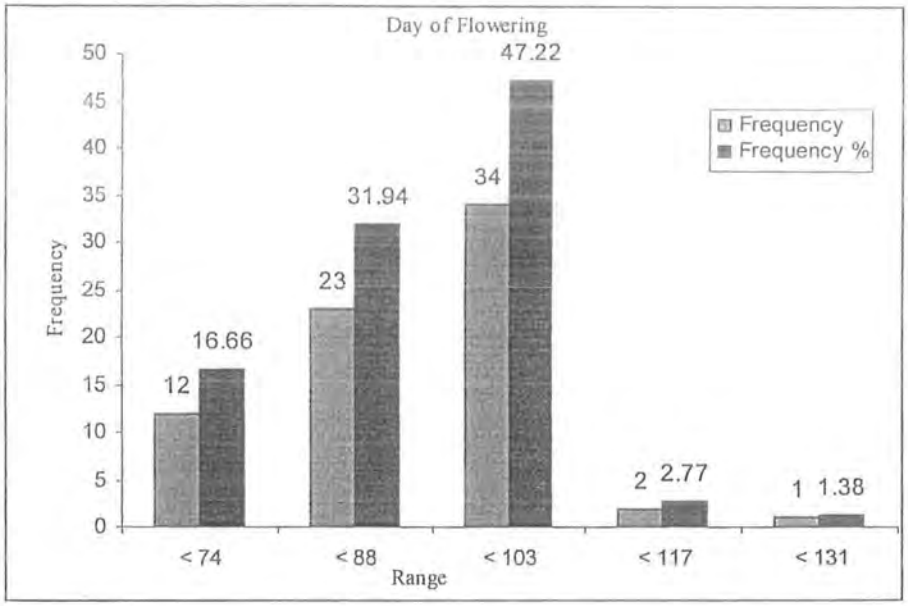


Figure 3.2 Frequency Distribution for Day of Maturity

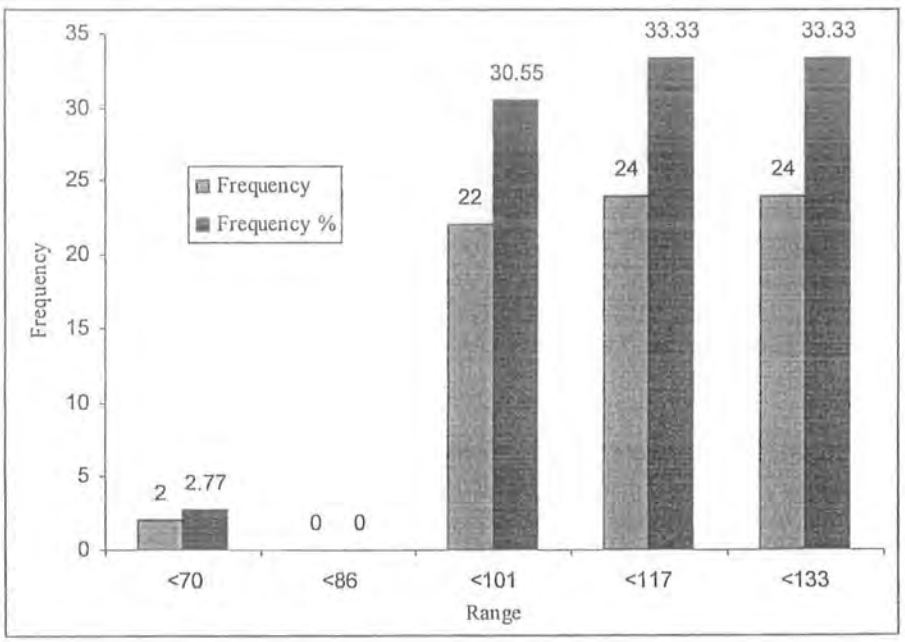


Figure 3.3 Frequency Distribution for Plant Height

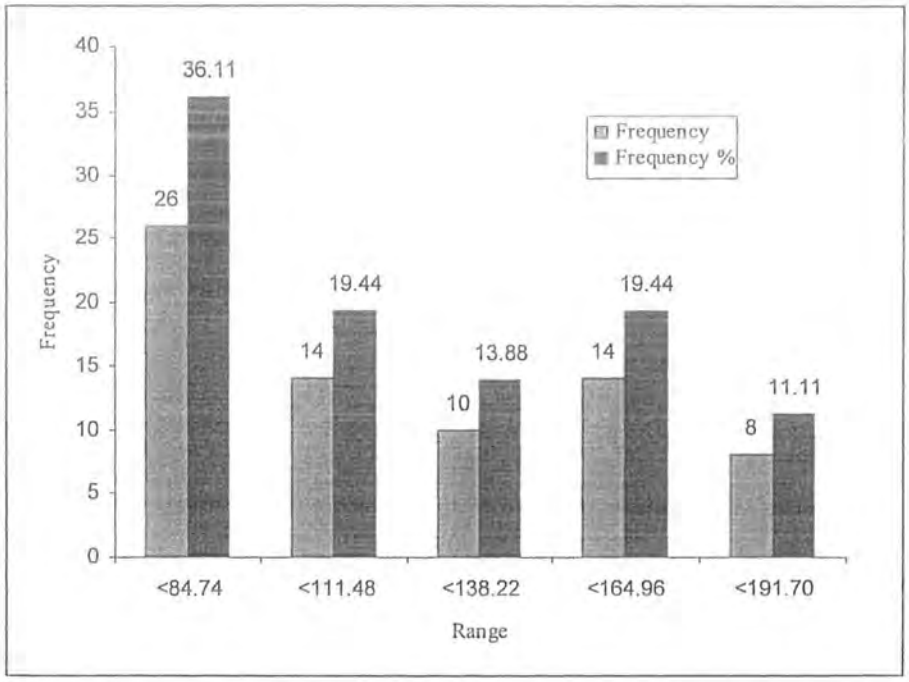
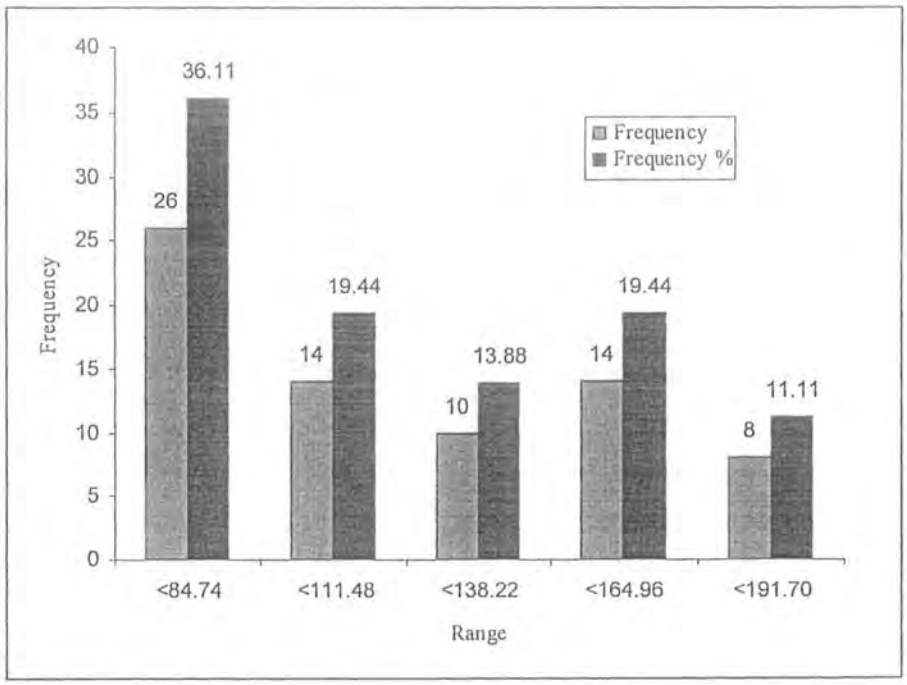


