Chapter 5

Discussion

In recent years, there are an increased number of releases of genetically related varieties by rice breeders, which resulted in limited levels of genetic diversity. Information on the extent of genetic diversity among cultivars is needed to exploit the available genetic resources to create new genotypes. The information on genetic diversity helps in choosing parents for generation of new varieties which needs continuous evaluation of germplasm for useful characters based on morphological data. Morphological markers reflect not only the genetic contribution of the cultivar, but also the interaction of the genotype with the environment (G x E) in which it is expressed. Hence the descriptions based on morphological data are inadequate in providing reliable information for the calculation of genetic distance or the validation of pedigrees.

Recent advances in genetics and molecular biology have provided descriptors based on protein markers (Isozymes) which were later replaced by DNA markers due to the availability of unlimited number of polymorphic loci which proved to be sufficient for cultivar identification and better understanding of pedigree.

The first generation DNA markers include RFLP markers, were used for evaluation of variation within cultivar of rice accessions (Olufowote et al., 1997). RFLP was later replaced by PCR based markers, as it is cumbersome, time consuming, labour intensive and costly affair. The PCR based markers proved to be highly evolved over RFLP, which include RAPDs, AFLPs and Microsatellites. RAPDs and AFLPs were used for assessing genetic diversity in rice (Fukuoka et al., 1992; 1994; Virk et al., 1994; Agarwal et al., 1999). The use of RAPDs is of less importance when compared to microsatellites and AFLPs as they are dominant and non-reproducible. AFLP technique is reaching better grounds at present but yet the drawback underlying behind their usage is that they are dominant markers but requires quality DNA to reproduce. Nowadays, microsatellite markers are gaining a lot of importance as they provide a rapid approach to analyse the genetic diversity and reflect the genetic relationships among the selected genotypes.
5.1.1 MORPHOLOGICAL TRAIT VARIABILITY

The characterization of the rice genotypes individually using either plant morphological characters or chemical or electrophoresis or combining all these parameters is possible. The morphological markers provide easy and convenient means of genotype identification and characterization. However, it is time consuming. Presence of differences among the individual of a species refers to variability. Differences may be either in the genetic constitution of the individual plants or in the environment in which they are cultivated. The nature of variability present in the gene pool for a given character decides the success of the breeding programme. Hence, it is of utmost importance of a plant breeder to assess the variability that is existing in the gene pool for any character of a crop species. In the present study, the morphological variation did exist among the 40 rice varieties with respect to the 14 quantitative (Table 3.3) and 22 qualitative traits (Table 3.4) recorded. The extent of variability was found to be maximum for height of the plant, days to flowering followed by yield and total duration with regard to quantitative traits (Table 4.3). Elemery et al., (1998) reported maximum variability for plant height. Yolanda and Das (1995) and Khanghah and Sohani (1999) reported maximum variability for yield. Among all the traits investigated, number of tillers recorded maximum value of coefficient of variation (28.98) (Table 4.3). However, the results obtained from the study are carried out to characterize 40 rice genotypes based on the morphological (qualitative and quantitative) traits (Table 4.5) and molecular markers are discussed here under.

Based on the plant height studied the rice genotypes varied significantly (Figure 4.1 & Table 4.2). The highest plant height was observed in Kichilisamba (174 cm) and the lowest plant height was observed in ADT 39 (65 cm) with a mean plant height of 135.05 cm (Table 4.2). Based on the average plant height, among the 40 genotypes, 3 genotypes had short, 19 genotypes had medium and 18 genotypes had tall plant height (Table 4.7), which suggested significant variation in plant height that could be used for identification of genotypes. Zafar et al. (2004) observed a significant amount of genetic variation for quantitative and qualitative
characters among the land races of rice. Bose and Pradhan (2005); Le and Yang (2005); Madhavilatha and Suneetha (2005) and Singh et al.,(2006) have observed the genetic divergence of rice accessions and reported that traits like plant height and days to 50 % flowering contributed to genetic divergence and important in the choice of parents for breeding programme.

