EVALUATION AND MOLECULAR CHARACTERIZATION OF ADVANCED MUTANT LINES IN RICE (*Oryza sativa* L.)

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By

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CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION AND MOLECULAR CHARACTERIZATION OF ADVANCED MUTANT LINES IN RICE (*Oryza sativa L.*)"** submitted by **Mr. MAHENDRAKUMAR, V. B.** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Raichur, is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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AFFECTIONATELY DEDICATED

TO

MY BELOVED PARENTS

BHUVANENDRAKUMAR, A. V. N.,
VANAJAKSHMI, C. S.

LOVELY SISTERS, DIVYA, BHAVYA,
BROTHER IN LAW, UMESH

&

ALL FRIENDS...

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With ever regardful memories……

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Raichur
July, 2014 ***(MAHENDRAKUMAR, V. B.)***

LIST OF ABBREVIATIONS

%	:	Per cent
bp	:	base pair
cm	:	Centimeter
dNTP	:	dinitro-triphosphate
EC	:	Electric conductivity
et al.	:	And others
Fig.	:	Figure
g	:	Gram
ha	:	Hectare
kb	:	kilo base pair
Kr	:	Kilo rad
µg	:	microgram
µl	:	microlitre
µM	:	micromolar
M	:	Molar
mg	:	Milligram
mM	:	Millimolar
No.	:	Number
ng	:	nanogram
pg	:	picogram
Pm	:	picomoles
t	:	Tonnes

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Introduction

I. INTRODUCTION

Rice (*oryza sativa* L.) is the major staple food for more than half of the global population and considered as the "global grain". By the year 2025, 21% increase in rice production will be needed over that of year 2000. Centre of origin of cultivated rice is the part of South East Asia, which is considered as the heartland of rice cultivation. About 92 per cent of the world's rice production and 90 per cent of global rice consumption is from Asia which accounts for 60 per cent of global population. Rice provides 30–75 per cent of the total calories to more than 3 billion Asians. In India, rice is grown on 44.40 million hectares, with an annual production of about 106.23 million tons and productivity of about 2395 kg ha^{-1} (FAO, 2013). India is the second largest country of rice production and rice continues to hold the key to sustain food production by contributing 20-25 per cent of agriculture and assures food security in India for more than half of the total population (Anon., 2013).

It is notable that the grain yield especially of rice has not been harvested in commensuration to its existing genetic potential in almost all rice growing ecosystems. One of the major reasons behind this failure is the sensitivity of this crop to abiotic stress particularly salinity (Grover *et al.*, 2000). Salt stress is one of the major abiotic stresses, which adversely affect the crop productivity (Yasseen *et al.*, 2010; Joseph and Jini, 2010). In India, salt affected area is about 6.73 mha. Salinity is one of the major obstacles in increasing production in rice growing areas worldwide, which is an ever-present threat to crop yield (Bhowmik *et al.*, 2009). It causes reduction of crop yield and alteration in plant metabolism, including reduced water potential, ion imbalances and toxicity and sometimes several salt stress may even threatens survival (Joseph and Jini, 2011). So the need of the hour is to develop plants with resistance to abiotic stress. The losses incurred by the farmers due to salinity were estimated at 28% ha^{-1}, if salinity increase from low salinity level to medium salinity level and 76% ha^{-1}, if it jumps from lower salinity to higher salinity level. Rice is most economically important cereal crop in many part of the world and considered as a salt sensitive species (Htwe *et al.*, 2011). Even a low concentration of 50 mM is not tolerable (Flower and Yeo, 1981). In order to produce new rice genotypes with better adaption to salinity, mutation techniques can be resorted (Mba *et al.*, 2007).

Generally, salinity tolerance is a polygenic trait. Development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline

area. The response of rice to salinity varies with growth stages. Several studies indicated that rice is tolerant during germination and becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage becomes sensitive during pollination and fertilization and then become increasingly more tolerant at maturity (Bhowmik *et al.,* 2009). Screening rice germplasm to locate salt tolerant genes for use in improving the currently grown varieties is of continuous importance to plant biotechnologist. One major approach in plant breeding is to maximize the genetic diversity between the parental genotypes for hybridization. There are rice varieties which yield high under normal condition; they fail to perform in salt affected soil. So genetic diversity screening for a trait of interest is an essential part of the commencement of breeding program. Babu *et al.* (2006) reported that genetic improvement mainly depends on the amount of variability present in the population. Hence, estimation of genetic diversity for salt tolerance parameters among the genotypes is important for planning the future breeding programme.