Figure 3.4 Frequency Distribution for Productive Tillers per Plant



Figures 3.5 Frequency Distribution for Panicle Length

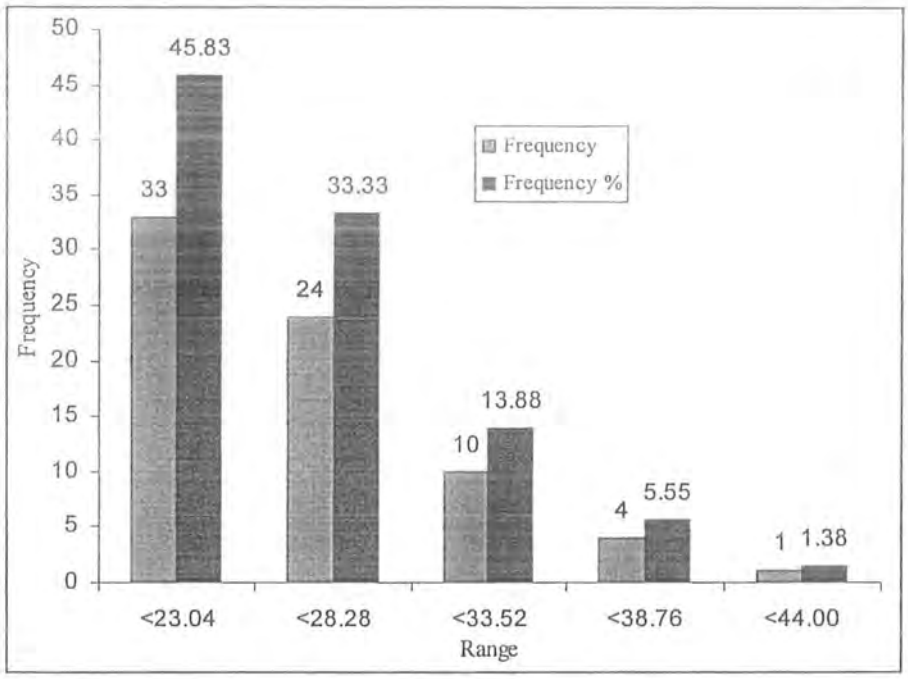


Figure 3.6 Frequency Distribution for Spikelets per Panicle

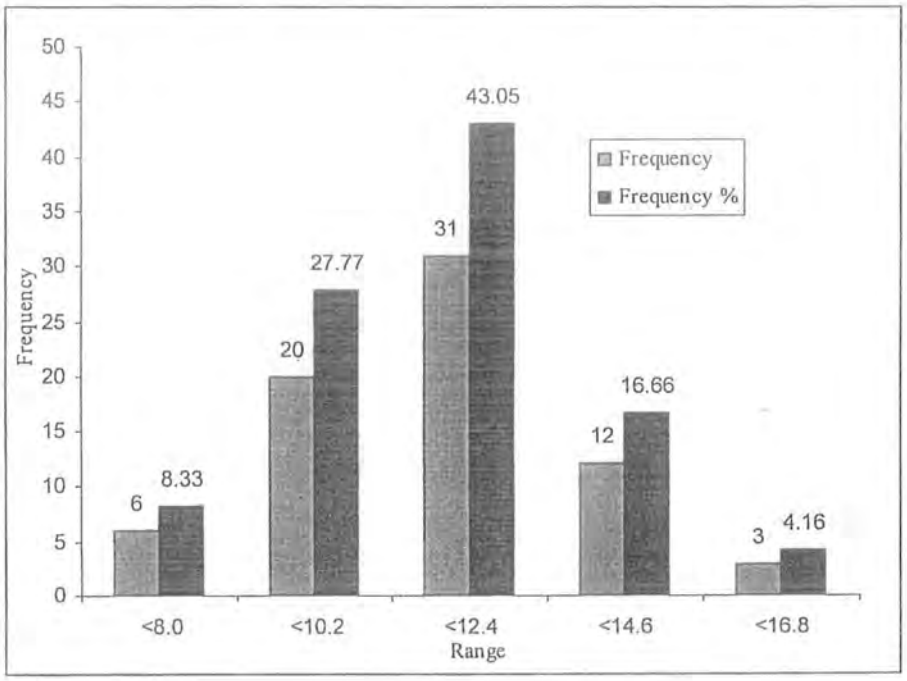


Figure 3.7 Frequency Distribution for Straw Yield

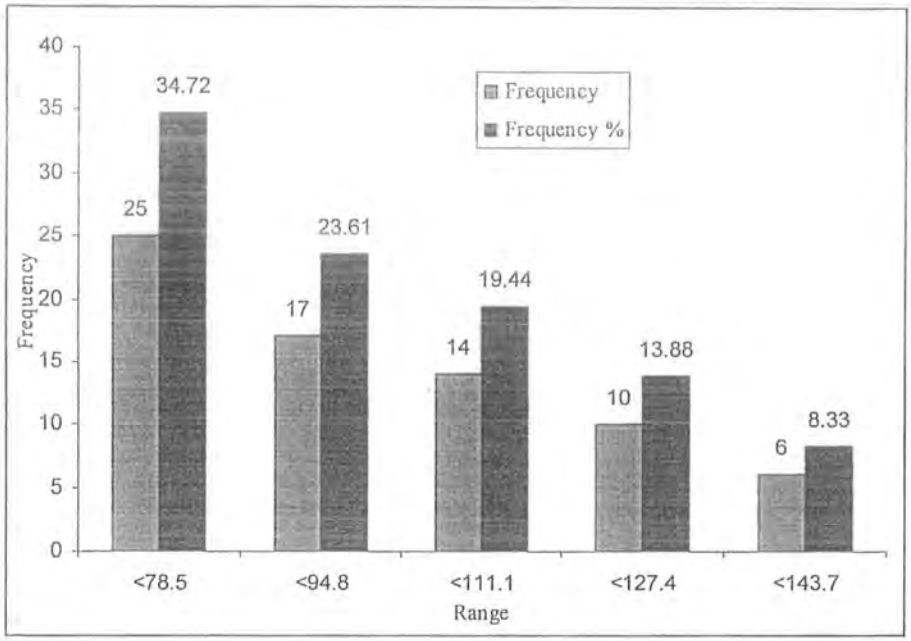


Figure 3.8 Frequency Distribution for Grain Yield

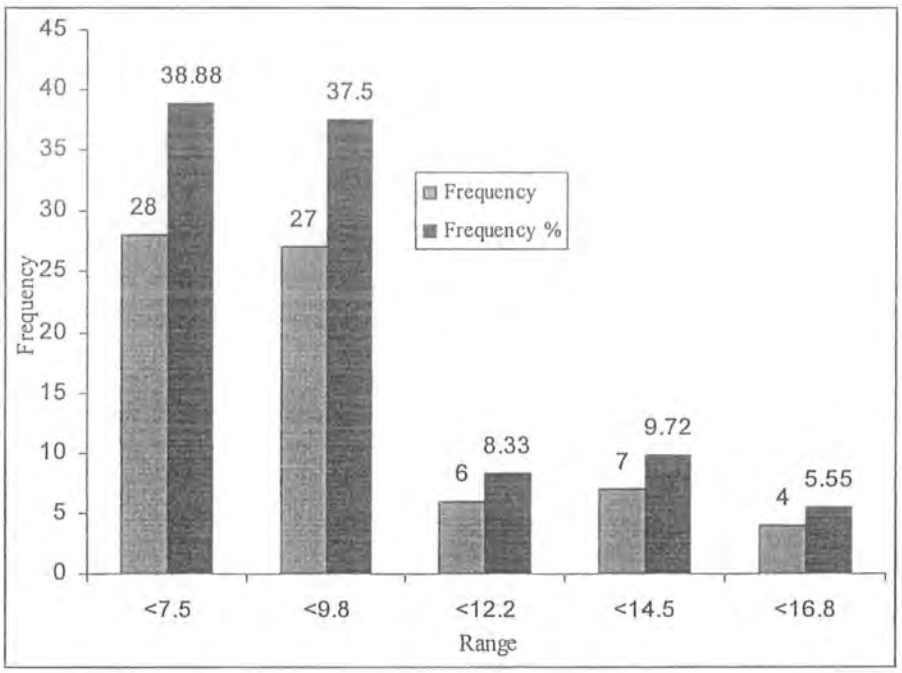


Figure 3.9 Frequency Distribution for Grain Length

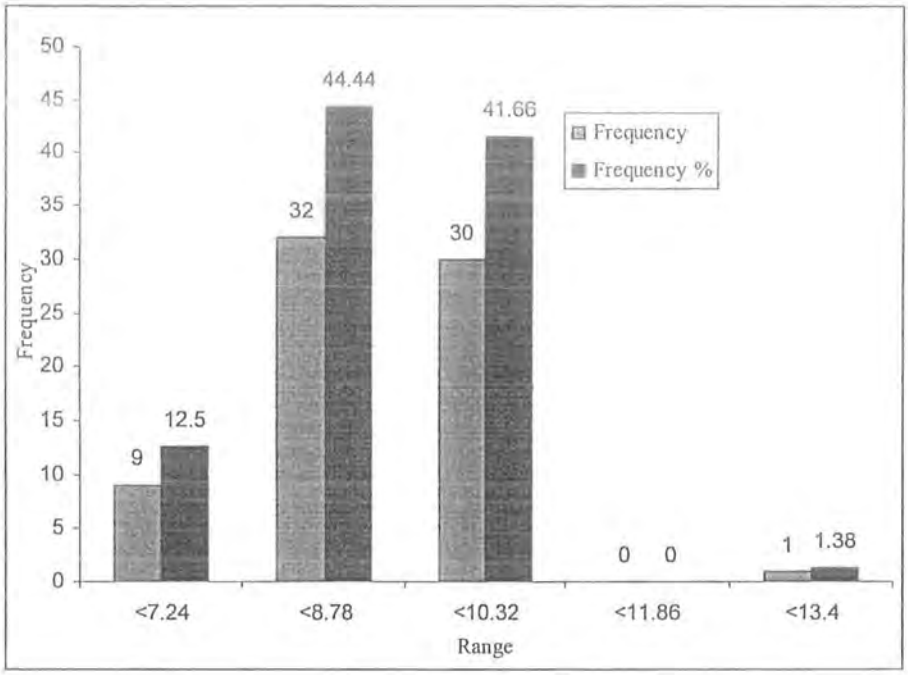


Figure 3.10 Frequency Distribution for Grain Width

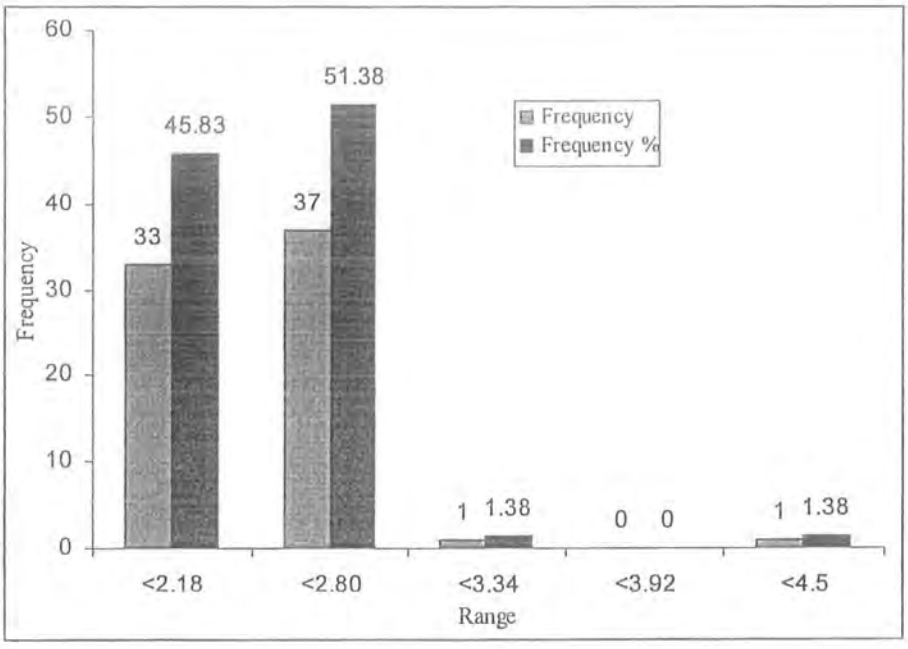


Figure 3.11 Frequency Distribution for Grain Length to Width ratio

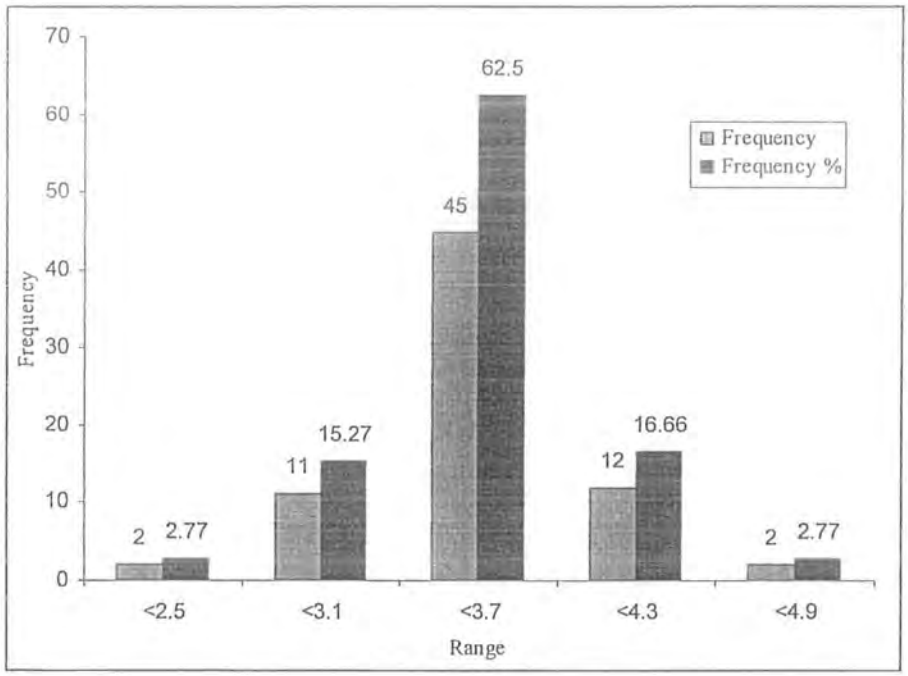


Figure 3.12 Frequency Distribution for 100 Seed Weight

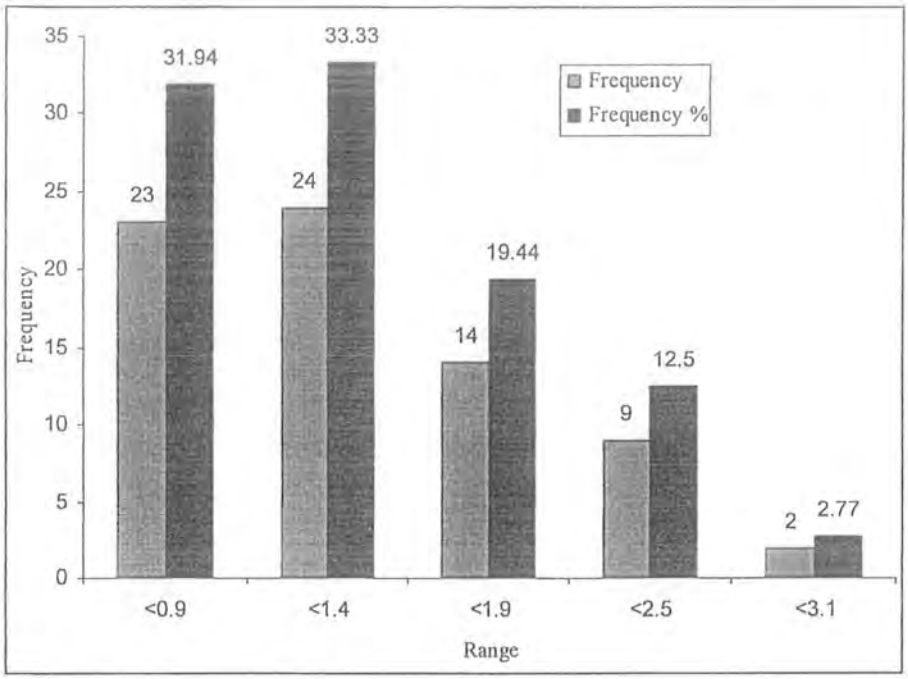


Table 3.34 Correlation Co-efficient of Quantitative Traits

	DF	DM	PH	PT/P	PL	S/P	GY	SY	100-SW	SL	SW	SL/SW
DF	1											
DM	0.65777	1										
PH	-0.0073	-0.01278	1									
PT/P	-0.05025	-0.00167	0.227718	1								
PL	-0.1947	-0.079	0.468334	0.036193	1							
S/P	-0.21708	-0.26981	0.010316	0.16754	-0.00733	1						
GY	-0.33672	-0.44138	0.170138	0.257181	0.06568	0.39789	1					
SY	-0.26129	-0.33911	0.39825	0.195691	0.318117	0.355552	0.866886	1				
100-SW	-0.23487	-0.26052	0.335786	0.213931	0.292633	0.300948	0.158404	0.221305	1			
SL	-0.01261	-0.08969	-0.07021	-0.28441	0.026509	0.192738	0.052765	0.128909	0.064599	1		
SW	0.05389	0.164294	-0.05697	-0.00928	0.007776	-0.19768	-0.18173	-0.19064	-0.06191	0.01285	1	
SL/SW	-0.20615	-0.28891	-0.00888	-0.27735	0.09592	-0.05978	-0.12302	-0.07534	-0.0561	0.258846	-0.0551	1

DF = Day of Flowering
DM = Day of Maturity
PH = Plant Height
PT/P = Productive tillers/Plant
PL = Panicle Length
S/P = Spikelets/Panicle
GY = Grain Yield
SY = Straw Yield
100-sw= 100 Seed Weight
SL = Seed Length
SW = Seed Width
SL/SW= Seed length/Seed width ratio

Correlation Co-efficient (ranged 1.0 to -1.0)

≤0.5 highly significant positive
≤0.1 significant positive
≥-0.1 significant negative
≥-0.5 highly significant negative

Molecular marker (SDS-PAGE) based Genetic Diversity

Genetic diversity on the basis of molecular marker SDS-PAGE was carried for all 72 accessions. Seed storage proteins were extracted and run on the gel. Rice protein consists of four fractions viz. Albumin, Globulin, Glutelin and Prolamin. Fig --- shows the SDS-PAGE protein profiles for rice with apparent Molecular Mass(MM) in Kilo Daltons (kD) resulting into a differential migration. The lane 1 shows the total protein profile, lane 2 shows the acid or alkali soluble protein fraction i.e. glutelin. It consists of a 57kD proglutelin or glutelin precursor. This 57kD polypeptide is cleaved to produce its mature subunits i.e. glutelin acidic subunit of 40kD and glutelin basic subunit of 20kD. Lane 3 shows the alcohol soluble prolamin fraction. It ranges from 16kD to 10kD, however, 13kD bands comprise the major polypeptides of this fraction. Lane 4 shows the water and salt soluble albumin and globulin fractions. Glutalin and prolamin are the major storage proteins comprising up to 75% and 25% respectively, of total seed storage proteins. Therefore variation in these two proteins is described.