In the present study, plant height (-0.397) and grain length (-0.322) showed significant and negative association with yield (Table 4.4). Agro morphological traits like height of the plant, weight of panicle, 1000 seed weight, length of the panicle also contribute towards genetic divergence (Singh and Singh, 2008). Zafar et al., (2004) observed significant and positive association with height of the plant and length of the panicle. A similar variation in plant height was reported by Rosta (1975), Anitalakshmi (2002), Nethra (2003) and Mageswaran (2010) grouped in to 10 rice genotypes based on this character. Zaman et al. (2004) evaluated 8 agro-morphological characters of 20 rice accessions and reported that days to flowering and height of the plant contributed to total divergence.

Significant difference was observed among the rice genotype for number of tillers per plant. The mean number of tillers per plant observed was 10.98, of which TKM 10 recorded 8 and ADT 45 recorded the maximum of 14 tillers per plant (Table 4.2). Masuthi (2011) reported significant difference among scented rice genotypes for number of tillers per plant. Suman et al. (2005) studied the genetic divergence in rice germplasm using 16 quantitative characters and found that harvest index contributed maximum to the divergence followed by seed density and total number of tillers per plant. Ogunbayo etal. (2007) characterized 96 rice landraces using 14 agro-morphological traits reported that total number of tillers was not a functional of yield but these traits were significantly associated with height of the plant and maturity date.

In the present study, out of 40 genotypes, 12 genotypes possessed dark green as blade colour and 27 genotypes possessed green blade colour and one possessed pale green blade colour (Table 4.8). Evera (2003) used the trait leaf blade colour to characterize twenty six cultivars of rice. Monika et al. (2007) also grouped nineteen varieties of rice based on intensity of green colour of leaf. Similar
type of work was carried out by Anitalakshmi (2002), Nethra (2003), Rimpi et al. (2008), Mageshwaran (2010) and Sarika et al. (2011).

The presence of pubescence on blade surface of leaf was seen among all the 40 rice genotypes (Table 4.8). Evera (2003) used pubescence on blade surface to characterize twenty six rice cultivars while Monika et al. (2007) and Bora et al. (2008) also characterized nineteen and eleven cultivars in rice respectively. And Mageshwaran (2010) grouped 10 rice genotypes based on this character.

Evera (2003) used this trait to characterize twenty six paddy cultivars while Monika et al. (2007) and Bora et al. (2008) used the same trait to characterize nineteen and eleven cultivars of rice respectively. Chaudhury and Sahai (1993) found high variability for stigma colour while evaluating 1270 Cambodian rice genotype. The presence of light green internode colour was seen among 38 genotypes except 2 genotypes which possessed green internode colour (Table 4.8). Masuthi (2011) reported that among the plant characters plant habit, stem length, stem thickness, internode colour, node anthocyanin colour, ligule colour, auricle colour, days to 50 per cent heading and time of maturity constituted some distinguishable characters.

Rosta (1975) suggested that length and width of blade were quite useful traits in varietal identification. In the present study among 40 genotypes, 15 genotypes possessed short leaf length, 4 genotypes possessed long leaf length and 21 genotypes possessed medium leaf length (Table 4.7). Monika et al. (2007) also grouped nineteen rice varieties based on length and width of the blade and suggested that high heritability and genetic advance (GA) was observed with reference to this character. In the present study among the various characters studied, leaf width is positively and significantly associated to the yield (Table 4.4).

Monika et al. (2007) in nineteen rice varieties used this parameter for characterization. Colour of stigma at the time of anthesis is an important character, used for varietal characterization. Based on the colour of stigma, Masuthi (2011) grouped the genotypes into five categories as white, light green, yellow, light purple and purple type. Rimpi et al. (2008) grouped nine of eleven varieties into white stigma category. Chaudhury and Sahai (1993) found high variability for stigma colour while evaluating 1270 Cambodian rice genotype. Similar type of
classification was reported by Rohini Devi (2000), Dhanaraj (2001) and Anitalakshmi (2002) in rice.