Screening is an essential part of the breeding programs and several screening and selection schemes have been proposed for salt tolerance improvement in wheat and other crops (Dewey, 1962, Kingsbury and Epstein, 1984 and Gorham, 1997). Also, selection for improved salt tolerance on the basis of seedling stage has been used in various crop species, for example in rice (Shannon *et al.,* 1998), maize (Rao and McNeilly, 1999) and wheat(Qureshi *et al*., 1990, Khan *et al*., 2003). Additionally, selection for salinity tolerance based on seedling response has suggested that the variation at this stage is genetically controlled (Maiti *et al.,* 1996). For enhancing salt tolerance in crop plants, it is very important to find sufficient variation and to devise such screening techniques which are reliable to identify tolerant genotypes. Variation for salt tolerance has been reported in many crop species, both between and within plant species of rice (Alam *et al.,* 2004), cotton (Noor *et al.,* 2001) and other crop plants. Since researches suggested that improvement in salt tolerance in rice would be possible through selection and breeding, therefore, the present study was aimed to generate information on the genetic variability for salinity tolerance at the early plant stage in rice genotypes mainly grown under saline soil conditions.

Salt tolerance is a complex quantitative genetic character controlled by many genes, using conventional breeding methods, plant selection for salt tolerance is not easy because of the large effect of environment and low heritability of salt tolerance. Because

of the complexity of the trait, it has been difficult to develop an accurate, rapid and reliable screening technique (Islam *et al.,* 2011). Among the several classes of available DNA markers, microsatellite or simple sequence repeat (SSR) markers are considered the most suitable due to their multi allelic nature, high reproducibility, co dominant inheritance, abundance and extensive genome coverage. A large number of SSR markers have been developed and mapped in rice (Temnykh *et al.*, 2000, McCouch *et al.*, 2002), which vary in the degree of polymorphism depending on their position in the coding or non coding segments, nature of their repeat motifs and the genome wide abundance. Therefore, an ideal set of SSR markers providing genome wide coverage will facilitate an unbiased assay of genetic diversity which in turn will provide a robust, unambiguous molecular description of rice cultivars.

In the present study, an attempt has been made to evaluate variability created by induced mutation. And keeping all these points in view, the present investigation was carried out with the following objectives

1. Assessing genetic variability in M_4 mutant lines for yield and yield components
2. Assessing genetic diversity in M_4 mutant lines for yield and yield components.
3. Screening of selected M_4 mutant lines for salinity tolerance.
4. Molecular diversity studies of salinity tolerant mutant lines**.**

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The literature relevant to experiment of this investigation is reviewed and presented under the following headings.

2.1 Assessing of genetic variability in M_4 Mutant lines for yield and yield components

2.2 Assessing of genetic diversity in M_4 mutant lines for yield and yield components

2.3 Screening of selected M_4 mutant lines for salinity tolerance

2.4 Molecular diversity studies of salinity tolerant mutant lines

2.1 Assessing of genetic variability in M_4 Mutant lines for yield and yield components.

Bhattacharya and Sowbhagya (1980) and Das *et al.* (1983) classified the rice genotypes using various grain characters (*viz.*, length, shape, 1000 grain weight or 100 grain weight and profile value).

Shivapriya (2000) reported moderate GCV and PCV for days to first flowering, plant height, days to maturity, grain breadth and L:B ratio in a study involving sixty eight local rice accessions.

Chaudary and Motiramani (2003) reported that a wide range of variation for most of the characters. Heritability in broad sense was very high for all the characters exhibited high heritability coupled with high genetic advance except harvest index. Grain yield per plant showed significant positive correlation with effective tillers per plant, spikelet density and biological yield per plant. Path analysis indicated a greater contribution of effective tillers per plant, spikelet density and biological yield per plant towards grain yield.