Glutelin

Rice glutelin consists of acidic (α) and basic (β) subunits which were composed of 4 and 3 bands respectively (Uemura *et al.*, 1996). The four bands of acidic glutelin (α) were assigned as α1, α2, α3 and α4 while that of basic (β) are assigned as β1, β2 and β3. It was revealed from the results that acidic glutelin subunits have apparent molecular mass approximately 40kD while basic glutelin subunits have molecular mass about 20kD, both migrated from heavier precursor having molecular mass 57kD during electrophoresis.

For acidic glutelin subunits variation was observed in α3 and α4 bands. The whole germplasm was divided into six groups. Group of type-1 showed low molecular mass (LMM) and Fast Migration (FM) for α3 band. Type-2 was of high molecular mass (HMM) and slow migration (SM) for α3 band. In type-3 α3 band has two sub-bands which revealed that it has intermediate molecular mass (IMM) or both fast and slow migrations (FSM). In type-4 α3 band was absent which showed that there was no migration (NM) and it remained attached at its precursor (57kD) during electrophoresis. Next two groups were assigned on the basis of α4 band migration. Type-5 contained low

molecular mass (LMM) and fast moving (FM) α4 band while type-6 contained high molecular mass (HMM) and slow moving (SM) α4 band.

It was clear from the results that type-1 with fast migration α3 band contained 13 accessions which were 18.05% of total germplasm. Type-2 with slow migration α3 consisted of 54 accessions with frequency percentage 75.00%. Type-3 having two bands of both fast and slow migration comprised 4 accessions which were 5.55% of total population. Type-4 showed no migration for α3 contained only one accession (PAK007963) with 1.38%. Results are given in table 3.34a, b.

Table 3.35a Frequency percentage for Acidic Glutelin (α3) by SDS-PAGE

Group	Frequency	Frequency %
Type-1 (FM)	13	18.05%
Type-2 (SM)	54	75.00%
Type-3 (FSM)	4	5.55%
Type-4 (NM)	1	1.38%

Table 3.35b Frequency Percentage for Acidic Glutelin (α4) by SDS-PAGE

Group	Frequency	Frequency %
Type-5 (FM)	42	58.33%
Type-6 (SM)	30	41.66%

For basic glutelin subunits variation was observed in β3 band which was clearly seen on electrophoretic gel. The whole germplasm was divided into three groups for basic glutelin subunit β3. Group of type-1 showed low molecular mass (LMM) and fast migration (FM) for β3 subunit. Type-2 showed high molecular mass (HMM) and slow

migration (SM) for β3 band. In type-3 β3 subunit showed absence of band which appeared that there was no migration (NM) from its precursor during electrophoresis.