Monika et al. (2007) grouped nineteen rice varieties based on stem length and reported high heritability of this trait. Out of 40 accessions studied 3 are of short plant height, 19 are of medium plant height and 18 accessions are of tall type (Table 4.7). Rimpi et al. (2008) grouped rice cultivars like Moniram, Monoharsali, Mahsuri and Satyaranjan into semi-dwarf and Bishnuprasad, Piyalee and Jyotiprasad into dwarf categories.

By using the trait anthocyanin colouration of nodes Monika et al. (2007) and Rimpi et al. (2008) characterized nineteen and eleven varieties of rice respectively. Similar type of work was carried out by Mageshwaran (2010) and Sarika et al. (2011). In the present study, 9 genotypes are of short panicle length, 22 genotypes are of medium panicle length, 7 genotypes possessed long panicle length and 2 genotypes possessed very long panicle length (Table 4.7). Leaf length, panicle length and days to 50% flowering were negatively and non-significantly associated with the yield (Table 4.4).

Similar type of classification was reported by Rohini Devi (2000), Dhanaraj (2001) and Anitalakshmi (2002) in rice. Ganesan and Subramanian (1994) and Verma et al. (2000) suggested that panicle length was influenced by both additive and non-additive gene expression. And Pusa 1460 genotype has the highest flag leaf length of 50.0 cm, whereas, Kagi Sali genotype was found to be the lowest in (20.5 cm) flag leaf length. The mean flag leaf length of blade observed was 28.6 cm. All the accessions possessed erect (1) flag leaf angle, except IR 50 which was intermediate (3), ASD 19 and TKM 1 showed horizontal flag leaf angle and none of the accessions are of descending type in the present study (Table 4.8). Masuthi (2011) based on the flag leaf length, grouped 13 genotypes had short type, 22 genotypes had medium type and 6 genotypes had long type flag leaf length. Sangeeta Das and Amitava Ghosh (2010) studied four hundred thirty one traditional rice cultivars from genotype collection of Rice Research Station, Chinsurah, characterization had been done on thirty one traits. Masuthi (2011) reported that characters like leaf blade colour, panicle exertion, stigma colour etc. showed moderate variability.
Out of 40 genotypes studied, 10 genotypes possessed few no. of grains per panicle, 18 genotypes grouped under medium and 12 were grouped under many (Table 4.7). Evera (2003) grouped CR 1009 as long duration variety and TKM 9 as short duration variety by using the same trait. Rimpi et al. (2008) also grouped eleven rice varieties using this character. Similar type of classification was reported by Rohini Devi (2000), Dhanaraj (2001) and Anitalakshmi (2002) in rice. Among the 40 rice accessions, 3 accessions have low yield, 21 accessions have medium yielding ability and 16 accessions have good yielding ability (Table 4.7). Masuthi (2011) observed significant difference among the scented rice genotype for plot yield and reported that out of 41 genotypes, 12 genotypes have less, 16 genotypes have medium, 11 genotypes have good plot yield. Though, this character is not influenced by environment for the characterization of the genotypes but farmers point of view these really have considerable value. Thus, the results suggested that the plant morphological characters could be used for broader classification of the genotypes. Several workers noticed similar observations for example Graham (1913) pointed out that colour of leaf sheath is usually associated with colour of the apiculus. Hector et al (1934) observed that colouration of apiculus is closely linked with internode and stigma colour. The colouration of nodes and internodes could be fairly used for identification. Awn length is highly variable character and it would vary from smallest possible tip to about three inches or more in same plant and hence may not be useful for classification in rice (Kashiram and Chetty 1934). The collection of 92 accessions (including 86 landraces and 6 cultivars) indicated considerable variability for growth and grain characters. Indigenous knowledge of such accessions as collected from the farmers revealed an array of specialty uses. Trait expression in these native genotypes is highly dependent upon local environment and has evolved over a long period of time through traditional and cultural cropping practices (Bhat and Gowda, 2004). Successful reports on plant characters for varietal identification was reported in crops like Vida laba (Bond and Crofton, 2001), sorghum (Thangavel, 2003), lucerne (Senthil kumar, 2003) and pearl millet (Arun Kumar et al., 2004), Oat (Sumathi, 2007), cotton (Manjunath Reddy, 2005; Sangeetha Macha, 2010 and Ameena, 2009), wheat (Romuald Kosina, 2010), rice (Anitalakshmi, 2002; Nethra, 2003; Rimpi et al., 2008; Mageshwaran, 2010 and Sarika et al., 2011), french bean (Chandrashekar, 2008), soybean (Chavan, 2010) and cowpea (Naima Ghalmi et al., 2010).
CHARACTERIZATION OF GENOTYPES BASED ON MOLECULAR MARKERS