Elayaraja *et al.* (2003) carried out investigation in Tamil Nadu, India during 2001 to study the nature and amount of induced genetic variability in *O. sativa*. The study consisted of a cv. PY 5 treated with physical mutagen *viz.*, gamma rays at 5, 10, 15 and 20 Kr. The maximum variability was observed in plant height (20 Kr), leaf area index (15 Kr), grain yield per plant (5 Kr), dry matter production and harvest index (10 Kr) in

M_2 generation. A high heritability associated with a high genetic advance as percentage of mean was observed for plant height, leaf area index, grain yield per plant and harvest index in all the treatments of M_2 generation.

Katoch *et al*. (2005) studied that locally adapted cultivar of *Basmati* rice (*Oryza sativa* L.), namely, T-23 was treated with ethyl methane sulfonate (EMS at 0.8, 1.0 and 2.0 %) and gamma rays (at 25, 30 and 35 Kr) in Hisar, Haryana, India. A number of various types of morphological macro mutations were induced in M_2 generation. These mutants were evaluated in M_3 generation in RBD with two replications. Estimates of variability, heritability and genetic advance were worked out for the mutants. Wide range of variability was observed for grain yield / plant, effective tillers / plant, days to flowering, days to maturity, 100 seed weight and protein content. High heritability coupled with moderate genetic advance was observed for days to flowering, days to maturity and plant height.

Panwar (2005) observed high heritability and genetic advance for grain yield per panicle, chaffy grains per panicle, grain yield per plant, filled grains per panicle and secondary branch number per panicle, indicating the effectiveness of selection for these characters.

Borbora *et al.* (2005) studied correlation and path analysis for 11 characters in 30 genotypes of rice comprising 16 local varieties and 14 high yielding varieties / advanced lines under two sowing dates. The highest positive direct effect on grain yield per plant was recorded for grain yield per panicle followed by secondary branch number per panicle and plant height under both the environments. Chaffy grain number per panicle showed the highest negative direct effect on grain yield per plant followed by panicle number per plant, days to 50 % flowering, primary branch number per panicle and 1000 grain weight. The characters filled grain number per panicle, primary branch number per panicle and 1000 grain weight showed highest indirect effects on grain yield per plant.

Sivakumar and Kannan (2005) assessed the nature and magnitude of association between grain yield and its component characters of wide compatible gene involving inter sub-specific rice hybrids. Most of the characters studied (number of tillers per plant, panicle length, pollen fertility, grains per panicle and 100 grain weight) exhibited positive correlation with grain yield at both phenotypic as well as genotypic levels.

Twelve F_1 hybrids of scented rice and their seven parents involving induced mutants and basmati varieties were evaluated by Hasib (2005) for eight important panicle characters. Character association analysis revealed significant positive association of all the panicle traits, except test weight with grain yield per panicle. Path coefficient analysis revealed that panicle weight had highest positive direct effect followed by panicle length and secondary branches per panicle. Selection on higher panicle weight and higher number of secondary branches per panicle could be effective for yield improvement in scented rice.

Shashidhar *et al.* (2005) reported grain yield showed positive association with plant height, productive tillers per plant, dry matter per plant, leaf weight per plant and harvest index both at phenotypic and genotypic levels.

Genetic variability, correlation and path coefficient were estimated from 20 low land rice varieties. Highest GCV was recorded in flag leaf, high density grains / panicle and panicle weight. High heritability (in broad sense) accompanied by high genetic advance was observed in flag leaf, spikelets, filled grains and high density grains / panicle. Grain yield/plant was positively and significantly associated with effective tillers / plant, panicle weight, spikelets and high density grains / panicle. High positive direct effects coupled with significantly positive associations of effective tillers / plant and high density grains / panicle with grain yield per plant was recorded by Monalisa *et al*. (2006).

Rita *et al*. (2006) reported high heritability coupled with high genetic advance for harvest index, total number of chaffy spikelet per panicle, grain yield per plant, total number of filled spikelets per panicle and spikelet fertility percentage indicating selection may be effective for these characters.