It was obvious from the results that Type-1 with fast migration β3 subunit consisted 39 accessions with frequency percentage 54.16%. In type-2 β3 subunit was slow migrated which contained 31 accessions with 43.05% of total germplasm. Typ-3 having no migration of β3 band consisted only two accessions (PAK007963, PAK007970) with frequency percentage 2.77%, results are given in table 3.35

Table 3.36 Frequency Percentage for Basic Glutelin (β3) by SDS-PAGE

Group	Frequency	Frequency %
Type-1 (FM)	39	54.16%
Type-2 (SM)	31	43.05%
Type-3 (NM)	2	2.77%

Prolamin

Major component of prolamin is 13kD polypeptide, which consists of 13a (15kD) and 13b (13kD) bands, Ogawa *et al.* (1987).

Based on the presence and absence or staining property of the bands prolamin diversity was catogorized into four types. Type-1 contained high intensity (HI) of both 15kD and 13kD subunits. Type-2 consisted of low intensity (LI) of 15kD and 13kD bands. Type-3 showed an intermediate band (IB) of 14kD in between 15kD and 13kD subunits. Type-4 was categorized having high intensity of 15kD subunit and low intensity of 13kD subunit, which was assign as high low intensity (HLI).

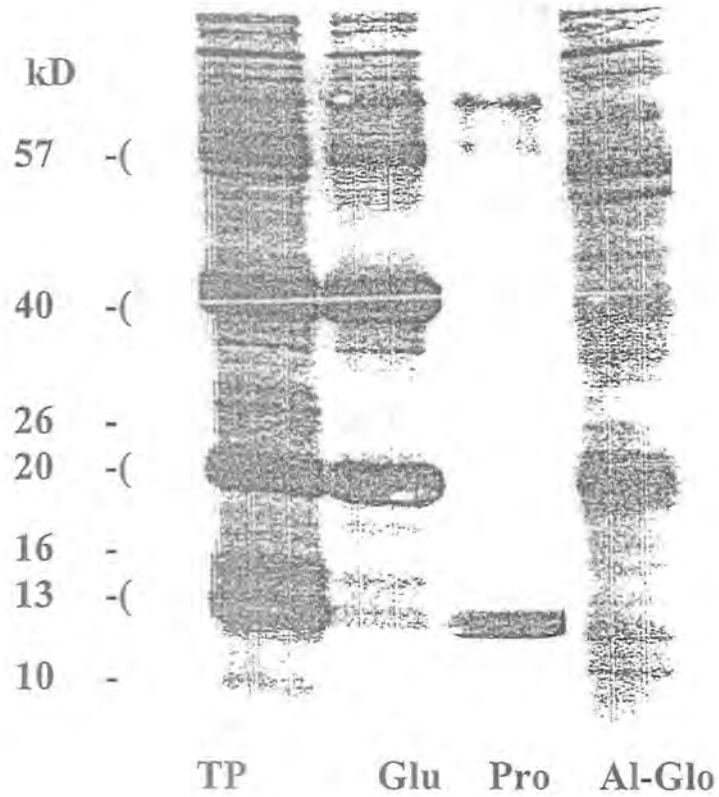
Results showed that type-1 with high intensity of both 13a (15kD) and 13b (13kD) contained 43 accessions with percentage 59.72% of total population. Type-2 having low intensity of both 13a and 13b subunits of prolamin contained 7 accessions with frequency percentage 9.72%. Type -3 with intermediate band (14kD) in between 13a and 13b contained only two accessions (PAK006634, PAK008005) which were 2.77% of total germplasm. Type-4 with low intensity of 13kD (13b) and high intensity of 15kD

(13a) bands consisted 20 accessions which were 27.70% of total population, (given in table 3.36).

Table 3.37 Frequency Percentage of Prolamin (13a, b) by SDS-PAGE

Group	Frequency	Frequency %
Type-1 (HI)	43	59.72%
Type-2 (LI)	7	9.72%
Type-3 (IB)	2	2.77%
Type-4 (HLI)	20	27.70%

Figure 3.13 SDS-PAGE protein profiles showing fractions based on solvent solubility.

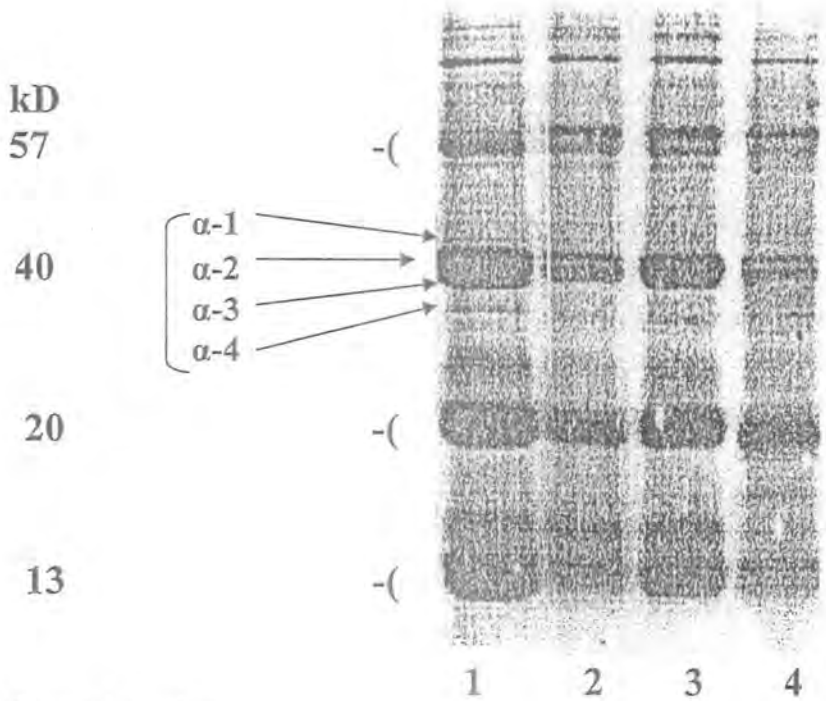


TP = Total protein

Glu = Glutelin (acid/alkali soluble)

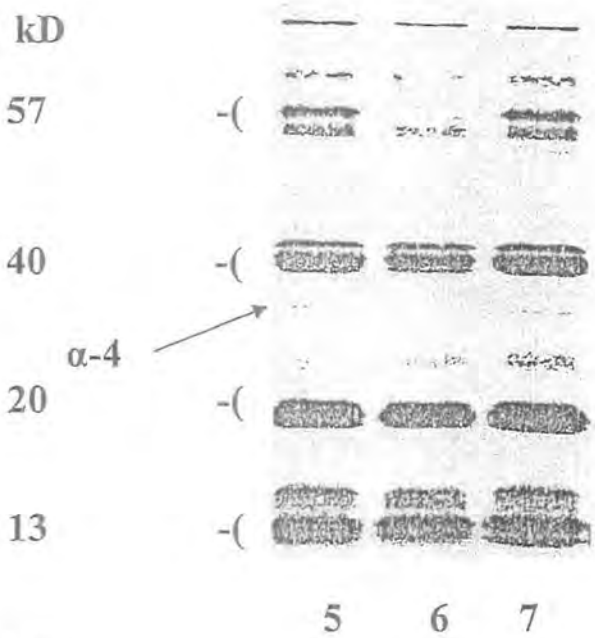
Pro= Prolamin (alcohol solube) Al-Glo = Albumine & Globulin (water/salt soluble)

Figure 3.14a Variation of Glutelin α-3 band by SDS-PAGE analysis



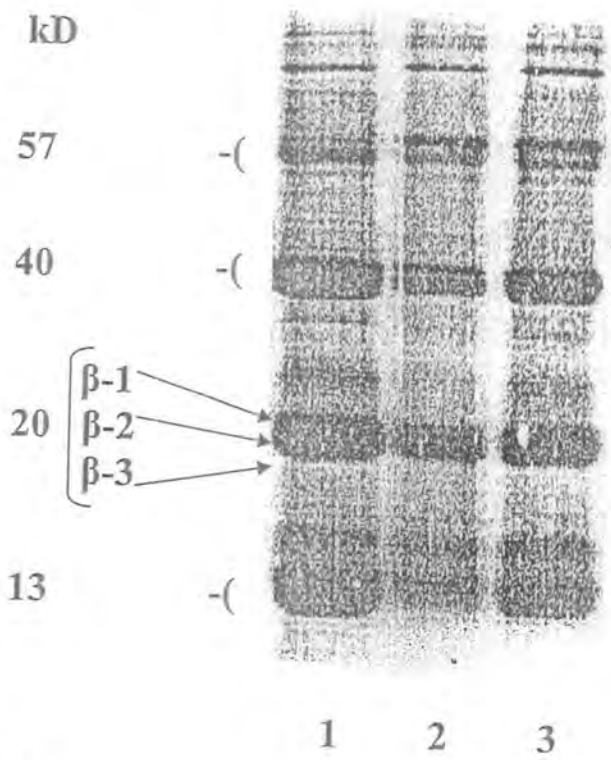
Lane 1: α-3 fast migration
Lane 2: α-3 slow migration

Lane 3: α-3 two bands
Lane 4: α-3 band absent
Figure 3.14b Variation of Glutelin (α-4) band by SDS-PAGE analysis



Lane 5,7: α-4 fast migration
Lane 6 : α-4 slow migration

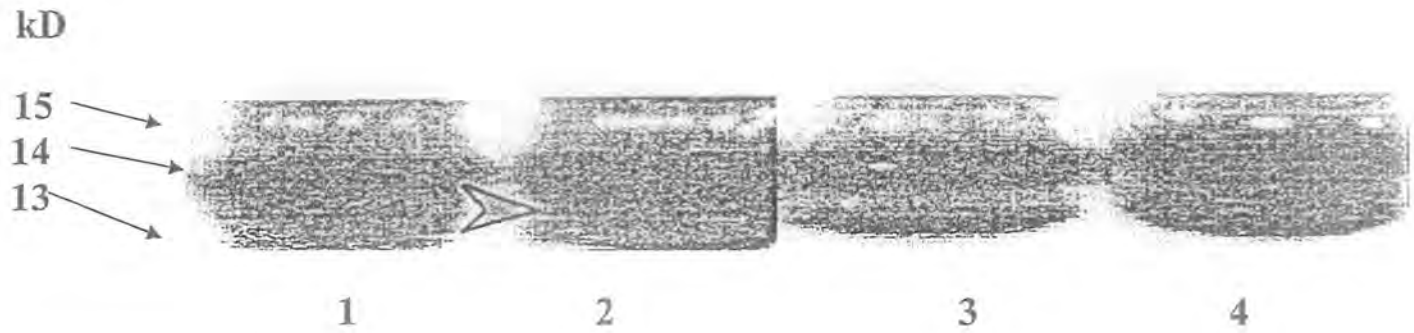
Figure 3.15 Variation of Glutelin (β-3) band by SDS-PAGE analysis