Genetic diversity at genomic level is a pre requisite for the selection of the variation. Though, a range of plant morphological traits is accurately used for distributing the genotypes, environment play an important role in influencing their expression. Thus as extremely powerful tool for genotype characterization and identification would be available in DNA polymorphism, which could be detected and assessed. Advances in molecular biology now allow such detection and it is becoming increasingly possible to identify variations between individuals of similar phenotypes at the DNA level. Better approach for the assessment of genetic diversity is based on molecular markers. It does not need field evaluation and has proven to be a powerful tool. Microsatellites or Simple Sequence Repeat (SSR) markers are widely used for diversity analysis (Gao et al., 2005; Zhang et al., 2007 and Thomson et al., 2009). Both morpho-agronomic and molecular analyses are done in genetic diversity studies (Kumar et al., 2009; Ghalmi et al., 2010 and Sharma et al., 2010). Among the molecular markers, SSR’s has proved to be the most powerful tool for variety identification in groundnut and has much potential in genetic and breeding studies. Among the 17 SSR primers used for assessing the genetic diversity, 6 primer pairs (24.0 per cent) were polymorphic (Shoba et al., 2010). In the present study using 22 SSR primers among 40 rice accessions including primitive types, a total of 174 alleles were found of which RM 6925 showed the highest number of alleles (16) and RM 6124 showed the least number of alleles (2), with an average of 8 alleles across 22 SSR primers (Table 4.15). Similar studies were conducted for the genotypes in Eastern Himalayan region of North East India where the highest number of alleles (21) was detected in the locus RM264 and the lowest (4) was in the locus RM130. The indigenous rice varieties were genetically variable, while agronomically improved varieties were monomorphic within varieties at all loci. (Choudhury et al., 2013).

These for DNA profile clearly suggested the presence of abundant polymorphism in rice genotypes. In the study 40 accessions were grouped into 12 clusters with 22 SSR markers as these markers showed superior for genetic
diversity and marker based applications in genotype enhancement approaches (Table 4.14). A total of 174 alleles were found in this study and the number of alleles generated by any single primer varied from 2 to 16 with an average of 8 alleles across 22 SSR primers (Table 4.15). This indicates a wide degree of diversity among the accessions. This was similar to those reported by Wang et al., (2009) and Jalaluddin et al., (2007) using a different set of rice germplasm. Yang et al. (1994) found 25 alleles for 10 markers among 238 rice accessions. The minimum genetic relatedness was noticed from genotype TKM 3 and Manjal Ponni. The highest genetic similarity (100%) was noticed between ADT 36 and Varapukudanchan (Fig.4.4). The similarity matrix was constructed using the SSR data to assess the genetic relatedness among the selected genotypes, which added a new dimension to genetic similarly prospective generated. The selected genotypes were grouped into 12 clusters, cluster-I has 21 genotypes, followed by cluster-V (4 genotypes), cluster-II (3 genotypes), cluster-III, cluster-VI and cluster-VII (2 genotypes) and cluster IV, VIII, IX, X XI and cluster XII (1 genotype) (Table 4.14). Similarity co-efficient varied from 0.75 to 1.00 for all the genotypes. The sub clusters have similar genotypes indicating their belonging to similar genetic background. Higher range of similarity index for genotypes indicated by the microsatellite markers provides greater confidence for assessment of genetic diversity and relationship, which can be used for further breeding programmes and assessing genetic purity. Manjal Ponni and TKM 3 showed significant difference from other clusters. It might be because of very specific characteristics present in Manjal Ponni and TKM 3 but absent in remaining 38 rice genotypes. The genotypes showing greater genetic distance among them could be used as parents for future improvement programmes. According to Figure 4.4, no cluster possessed genotypes with all the desirable traits which could be directly selected and utilized. All the maximum and minimum cluster mean values were distributed in different clusters. The clustering proved the existence of significant amount of variability. The clustering did not follow any particular pattern in grouping the genotypes with respect to the origin. Similar clustering pattern was reported by Ushakumari and Rangaswamy, 1997. Similar studies have been made by different authors using SSR markers (Panaud et al., 1996; Neelu et al., 2006; Anandhan et al., 2010; Daniela et al., 2010; Suprava Mohanty, 2010 and Gowda et al. 2011). Highest PIC value (0.9012) obtained for RM 6925 and lowest PIC value (0.0696) for RM 6124
indicating higher diversity with higher PIC value in the present study. Rahman et al. (2006, 2008) reported 18 and 78 alleles with 3 and 5 primers respectively and also reported highest PIC value (0.910) for RM 335 and lowest PIC value (0.670) for RM 11. Thus, the study provided a detailed analysis and quantifications of genetic diversity in selected genotypes of North-Eastern Zone of Tamil Nadu. The data also reaffirm the powers of SSR markers to distinctly group closely related landraces.