Karim *et al*. (2007) studied the variability and genetic parameter analysis in 41 aromatic rice genotypes. Significant variations observed the phenotypic variance was higher than the corresponding genotypic variance for the characters. These differences were in case of number of panicles per hill, number of primary branches, number of filled grains per panicle, spikelet sterility (%) and grain yield per hill indicating greater influence of environment for expression of these characters.1000 grain weight and days to maturity showed least difference between phenotypic and genotypic variance, which indicated additive gene action for expression of the characters. Considering genetic parameters, high genotypic coefficient of variation (GCV) value was observed for 1000

grain weight followed by spikelet sterility (%), grain yield per hill and number of filled grains per panicle, whereas days to maturity showed very low GCV. High heritability with high genetic advance in percent of mean (GAPM) was observed for 1000 grain weight followed by spikelet sterility (%) and number of filled grains per panicle indicated that these characters were under additive gene control and selection for improvement might be effective. Days to maturity showed high heritability but low genetic advance (GA %), which indicated that non additive gene effects were involved for phenotypic expression of this character.

Mamta *et al*. (2007) studied thirty four genotypes for their genotypic, phenotypic and environmental coefficient of variation during wet season 1999–2000. Results indicated that phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) for all the traits. PCV was highest in grain yield (33.15 %) followed by biological yield (26.67 %), effective tillers/plant (25.87 %) and spikelets / panicle. GCV was highest for grain yield/plant (26.19 %) followed by effective tillers / plant (21.46%) and biological yield plant^{-1} (20.47 %). GCV and PCV were lowest for panicle length followed by days to 50% flowering and plant height. High heritability coupled with high genetic advance was recorded for spikelet / panicle. High heritability was observed for 50 % flowering, test weight and panicle length. Moderate heritability and low genetic advance was recorded for total number of tiller plant-^{1} and effective tiller plant-^{1}. Days to 50 % flowering, plant height, seed setting percentage, grain yield and harvest index showed moderate genetic advance. Genetic advance in per cent of mean ranged from 8.39 to 40.19.

Mustafa *et al*. (2007) evaluated fourteen rice (*Oryza sativa* L.) genotypes at the Gezira Research Station Farm (GRSF) for genetic variability and correlations between yield and yield components using phenotypic markers and polygenic trait analysis. A wider genetic variability was observed using the genotypes for most of the characters studied. The highest genotypic coefficient of variation was recorded for grain yield, percent unfilled grain / panicle, number of grains / panicle and number of filled grain panicle^{-1}. Phenotypic correlations between grain yield and number of filled grain panicle^{-1}, number of panicle / m^{2} and 1000 grain weight were 0.52, 0.36 and 0.27, respectively. These results suggested that improvement in yield could be attained by selecting rice plants for higher number of filled grain / panicle, number of panicle m^{-2}, and 1000 grain weight. The path analysis reveled that number of filled grains panicle^{-1} had direct positive

(0.87) contribution to the grain yield ha^{-1} and positive (0.33) indirect effect on grain yield ha^{-1} through days to 50 % maturity and number of grains $panicle^{-1}$ (0.089); while number of filled grains per particle had negative (-0.30) and (-0.21) indirect effect on grain yield ha^{-1} through number of tillers $plant^{-1}$ and number of panicles m^{-2}, respectively. The relative contribution of characters towards variability and results of correlation and path coefficient indicated the importance of number of grain $panicle^{-1}$, number of filled grain $panicle^{-1}$ and number of panicle m^{-2}. Genotypes having these characters would offer a good possibility for the improvement of rice through conventional selection.

Neelima *et al.* (2007) investigated the correlation and path coefficient analysis for various growth characters (plant height, tiller number and total dry matter production) and yield attributes (productive tillers, length of panicle, grains per panicle, filled grains per panicle and test weight), the systematic inter-relationship between the variables can be understood. Path coefficient analysis helps in separating the direct effect from indirect effect by partitioning the correlation coefficients. It clearly showed that all the characters except number of leaves, productive tillers, length of panicle and grains per panicle were highly significant ($p = 0.01$) and found to be positively correlated with grain yield of rice. Straw yield ($r = 0.91$), plant height and dry matter production ($r = 0.88$) and LAI ($r = 0.84$) had higher correlation coefficients but their direct effects varied. The direct effect was highest for straw yield (0.589). Though number of leaves and productive tillers showed a positive correlation with seed yield of rice their correlation coefficients were less ($r = 0.40$ and 0.37, respectively). The information on correlation and path coefficient studies in regular agronomic experiments is meagre. Hence this investigation was undertaken to assess and measure the magnitude of relationship between grain yield of rice and other characters.