Lane 1: β-3 fast migration

Lane 2: β-3 slow migration
Lane 3: β-3 absent

Figure 3.16 variation in prolamin polypeptide bands by SDS-PAGE analysis



Lane 1: 13kD low intensity
Lane 2: 13/15kD low intensity
Lane 3: 14kD band present
Lane 4: 13kD same as 15kD band

CHAPTER-4

Discussions and Conclusion

Germplasm is a vital source in generating new plant types having desirable traits that help in increasing crop production and thus improves the level of human nutrition. It serves as base for developing new high yielding varieties resistant to biotic and abiotic stress (Mulehlbauer, 1991). In order to maintain, evaluate and utilize germplasm efficiently and effectively, it is important to investigate the degree of genetic diversity it contains (Smith & Smith, 1989). Genetically heterogeneous population produces more and stable yield than genetically homogenous lines (Simmonds, 1979). The identification of plants with suitable combination of characters from a population with genetic diversity is depending upon the knowledge of breeder on that population. This knowledge is utilized to decide a selection criterion, which is expected to prove effective for yield improvement together with other traits, which is resistant to all kind of biotic and abiotic stresses. Smith & Smith (1989) considered morphological characterization as first step in description and classification of crop germplasm.

4.1 Genetic diversity based on Morphological characters

4.1.1 Qualitative Traits

In order to evaluate, maintain and utilize germplasm effectively and efficiently it is important to investigate the extent of genetic diversity it contains. Out of all 72 accessions most significant level of diversity was observed in seed coat color (frequency 8, 11.11%). Significant level of variability was seen in flag leaf (Frequency 4, 5.55%) and panicle exertion (frequency 4, 5.55%). Least significant level of diversity was seen in lodging (frequency 2, 2.77%), panicle type (frequency 3, 4.16%), awning (frequency 3, 4.16%) and awn color (frequency 3, 4.16%).

It was concluded that traits of seed coat color, flag leaf and panicle exertion were under the control of multiple alleles, which were alternative forms of a single gene pair located on homologous chromosomes and diversity in these traits was obvious. The traits of lodging, panicle type, awning and awn color did not revealed any diversity and these traits were under the control of single gene pair having no alternative forms. However,

panicle type, awning and awn color showed incomplete dominance having three different phenotypes while lodging revealed complete dominance having two clear cut phenotypes.

4.1.2 Quantitative Traits

Descriptive Statistics for Quantitative Traits

Diversity in rice germplasm was categorized on the basis of 'co-efficient of variance percentage' into three categories. First highest degree of variation was >15%, second low degree of variation was >10% to ≤15% and third least degree of variation was ≤10%.

The highest degree of variation was seen in six quantitative traits out of total twelve characteristics. Mean value for plant height was observed 113.7 ± 33.72%, minimum value for this trait was 58.0cm shown by accession No. PAK007983 and maximum value was 191.7cm shown by accession No. PAK007060. The trait of productive tillers per plant had mean value 7.5 ± 37.25%, minimum value 2.2 was shown by accession No. PAK007916 and maximum value by accession No. PAK007060. Panicle length also revealed highest degree of variation with mean value 25.3 ± 19.21%, minimum value 17.8cm (accession No. PAK007060) and maximum value 44.0cm (accession No. PAK007896). Mean value for spikelets per panicle was 11.5 ± 17.7%, minimum value for this trait was 5.8 shown by accession No. PAK007923 and maximum value was 16.8 shown by accession No. PAK007959. The trait of 100-seed weight was also highest diversified. Mean value for this trait was observed 1.3 ± 46.16% which was the highest degree of variation in the whole germplasm. Minimum value for 100-seed weight was 0.3mm seen in accession No. PAK007995 and maximum value was 3.1 mm seen accessions No. PAK007998. The trait of straw yield was also included in the category of highest diversity because it had mean value 95.2 ± 18.8%, minimum value 62.2g was shown by accession No. PAK007940 and maximum value 143.3g was shown by accession No. PAK008015.

Highest variance (diversity) was observed for the above characters in all accessions collected from various parts of Pakistan. High variance was because of diversity in collection/selection criteria, having strong genetic based diversity and strong environmental acceptability. Highest diversity for these traits indicates the importance of

this germplasm and further improvement through simple selection from these materials. In general the selection for these traits is effective in developing high yielding varieties of rice from the present material. These characters can also be used for further evaluation and breeding programs. Subdividing the variance into its components assists genetic resources conservation, utilization and enables planning for use of appropriate gene pool in crop improvement for specific plant attributes (Bekele, 1984). In the whole germplasm some accessions showed best performance for two or more traits i.e. PAK007060 showed best performance for plant height, productive tillers per plant and panicle length, hence these could be directly utilized or included in hybrid program for varietal development. Selection on the basis of best performance has already been suggested by many researchers; Donald (1962), Lal (1967), Singh (1977), Wallace and Munger (1966) and Khan & Malik (1989). According to Ghafoor *et al.*,(1989) highest yielding accessions selected from the local germplasm might prove their superiority in advance testing under various agro ecological conditions.

Low degree of variation on the basis of descriptive statistics was observed in four traits. Day of flowering revealed mean value 86.9 ± 13.15%, minimum value 54 was shown by two accessions PAK007961 and PAK007962, maximum value 131 was observed in accession No. PAK007916. Day of maturity also showed low degree of variation with mean value 110.1 ± 13.61%, Minimum value 60 seen in accession No. PAK007961, PAK007962 and maximum value seen in accession No. PAK007916, PAK007998. Seed width had mean value 2.6 ± 13.13%, minimum value 1.6mm shown by accession No. PAK007060, maximum value 4.5mm shown by accession No. PAK007962. Seed length to width ratio was also included in this category. Mean value for this trait was 3.5 ± 13.38%, minimum value 2.0 was seen in accession No. PAK007962 and maximum value 4.9 was seen in accession No. PAK008010. Low variance was observed in these traits. These characters need to be improved by acquiring germplasm from other sources or collecting germplasm from center of diversity. Breeding technique like wide hybridization and mutation can also be implied to induce genetic variance. Low variance for these traits is supposed to be related to narrow genetic base as these characters are least affected by the environmental stress. Here, also some accessions showed best performance for more than one traits. Accession No. PAK007961

and PAK007962 revealed minimum values for day of flowering and day of maturity. Accession No. PAK007916 showed maximum values for both day of flowering and day of maturity. In all these three accessions plants were matured in a short interval after day of flowering which is a best selection for plant breeders. Accession No. PAK007962 also revealed maximum value for seed width and minimum value for seed length to width ratio which again seeks attention of breeders for development of varieties.

Least degree of variation for descriptive statistics was observed only in two traits; seed length and grain yield. The trait of grain yield revealed mean value 8.98 ± 2.14% which was the least degree of variation as compared to other quantitative traits. Minimum value of grain yield per plant was 5.2g shown by accession No. PAK007999 and maximum value 16.8g by accession No. PAK008015. Seed (grain) length also showed the least degree of variation having mean value 8.8 ± 10.3% with minimum value 5.7mm shown by accession No.PAK007060 and maximum value 13.4mm shown by accession No.PAK007567. The characters having least degree of variation need full attention by breeders. Interbreeding is required to produce variability in these characters. Least variance in these traits also indicates that these are under the influence of less gene pairs as compared to other quantitative traits. Grain yield per plant is an important character for improvement and it seeks more attention as compared to other quantitative traits.

Frequency Distribution for Quantitative Traits

The germplasm of *Oryza sativa* was having considerable degree of variation for quantitative traits. The frequency distribution for various traits was presented in tabulated as well as graphic form. Important quantitative traits studied were day of flowering, day of maturity, plant height, productive tillers per plant, panicle length, straw yield per plant, grain yield per plant, 100-seed weight, seed length, seed width, seed length to width ratio.

For day of flowering maximum value calculated was 34 with 47.22% of total germplasm (72 accessions). It ranged ≤103 day of sowing. Results revealed that most of the plants were put in this category. However, 12 accessions which were 16.66% of total germplasm showed minimum range for day of flowering which may help breeders and researchers for their studies in early flowering of rice. Out of these 12 accessions two accessions (PAK007961, PAK007962) produced flowers just within 54 days. These two accessions are direct source of study for breeders and biotechnologists. Only one

accession (PAK007916) took maximum time of 131 days to produce flowers. It is late flowering type and it may also be helpful to some extent.

For day of maturity maximum value 24 with frequency percentage 33.33% was seen in two categories; ≤117 and ≤133 both of which were late maturing categories. So, results revealed that most of accessions were late maturing and rice plant takes more time to mature after flowering. However, two accessions (PAK007961, PAK007962) having percentage 2.77% of total germplasm took minimum time to mature and fall under category <70 days. Both accessions took 60 days each for maturity after sowing. Once, again these two accessions seek attention from breeders and researchers. Less time for maturity and early flowering is one of the objectives in rice crop research (IRRI, 2003).

Maximum frequency for plant height was observed in 26 accessions which were 36.11% of total germplasm having range ≤84.7cm. Minimum frequency for this trait was observed in 8 accessions with percentage 11.11% and height ≤191.7cm. As we have a careful view on the results it is clear to us that maximum accessions fall in the category having plant height ≤84.7cm which is the minimum range for plant height. Less plant height is preferred by plant breeders because they want more grain yield and less straw yield. So, germplasm used in this study is best suited for breeders research as most of the accessions in this germplasm already contain less plant height. Minimum value for plant height 58.0cm was seen in accession No.PAK007983 which is a direct source of study for researchers and plant breeders.