5.1.2. CORRELATION STUDIES

The nature and extent of relationship among the traits was obtained by correlation studies. The estimation of correlation coefficient among the different characters indicates the extent to which it was associated. (Falconer, 1981). Yield is a major important trait which is influenced by a large number of other component traits. A knowledge of the association between yield and its component traits and also between the component traits helps in improving the efficiency of crop improvement.

In the present investigation, correlation coefficients were worked out between fourteen quantitative characters. The highly positive and significant correlation value was recorded for number of grains per panicle (0.740), number of tillers (0.705), number of productive tillers (0.689) and leaf width (0.318) with yield (Table 4.4). It indicates that the selection in any one of these yield attributing traits will lead to increase in the other traits, there by finally enhancing the yield of the grain. Karmarkar et al., (1998) reported that seed length, width and thickness showed strong and positive correlation with seed weight.

5.1.3 MORPHOLOGICAL TRAIT DIVERSITY AND CLUSTER ANALYSIS

The clustering of genotypes based on morphological (quantitative) traits resulted in nine clusters (Table 4.6). Maximum number of varieties were included in cluster I, V, VI (7 varieties) and the minimum number is in cluster VIII (1 variety). The cluster I consisted of ADT 36,TKM 4 ,Odagathur local ,Odagathur Ponnni , UMA , IW Ponni and Varapukudanchan , cluster V consisted of ADT 39 , BPT 5204, TKM 8 , ADT 42, CR 1009 , ADT 43 and CO 43 and cluster VI
consisted of TKM 1, TKM 6, Manjal Ponni, TKM 7, TKM 5, Kattasamba, Seeragasamba. These varieties included in the Cluster I having medium duration, tall and short bold grains. Similarly the cluster II consisted of IR 20, Bavani, ASD 19 and TKM 9 which are of medium duration having medium to (tall nature) long slender grains. The cluster III consists of ADT 37 and ADT 45 with short duration, semi dwarf nature with medium slender grains, high yielding and Annaikomban resistant. The cluster IV consisted of ADT 38, TRY 1, IR 50, ADT 44 and ADT 46 with (medium duration, dwarf in nature and medium slender grains). The cluster V consisted of ADT 39, BPT 5204, TKM 8, ADT 42, CR 1009, ADT 43, and CO 43 (with short duration, semi dwarf nature and long slender grains). The varieties having long duration, tall compact and short bold grains are included in the cluster VI (TKM 1, TKM 6, Manjal Ponni, TKM 7, TKM 5, Kattasamba, Seeragasamba. The cluster 7 included TKM 3, Kichilisamba, TKM 11, TKM 12 and Kuzhivedichan. The cluster 8 consisted of TKM 10 and cluster 9 consisted of Poongar and Kullakar. Earlier Jaylal (1994) grouped forty genotypes of rice into nine clusters on the basis of yield attributes.