The variability, correlation and path analysis for yield and quality traits in twenty five indigenous aromatic rice genotypes were estimated and reported high heritability (broad sense) coupled with high genetic advance for yield $plant^{-1}$, number of panicle bearing tillers and number of grains $panicle^{-1}$. Number of panicle bearing tillers and days to 50 % flowering exhibited highest positive direct effect along with positive association with yield $plant^{-1}$. Kernel length after cooking showed highest positive direct effect on kernel length (Jaiswal *et al.,* 2007).

Estimates of heritability in forty four extra early and early maturing rice genotypes in broad sense were observed to be high for flag leaf area, 1000 grain weight, grain L/B

ratio, days to 50 per cent flowering and grains panicle^{-1}. High heritability coupled with high genetic advances as per cent mean were recorded for grain yield plant^{-1}, flag leaf area, grain L/B ratio, spikelets panicle^{-1} and days to 50 per cent flowering, grain yield palnt^{-1} exhibited highly significant and positive correlation with fertile grains panicle^{-1}, grains panicle^{-1}, 1000 grain weight, panicle length, flag leaf area and spikelets panicle^{-1} (Sharma and Sharma, 2007).

Correlation studies revealed positive and significant association of plant height, productive tillers per plant, panicle length and filled grains per panicle with grain yield per plant. Selection based on productive tillers per plant and filled grains per panicle would be more useful for improvement of grain yield in rice hybrids, because of their high and positive direct effect of grain yield (Eradasappa *et al*., 2007).

Kole *et al*. (2008) studied the variability, correlation and path coefficients for twelve morphological characters on 18 morphologically distinct mutants in M4 generation along with their two mother genotypes (IET 14142 and IET 14143). Genotypic and phenotypic coefficients of variation were high for flag leaf angle and panicle number; moderate for grain number per panicle, straw weight, harvest index and grain yield per plant and low for days to flower, plant height, panicle length, spikelet number, spikelet fertility (%) and test weight. High heritability accompanied by high to moderate genetic advance for flag leaf angle, panicle number, grain number, straw weight and grain yield indicated the predominance of additive gene action for the expression of these characters. Grain yield was found to be positively and significantly correlated with plant height, panicle number per plant, straw weight and harvest index at both genotypic and phenotypic levels indicating the importance of these characters for yield improvement in this population. The results of genotypic path analysis revealed that panicle number had the highest positive direct effect followed by grain number, test weight, plant height, days to flower and straw weight. The overall results indicated that selection favoring higher panicle number per plant, test weight and straw weight and medium plant height with a reasonable balance for moderate grain number would help to achieve higher grain yield in this population of aromatic rice.

Jayasudha and Sharma (2010) conducted studies on genetic variability, character association and path-coefficient analysis on forty seven (47) rice genotypes including thirty three hybrids and fourteen parents for grain yield and some physiological traits. Analysis of variance revealed considerable variability among the genotypes for all the

characters. A high genotypic and phenotypic coefficient of variation was observed for grain yield per plant, harvest index, pollen fertility (%) and spikelet fertility (%). Characters like pollen fertility (%), spikelet fertility (%), days to 50% flowering and grain yield per plant showed high value of heritability coupled with high genetic advance. Spikelet fertility (%) and harvest index showed positive and significant correlation with seed yield per plant both at genotypic and phenotypic levels. Results of path-coefficient analysis revealed that productive tillers per plant had the highest positive direct effect on grain yield followed by harvest index, spikelet fertility (%), pollen fertility (%) and plant height. The study revealed that genetic improvement of grain yield in rice is admissible by selecting characters having high positive correlation and positive direct effect.