For productive tillers per plant maximum frequency was 35 with percentage 48.61% having range ≤10.92 followed by the category with frequency 32, percentage 44.44% having range ≤6.56. Only one accession (PAK007060) produced maximum tillers which was 1.38% of total germplasm ranged upto 24.0 tillers per plant. Productive tillers per plant have direct relation with grain yield. More the number of productive tillers more is the yield per plant. In the whole germplasm most of the accessions produced less tillers per plant which reveals that this trait is not so much developed in rice crop as it should be. This trait wants more attention from breeders and research workers. The only accession PAK007060 producing maximum tillers (upto 24) may prove direct source to improve this character in rice germplasm.

For panicle length maximum frequency seen was 33 with percentage 45.23% and range 23.0cm followed by the category having frequency 24 with percentage 33.33% and range ≤28.28cm. Only one accession (PAK007896) showed maximum panicle length 44.0cm with frequency percentage 1.38% of total germplasm. Most of the plants in the whole germplasm showed less panicle length. More panicle length is not preferred by plant breeders as it produces more straw as compared to less panicle length. So, most of accessions in the germplasm already contain less panicle length which is an ideal condition for breeding work. There is less or no need to improve this trait in rice germplasm.

Maximum value for spikelets per panicle was 31 with percentage 43.05% having range ≤12.4 followed by the category with 20 accessions, percentage 27.7% having range ≤10.2 and category having minimum value 3 with percentage 4.16% ranged ≤16.8. Results reveal that most of the accessions have less number of spikelets per panicle. Only three accessions (PAK007959, PAK007953, PAK007956) produced maximum spikelets per panicle which fall in the category upto 16.8. More the number of spikelets per panicle more grains are produced and grain yield per plant inhances consequently which is major objective of rice crop research (IRRI, 2003). We conclude from the results that most of the germplasm contains less number of spikelets per panicle so it needs more attention by breeders to be improved. Accession No. PAK007953, PAK007956, PAK007959 are direct source of breeding material for research.

Maximum frequency for straw yield was observed 25 with percentage 34.72% ranged ≤78.5g. Next category contained 17 accessions with percentage 23.61% ranged ≤94.8g. Minimum frequency 6 with percentage 8.33% was observed in category having maximum production of straw 143.7g. Most of the germplasm fall in the category having less straw yield. Less straw yield is preferred by plant scientists. So germplasm already contains most of accessions with less yield of straw therefore, little or no need to improve this trait. Maximum production of straw was seen in accession No.PAK008015 and minimum production 62.2g of straw was noted in accession No.PAK007940 which can directly be used in improvement program.

Grain yield per plant showed maximum value 28 with percentage 38.8% having range ≤7.5g which was the least production of all categories. Minimum frequency 4 was

showed by another category having percentage 5.55% and ranged ≤16.8g which was the highest production of all categories. Results revealed that most of plants were having less production categories. Grain yield is an important trait and studied by many scientists (Choudhary & Khan 1953, Mulehbauer, 1991, Smith & Smith 1989, Simonds 1979). To increase grain yield per plant is an important objective for breeding program of rice crop (IRRI, 2004). Grain yield per plant is directly related with our food needs. The germplasm used in this study showed less production of grains per plant which concludes that there is need for more breeding work to improve grain yield per plant. Less number of accessions fall in categories having maximum production. Only four accessions (PAK008015, PAK007953, BAS-385, DR-92) were categorized for maximum seed production per plant (upto 16.8g). Accession No.PAK008015 gave maximum grain production 16.8g per plant which seeks special consideration during research work.

For the trait of grain length maximum number of accessions 32 with frequency percentage ≤44.44% were categorized in a group with 8.78mm grain length, followed by the group with 30 accessions 41.62% of total germplasm having length ≤10.32mm. It was clear from the results that most of the accessions in germplasm contained intermediate length. Few accessions with less grain length and very few with higher value. Grain length is an important character of rice and market value of rice varieties depend upon grain length. More the grain length higher the value in market, moreover, grain length also affects nutritional value of rice. So, there is need to enhance this character in the germplasm under study. Maximum value of grain length 13.4mm was seen in accession No.PAK007567 which may prove direct source of information for researchers.

For grain width maximum accessions 37 with percentage 51.38% were observed in a category having seed width ≤2.80mm followed by the group consisting 33 accessions with 45.8% having seed width ≤2.18mm. Other groups with greater seed width consisted few number of accessions. Results showed that most of the plants in whole germplasm contained less grain width. Grain width has same importance as grain length. Both characters have same nutritional and market value. There is need to exploit more grain length in rice germplasm to fulfill our needs. Maximum grain width 4.5mm was observed in accession No.PAK007962 which seeks attention of plant breeders and researchers.

Grain length to width ratio is an other important trait related with seed length and seed width. Group having length to width ratio ≤3.7 contained maximum number of species that was 45 with 62.5% of total germplasm. The group with minimum length to width ratio (≤2.5) contained only two accessions (PAK007962, PAK007577) while group with maximum length to width ratio (≤4.9) also contained two accessions (PAK008010, PAK006542). Greater the seed length to width ratio lower is the nutritional and market value of crop (Malhorta. *et al.*, 1974). So there should be minimum seed length to width ratio for better quality of seed. The germplasm in this study mostly contained intermediate value of length to width ratio. There is need to improve this trait in rice germplasm. Minimum value (2.0) for seed length to width ratio was observed in accession No.PAK007962 which is a direct source of genetic study for further investigation.

100 seed weight is an important trait which represents average seed yield of an accession. Maximum accessions for 100-seed weight were 24 with percentage 33.33% having ≤1.4g weight followed by the category having 100-seed weight ≤0.9g with frequency 23 and percentage 31.94%. Results revealed that most of the accessions in the whole germplasm were having less value for 100-seed weight. Increase in average seed yield is one of the most important objectives in rice crop research (IRRI, 2003). 100-seed weight directly affects per acre yield of a crop. More is the seed weight higher is the crop production per acre which is an important factor to fulfill our food needs. Germplasm studied in this research work contained most of accessions with less weight of 100-seeds. More work is needed to enhance seed yield by maximizing 100-seed weight. Accessions having >1.9g of 100-seed weight seek more attention. There were 11 accessions in two categories having 100-seed weight ≤2.5g and ≤3.1g respectively. Two accessions in the category having maximum 100-seed weight were PAK007998 and PAK007567 with 3.1g and 2.6g production respectively, seek special attention for further studies.

4.1.3 Correlation

A critical review of the results showed that correlation between day of flowering and day of maturity was highly significant (0.657) positively correlated and day of flowering with seed weight was significant positive (0.0538) which reveals that these traits are directly proportional to day of flowering. High significance for day of maturity shows that both have very close positive relation. Day of flowering was negatively significant correlated with plant height (-0.007), panicle length (-0.194), productive tillers per plant (-0.050) and seed length (-0.0126) while negatively highly significant for spikelets per panicle (-0.217), grain yield (-0.336), straw yield (-0.261), 100-seed weight (-0.234), seed length to width ratio (-0.206) which reveals that increasing the day of flowering decreases values for these traits. Day of flowering should be minimized to enhance values of these traits.

Day of maturity is positively correlated only with seed width (0.164) and day of flowering (0.657) which reveals that these two traits have direct relation with day of maturity. It showed negative correlation with plant height (-0.0127), productive tillers per plant (-0.0016), panicle length (-0.0789) and seed length (-0.089) which was negatively significant. Day of maturity also showed negative correlation with spikelets per panicle (-0.269), grain yield (-0.441), straw yield (-0.339), 100-seed weight (-0.260) and seed length to width ratio (-0.288) which was highly significant. Most of the traits were negatively correlated with day of maturity which indicates that rice plant should not be late matured. Among all traits grain yield per plant, 100-seed weight and seed length are the most important to maximize per acre yield. All these traits are negatively correlated with day of maturity which shows that day of maturity should be minimized which seeks special attention by plant breeders and researchers.

Plant height was positively correlated with Productive tillers per plant (0.227), panicle length (0.468), straw yield (0.398) and 100-seed weight which were highly significant for this trait. Plant height was also positively correlated with spiklets per panicle (0.0103) and grain yield (0.170) which showed significant positive correlation. It shows that more plant height higher is straw yield and grain yield. But greater straw yield is not preferred therefore, less plant height is considered better. Plant height was significantly negative for day of flowering (-0.0072), day of maturity (-0.00166), seed

length (-0.0702), seed width (-0.0569) and seed length to width ratio (-0.0088) which reveals that increasing plant height decreases value of these traits. As we need more length and greater width of seed for better crop yield therefore plant height should be minimized by further research.

The trait of productive tillers per plant showed positive correlation with grain yield (0.257), plant height (0.227), 100-seed weight (0.213) which were highly significant and with panicle length (0.0361), spikelets per panicle (0.167), straw yield (0.195) which were significantly correlated to this trait. It is clear from these results that more is the number of productive tillers per plant more is the grain yield, 100-seed weight and straw yield, so accessions having greater number of productive tillers per plant are preferred over those having less number and may prove sole material to develop new varieties. It showed negative correlation with day of flowering (-0.0502), day of maturity (-0.0016), seed weight (0.0093) which were significantly correlated and seed length (-0.284), seed length to width ratio (-0.277) which were highly significant negative for this trait. It can be concluded that less flowering and maturity time automatically enhance number of productive tillers per plant which may prove helpful during research work.

Panicle length also showed both positive and negative correlations with other traits. It was positively correlated with plant height (0.468), straw yield (0.318), 100-seed weight (0.292) which were highly significant while seed length (0.0265), seed width (0.0077), seed length to width ratio (0.0959), grain yield (0.0656) were positively significant which revealed that more is the panicle length greater is seed yield, straw yield and seed length, so accessions having greater panicle length are preferred in research work. Panicle length showed negatively significant correlation with day of flowering (-0.194), day of maturity (-0.0789), spikelets per panicle (-0.0073) which indicates that less is the time for day of flowering and maturity more is the panicle length which directly enhances crop yield.