5.2.1 GENETIC DIVERSITY STUDIES USING SSR MARKERS

Genetic diversity was lower (0.0722) for RM 6124 of genotype ADT 37 and higher (0.8997) for RM 24260 of genotype ASD 19 and also PIC value was lower (0.0696) for RM 6124 of genotype ADT 37 and was higher (0.9012) for genotype TKM 1 for RM 6925 (Table 4.15). Masood et al., (2013) reported an average genic diversity for 40 rice accessions was 0.4477, ranging from 0.0488 to 0.6638 and PIC value ranged from 0.0476 (RM 315) to 0.5993 (RM 252), with an average of 0.3785 per marker. Microsatellites are found to provide high PIC and found to be highly efficient and cost effective for cultivar identification and hence chosen as efficient markers for evaluating the heterogeneity of rice accessions (Yang et al., 1994; Olufowote et al., 1997; Bligh et al., 1999). The SSR markers can detect discrete loci, they are co-dominant, segregate in a Mendelian fashion and an ideal genetic markers.

5.2.2 CLUSTER ANALYSIS BASED ON SSR MARKERS

The similarity matrix was computed using SSR markers based on Jaccard’s coefficient following the UPGMA method using SHAN programme of NTSYS-pc. The 40 genotypes formed 12 clusters at nearly 9% similarity levels. Among the
different clusters, the cluster size varied from 21 (cluster I) to 1 (Cluster VIII, IX, X, XI, XII). The list of all the 12 clusters along with the list of genotypes included is presented in Table 4.14.

The cluster I consisted of Manjal Ponni, Odugathur Ponni, CO 43, ADT 46, TKM 9, TRY 1, ADT 43, ADT 42, TKM 6, TKM 5, TKM 1, ASD 19, Bavani, TKM 8, IR 20, ADT 38, IR 50, UMA, BPT 5204, Varapukudanchan, and ADT 36. The cluster II consisted of ADT 37, ADT 45, and TKM 4. The cluster III consisted of varieties ADT 44 and Poongar. The cluster IV consisted of Seeragasamba. The cluster V consisted of genotypes CR 1009, TKM 10, Kichilisamba, Odugathur local. The cluster VI consisted of genotypes IW Ponni and TKM 11. The cluster VII consisted of genotypes TKM 12 and Kuzhivedichan. The cluster VIII consisted of TKM 7, cluster IX consisted of Kattasamba, cluster X consisted of ADT 39, cluster XI consisted of Kullakar and cluster XII consisted of TKM 3. The Jaccard’s similarity coefficient for the SSR data set varied from 0.75 to 1.0. The SSR marker profiles resulted in twelve clusters at nearly 9 % similarity.

Wang et al., 1992 constructed dendrogram based on SSR markers using 129 accessions of rice which showed wide genetic variation in the rice accessions.

The 21 accessions Manjal Ponni, Odugathur Ponni, CO 43, ADT 46, TKM 9, TRY 1, ADT 43, ADT 42, TKM 6, TKM 5, TKM 1, ASD 19, Bavani, TKM 8, IR 20, ADT 38, IR 50, UMA, BPT 5204, Varapukudanchan, and ADT 36 were grouped into a single cluster (Cluster I) based on SSR profile with the similarity level. These results indicated that there is a narrow genetic base of above accessions based on SSR profile. Further the narrow genetic base of these accessions also evidenced from the cluster II which consisted of concessions ADT 37, ADT 45 and TKM 4.

Highest diversity was found between Manjal Ponni and TKM 3 and lowest diversity was found between ADT 36 and Varapukudanchan. The SSR marker data able to differentiate the accessions into 12 separate clusters when compare to the morphological data. This shows the potentiality of SSR markers for the characterization of germplasm accessions. Ghatge and Kadu (1993) studied 48 rice genotypes from different eco-geological regions of India and grouped into seven clusters revealing that genetic diversity was not associated with geographical diversity.