The trait of spikelets per panicle was positively correlated with grain yield (0.397), straw yield (0.355), 100 seed weight (0.300) which were highly significant for this trait while seed length (0.192), plant height (0.0103), productive tillers per plant (0.167) were significant. It is clear from the results that greater number of spikelets per panicle causes more production because of it's positive correlation with seed yield and

seed weight. So, varieties having more number of spikelets per panicle should be developed by breeders. Negative correlation of this trait was seen with seed width (-0.197), seed length to width ratio (-0.0597) and panicle length (-0.0733) which were significant while day of flowering (-0.217) and day of maturity (-0.269) showed highly significant negative correlation. Inverse and highly inverse relation of this trait with seed length to width ratio and day of flowering and maturity respectively is also an other evidence that number of spikelets per panicle should be enhanced during breeding programs.

Grain yield was positively correlated with productive tillers per plant (0.257), spikelets per panicle (0.397), straw yield (0.866) which were highly significant for this trait and plant height (0.170), panicle length (0.0656), 100 seed weight (0.158), seed length (0.0527) were significant. It is clear from the results that grain yield per plant can be maximized directly if traits of productive tillers per plant, spikelets per panicle, straw yield and panicle length are exploited by research work because of their highly significant direct relation. Negative correlation of grain yield was seen with seed width (-0.181), seed length to width ratio (-0.123), day of flowering (-0.336), day of maturity (-0.441) which were both significant and highly significant. So, there should be less time for flowering and maturity minimum seed length to width ratio to maximize grain yield.

The trait of straw yield was also correlated with other traits both positively and negatively. Positive correlation of straw yield was observed with plant height (0.398), panicle length (0.318), spikelets per panicle (0.355), grain yield (0.866), 100 seed weight (0.221) which were highly significant and seed length (0.128), productive tillers per plant (0.195) which were significant. To maximize straw yield these traits are exploited. Straw yield showed negative correlation with seed width (-0.190), seed length to width ratio (-0.075) which were significant and day of flowering (-0.261), day of maturity (-0.339) which were highly significant for negative correlation. Normally, more straw is not preferred by breeders and researchers because of it's less uses. To minimize straw yield values of seed width and seed length to width ratio can be enhanced by further research work but it should be kept in mind that it also influences grain yield negatively.

100 seed weight was positively correlated with plant height (0.335), productive tillers per plant (0.213), panicle length (0.292), spikelets per panicle (0.300), straw yield

(0.221) which were highly significant and grain yield (0.158) which was significantly correlated with this trait. More is the seed weight greater is crop yield so seed weight should be maximized exploiting any one or all traits which have positive correlation with it. It showed significantly negative correlation with day of flowering (-0.234), day of maturity (-0.260), seed width (-0.0619) and seed length to width ratio (-0.0560). It is clear from results that seed weight can be improved if plant takes less time for flowering and maturity, have less seed width and seed length to width ratio.

Seed length also revealed positive and negative correlations with other traits. It was positively correlated with seed width (0.428), seed length to width ratio (0.258) which were highly significant and panicle length (0.0265), spikelets per panicle (0.192), grain yield (0.0527), straw yield (0.128), 100 seed weight (0.0645) which were significantly positive. To have more crop yield seed length should be maximized by research activity. It also influences market value if rice is sown as cash crop. So, one or all the above traits having positive correlation with seed length can be enhanced to have maximum seed length. Negative correlation of seed length was seen with day of flowering (-0.0126), day of maturity (-0.0896), plant height (-0.0702) and productive tillers per plant (-0.284) which were significantly negative correlated. To maximize seed length time for flowering and maturity and plant height should be minimized.

The trait of seed width was positively correlated with seed length (0.0128), day of flowering (0.0538), day of maturity (0.164) and panicle length (0.0077) which showed significantly positive correlation with this trait. It was negatively correlated with plant height (-0.0569), productive tillers per plant (-0.0092), spikelets per panicle (-0.197), grain yield (-0.181), straw yield (-0.190), 100 seed weight (-0.0619) and seed length to width ratio (-0.0551) which showed significantly negative correlation with this trait. Most of the important traits which take part in higher crop yield i.e. grain yield, seed weight, productive tillers per plant etc. are negatively correlated with seed width which indicates that seed width should not be maximized. On the other hand, it's positive correlation with seed length and panicle length indicates that it should not be minimized. So, we conclude that this trait is not as important as others, however, it's positive and negative correlation with other traits may help in research activities.

It was observed that seed length to width ratio was positively correlated with panicle length (0.0959) and seed length (0.258) which showed significant and highly significant positive correlation respectively. All other traits were negatively correlated with seed length to width ratio out of which day of flowering (-0.206), day of maturity (-0.288), productive tillers per plant (-0.277) were highly significant and plant height (-0.0088), spikelets per panicle (-0.0597), grain yield (-0.0123), straw yield (-0.753), 100 seed weight (-0.0560), seed width (-0.0551) were significantly negative. Most of the traits are negatively correlated with seed length to width ratio. Negative relation of grain yield, straw yield, seed weight etc. indicate that seed length to width ratio should be minimum for higher crop yield. Accessions having less seed length to width ratio are more important than others which may help in further research activities.

4.2 Genetic Diversity based on SDS-PAGE analysis

SDS-PAGE is considered a reliable method because storage proteins are largely independent of environmental fluctuations (Gepts, 1989; Murphy *et al.*, 1990). Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky and Hymowitz, 1979; Murphy *et al.*, 1990; Khan, 1992; Das and Mukarjee, 1995). Varieties discrimination and identification have been achieved in a range of agricultural crops by means of electrophoretic technique (Mooler and Spoor, 1993). Moreover, because proteins are the primary gene product, they are providing valuable means of making genetic systems. The variation in protein composition is the reflection of genotypic variation. However, few studies indicated that cultivar identification was not possible with the SDS-PAGE method, as the electrophoretic patterns of proteins were similar among the cultivars (Ladizinsky and Alder, 1975; Raymod *et al.*, 1991; Ahmed and Slinkard, 1992; de Varies, 1996). Glutalin and prolamin are the major storage proteins comprising up to 75% and 25% respectively, of total seed storage proteins of rice. Therefore variation in these two proteins is described.

4.2.1 Glutelin

Rice glutelin consists of acidic (α) and basic (β) subunits which were composed of 4 and 3 bands respectively (Uemura *et al.* , 1996). The four bands of acidic glutelin (α) were assigned as α1, α2, α3 and α4 while that of basic (β) are assigned as β1, β2 and β3.

For acidic glutelin subunits variation was observed in α3 and α4 bands. The whole germplasm was divided into six groups. Group of type-1 showed fast migration (FM) for α3 band which reveals that α3 band is low molecular weight in these accessions. This group was 18.05% of total germplasm which also indicates that it has less range of distribution in Pakistan. Type-2 was of slow migration (SM) for α3 band which reveals that α3 band contained high molecular mass in these accessions. These accessions were 75% of total germplasm which clearly indicates wide distribution of this group in Pakistan's environment from Indus delta to northern mountains. In type-3 α3 band has two sub-bands which revealed that it has both fast and slow migrations (FSM) in other words α3 band showed intermediate migration in these accessions. This group comprised

only 5.5% of total germplasm. It can also be concluded that accessions of this group are recombinants of those in type-1 and type-2. In type-4 α3 band was absent which showed that there was no migration (NM) and it reveals that α3 band remained attached at its precursor (57kD) during electrophoresis. Only one accession (1.38%) showed this type of behavior. Next two groups were assigned on the basis of α4 band migration. Type-5 contained fast moving (FM) α4 band while type-6 contained slow moving (SM) α4 band which reveals that both low molecular and high molecular α4 bands are present in the germplasm. Fast moving and slow moving α4 band accessions were 58.33% and 41.66% of the total germplasm respectively. Only two types of α4 band indicate that there is no recombination of fast and slow migration bands in this type of glutelin, otherwise, there would be an intermediate band.

For basic glutelin units variation was observed in β3 band which was clearly seen on electrophoretic gel. The whole germplasm was divided into three groups. Group of type-1 showed fast migration (FM) for β3 subunit which indicates that accessions in this group contain low molecular mass of β3 subunit. It was 54.16% of total germplasm which reveals it's wide range distribution in agro-climatic zones of Pakistan. Type-2 showed slow migration (SM) for β3 band. It's sluggish motion in electrophoretic gel shows that accessions of this group have high molecular mass β3 band. These accessions were 43.05% of total germplasm which indicates that this type is also widely distributed in Pakistan. In type-3 β3 subunit showed absence of band which appeared that there was no migration (NM) from its precursor during electrophoresis. Only two accessions (2.77%) showed this type of migration. Absence of β3 band on gel does not mean that it was totally absent in these accessions but we say that there was some problem during electrophoresis because of that β3 band could not move. During experimental work it was observed that immature seed or improper crushing may cause these problems. Absence of two sub bands for β3 indicates that recombination has not occurred in slow migration and fast migration types.

4.2.2 Prolamin

Major component of prolamin is 13kD polypeptide, which consists of 13a (15kD) and 13b (13kD) bands, Ogawa *et al.* (1987).

Based on the presence and absence or staining property of the bands prolamin diversity was catogorized into four types. Results showed that type-1 with high intensity of both 13a (15kD) and 13b (13kD) was 59.72% of total population. High intensity of 15kD and 13kD bands in these accessions indicates that these are fully saturated with prolamin. Another interesting factor is that higher percentage indicates that these are widely distributed in agro-climatic zones of Pakistan. So we conclude that most of germplasm in Pakistani rice has higher value of prolamin which increases it's nutritional importance. Type-2 having low intensity of both 13a and 13b subunits of prolamin comprised 9.72% of total germplasm. Low intensity of 15kD and 13kD bands in this type indicates that accessions in this group contain less amount of prolamin. Less percentage (9.72%) also indicates limited distribution of these species in different environments. Type -3 with intermediate band (14kD) in between 13a and 13b contained only two accessions (PAK006634, PAK008005) which were 2.77% of total germplasm. Presence of intermediate band reveals that recombination has occurred in 13kD and 15kD bands which resulted in extra band. These two accessions are direct source of further studies and may prove helpful for improvement of varieties by genetic engineering and conventional breeding. Type-4 with low intensity of 13kD (13b) and high intensity of 15kD (13a) bands consisted 27.70% of total population. This type may also be categorized as group having intermediate intensity. Low intensity of 13kD indicates less quantity of this prolamin in the accessions of this group which can be improved by further research work. Deep analysis of whole germplasm shows that it contains greater quantity of prolamin which determines higher nutritional value of Pakistani rice.

It is concluded that SDS-PAGE analysis showed stability of the characters under consideration i.e. free from environmental stresses, phylogenetic relationship among the accessions, diversity among accessions at gene level (because protein is the primary product of gene), sorting the accessions of the same locality, variation occurred in the accessions by out breeding, intimation for the selection of accessions with best performance (diversity level).

CHAPTER-5

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APPENDIX: Data of quantitative characteristics studied along with Accession Registration numbers.

Acc. No.	Accession Registry	Grain yield	Straw yield	100-SW	SL	SL/SW	SW
1	006542	9.1	121.4	1.7	9.7	4.3	2.3
2	006549	7.3	98.7	2	8.5	3.1	2.8
3	006593	8.7	100.2	2	9.1	3.3	2.7
4	006597	5.4	78.5	1.9	8.9	3.4	2.6
5	006634	9.4	118.4	1.9	9.2	4	2.3
6	006678	8.5	99.6	1.9	8.9	3.5	2.5
7	006681	9.7	102.3	1.4	8.5	3.4	2.5
8	007060	12.1	98.6	1.8	5.7	3.5	1.6
9	007064	8.6	94.4	1.3	8.6	3.5	2.5
10	007092	9	124.7	1.5	9.1	3.3	2.8
11	007483	9.3	112.4	1.4	9.3	3.2	2.7
12	007498	9.6	123.4	1.4	9.6	4.8	2.8
13	007435	8.7	108.7	1.8	8.7	2.5	2.6
14	007566	9.1	110.4	2	9.1	3.3	2.9
15	007567	13.4	122.4	2.5	13.4	3.2	2.8
16	007577	6.9	79.8	1.9	6.9	2.6	2.8
17	007581	9	92.6	0.5	9	3.3	2.7
18	007588	8.2	88.8	1	8.2	3.3	2.6
19	007859	6.4	73.4	2.4	6.4	3.5	2.5
20	007855	9.3	131.4	1.8	9.3	3.2	2.8
21	007857	8.9	97.7	0.4	8.9	3.6	2.7
22	007878	9	94.6	0.5	9	3.3	2.6
23	007880	8.7	103.9	0.6	8.7	2.9	2.7
24	007884	8.7	72.8	1.4	8.7	3.4	2.5

25	007896	8.8	114.4	1.6	8.8	3.3	2.7
26	007899	8.1	102.4	1.4	8.1	3.2	2.8
27	007916	8.5	89.7	1	8.5	3.4	2.5
28	007980	8.4	92.6	0.7	8.4	3.6	2.6
29	007923	5.4	67.3	0.9	8.7	3.3	2.8
30	007924	5.7	69.4	1.2	8.8	3.7	2.6
31	007938	6.4	74.5	1.3	9.1	4	2.6
32	007940	5.5	62.2	0.9	8.9	3.9	2.7
33	007941	5.9	68.4	0.6	9.3	3.9	2.5
34	007942	13.7	141.3	1.7	9.1	3.1	2.3
35	007946	13	108.5	2.1	8.8	4	2.3
36	007947	14.7	139.2	2.7	8.6	3	2.2
37	007948	5.4	73.4	1.3	8	2.9	2.6
38	007951	14.2	134.7	2.2	8.3	4.2	2.1
39	007952	7.8	74.4	1.7	8.5	3.3	2.8
40	007953	15.5	140.2	1.4	7	3.2	2.5
41	007956	14	129.3	1.3	9.4	2	2.2
42	007959	11.2	110.8	1.4	8.5	3	2.6
43	007961	13.1	122.4	1.1	8.8	3.4	2.8
44	007962	10.9	90.7	1.6	9	3.6	4.5
45	007963	10.4	94.2	1.7	7.6	3.4	2.5
46	007970	6.5	73.6	1.2	8.2	3.5	2.4
47	007071	7.1	77.8	1.1	9.6	3.4	2.8
48	007072	11	102.5	1	9.1	3.1	2.7
49	0007973	7.5	82.4	0.9	9.2	3.9	2.5
50	007975	9.1	94.2	0.3	7.8	3.2	2.3
51	007976	6.6	74.7	1	8.2	3.2	2.7

52	007977	6.3	75.5	0.6	9.9	3	2.5
53	007978	6.1	69.9	2.9	9.8	3.1	3
54	007979	7.2	83.6	0.8	8.8	3.6	2.8
55	007980	12.1	114.3	1.1	8	3.5	2.6
56	007983	6.4	69.6	0.9	8.7	4.3	2.8
57	007986	8.1	90.4	0.9	9.1	4	2.5
58	007989	6.8	73.3	0.4	8.9	3.4	2.6
59	007990	8	78.1	0.9	8.9	3.2	2.1
60	007992	7.6	73.2	2.5	9.1	3.5	2.3
61	007993	5.9	67.5	0.8	9.9	4.1	2.9
62	007994	7.4	81.2	0.6	7.5	3.6	2.3
63	007995	7.8	84.3	0.9	9.5	3.6	2.7
64	007998	6.4	71.7	3.1	9	3.7	2.2
65	007999	5.2	64.9	0.8	8.8	3	2.5
66	008003	6.9	70.8	1.4	7.5	4.9	2.1
67	008005	8.5	84.8	0.7	9	4	2.5
68	008006	8	81.2	0.8	7.3	3.7	2.4
69	008010	6.9	71.3	1.4	9.7	3.3	2
70	008015	16.8	143.7	0.9	9.3	3.1	2.3
71	DR-92	15.3	123.6	1.2	9.1	3.3	2.5
72	BAS-385	15.9	127.2	1	9	3.4	2.8

Acc. No.	*Accession Registry*	*DF*	*DM*	*PH*	*PT/P*	*PL*	*S/P*
1	006542	94	119	180.2	11.8	29.4	11.2
2	006549	90	122	180	9.4	34.4	14.2
3	006593	97	124	175.8	11.6	29.8	12.2
4	006597	80	128	170.6	7.2	34.4	10.8
5	006634	82	114	162.4	9.8	31.4	13
6	006678	71	112	158	4.4	30.8	12.8

7	006681	103	97	136.8	3.6	25.8	9.2
8	007060	96	121	191.7	24	17.8	11
9	007064	93	119	90.4	10	22.8	12.2
10	007092	104	101	153.8	9	27	11.2
11	007483	88	102	161.4	6.4	29.2	13.6
12	007498	90	114	152.8	7	30.8	12.6
13	007435	70	97	134.6	4.6	26.4	9
14	007566	68	98	134	7.6	25.8	12
15	007567	79	99	148.8	6.6	24.4	13.8
16	007577	63	93	133.3	10.3	25	9.7
17	007581	96	124	141.2	4.8	21.6	12
18	007588	93	126	147.8	7.4	25.8	12
19	007859	86	112	168.2	6.6	34.6	10
20	007855	71	97	170.6	5.8	36.4	11.6
21	007857	93	129	146.8	6	28	10.6
22	007878	80	101	166.6	6.2	26.2	10.4
23	007880	94	124	77.2	5.8	19.2	9.6
24	007884	102	97	130.2	8.6	26.6	13.2
25	007896	93	121	149.4	10.2	44	8
26	007899	90	123	152.8	6.2	25.8	10.6
27	007916	131	132	141	2.2	18.2	10.2
28	007980	90	117	125.2	2.8	21.2	10.6
29	007923	101	131	128.6	4.8	23.8	5.8
30	007924	88	98	170	6.2	19	9.4
31	007938	84	107	110.8	7	19.8	12.4
32	007940	100	121	89.2	5.4	30.6	7.6
33	007941	93	119	81.2	5.4	29.6	9.2
34	007942	84	96	161.8	11.8	28	12.6
35	007946	64	93	134.2	7.8	21.8	13.6
36	007947	65	94	144.8	7.6	28.8	13.2
37	007948	97	122	93.6	8.8	22.4	9.8
38	007951	86	109	100.6	6	26.2	12.6
39	007952	78	113	94.2	8	26.6	12.6
40	007953	84	107	89.8	6.6	25.8	15.4
41	007956	85	106	88.2	9.2	26.2	15.4

42	007959	79	99	96.2	6.8	25.4	16.8
43	007961	54	60	91.2	8	31.8	14.2
44	007962	54	60	97.4	7.8	22.6	12
45	007963	71	97	135	9.2	23	12.6
46	007970	90	112	80.8	5.8	21.4	10.4
47	007071	84	108	83.4	6	21	13.6
48	007072	90	117	84.2	9.6	25.2	11.2
49	0007973	93	120	75.4	8.2	23	11
50	007975	85	114	78.2	7.6	20.2	12.2
51	007976	93	114	72.4	4.8	21	11.6
52	007977	89	115	62	5.8	19	9.8
53	007978	86	110	67.8	6.8	23.8	11.2
54	007979	91	94	65.2	7.2	20.2	8.2
55	007980	106	124	94.5	9.8	24.4	11.8
56	007983	94	129	58	6.4	19	9.4
57	007986	79	107	78.2	5.8	24.6	10.4
58	007989	92	128	69.9	7	23.2	9.6
59	007990	80	101	82	6.8	23	12.6
60	007992	93	116	64	6.2	22.4	12.8
61	007993	91	122	73.6	7.8	23	12
62	007994	86	133	84.2	10	19.2	12.2
63	007995	93	110	80.6	7.6	22.6	13.8
64	007998	94	96	63	8.8	20.8	14.4
65	007999	84	98	76	6	26.8	13.8
66	008003	68	112	82.2	7.4	27.8	7
67	008005	74	126	71.2	7.6	23	11.4
68	008006	84	99	81	7.8	29.6	8.4
69	008010	96	128	61.2	8.2	21.6	12.6
70	008015	79	94	91.8	8.6	22.4	11
71	DR-92	73	93	116.8	5.3	23	9.4
72	BAS-385	70	91	102.8	11.4	26.8	12.6