Siddiqui and Sanjeeva (2010) observed the mutagenic effect of gamma rays, Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) used either singly or in combination for induced genetic variability for yield and yield traits in M_2 and M_3 generation in the two cultivars of Basmati rice. The observation mean range and coefficient of variation (CV) suggest that the mutagen treatment had wider values than the control. The mean performance showed improvement in most of the mutagenic treatments in M_3 as compared to the corresponding treatments in M_2 generation. The magnitude of CV in the treated populations as generally higher than the control for most of the traits in both the generations for both the varieties. All the three mutagens were quite effective in inducing genetic variability in Basmati rice.

Selvaraj *et al.* (2011) investigated twenty one rice genotypes screened under artificially controlled conditions to identify the rice blast disease reaction. Sixteen genotypes which were already reported to have resistance genes reacted negatively to the blast disease. Four genotypes were found to be susceptible. All the 21 genotypes along with 64 hybrids were evaluated for nine traits in a randomized block design over five replications. Genetic variability, character association and path-coefficient analysis were studied. Grain yield was kept as a dependant character and the results were analyzed. Analysis of variance revealed considerable variability among the genotypes for all the characters. The phenotypic correlation coefficient (PCV) values were slightly greater than genotypic correlation coefficient (GCV), revealing negligible influence of environment in character expression. Results of path-coefficient analysis revealed that, test weight exhibited maximum positive direct effect on grain yield / plant followed by filled grains / panicle, plant height, panicle length, number of tillers / plant and days to 50% flowering

and they contributed primarily to yield and could be relied upon for selection of genotypes to improve genetic yield potential of rice.

Akinwale *et al.* (2011) estimated the phenotypic and genotypic coefficients of variation, broad sense heritability, genetic gain and correlations in rice (*Oryza sativa* L.). Genotypes differed significantly at ($p>0.001$) for all the traits studied, which implies that the genotypes constitute a pool of germplasm with adequate genetic variability. Genotypic coefficients of variation were lower than the corresponding phenotypic coefficients in all the traits studied, indicating considerable influence of the environment on the expression of the traits. High to medium broad sense heritability estimates observed for days to heading, days to maturity, plant height, grain yield and number of grains per panicle, panicle weight, number of panicles per m^2 and panicle length suggests high component of heritable portion of variation, which is the portion exploited by breeder and that selection for these traits can be achieved directly based on their phenotypic performance. High to medium heritability and genetic advance were recorded for the number of grains per panicle, grain yield, panicle weight and the number of panicles per plant. This suggests that these traits are primarily under genetic control and selection for them can be achieved through their phenotypic performance. Grain yield exhibited significantly positive correlation with the number of tillers per plant ($r = 0.58^{**}$), panicle weight ($r =0.60^{*}$) and number of grains per panicle ($r= 0.52^{*}$). Therefore, the results suggest that these traits can be used for grain yield selection.

Singh *et al.* (2011) studied eighty one rice (*Oryza sativa* L.) genotypes during *kharif* 2010 for thirteen quantitative traits to examine the nature and magnitude of variability, heritability (broad sense) and genetic advance. Analysis of variance revealed that the differences among eighty one genotypes were significant for all the characters except flag leaf width. Among all the traits number of spikelets per panicle exhibited high estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) followed by harvest index, grain yield per hill and number of panicles per hill. Broad sense heritability was highest for biological yield per hill, which suggested that this trait would respond to selection owing their high genetic variability and transmissibility. Maximum genetic advance as per cent of mean was recorded for number of spikelets per panicle with high value of heritability.

Idris *et al.* (2011) studied genetic variability and correlation between yield, yield components in some rice genotypes. Seven characters were measured including

yield, yield components. Phenotypic (PCV) % and genotypic variances (GCV) %, coefficients of variation were estimated. Phenotypic and genotypic correlation between characters was determined. There were highly significant differences ($p<=0.01$) between the most of the character, except for percentage of unfilled grains per panicle (%). The highest values of phenotypic and genotypic variance were recorded by yield $kgha^{-1}$. Also grain yield was attained the highest values of phenotypic and genotypic coefficients of variation. Positive phenotypic and genotypic correlation coefficient was detected between grain yield and number of filled grains per panicle, harvest index, panicle length and number of grains per panicle. This revealed that there was high genetic variability among the tested genotypes, indicating that it could be used for further improvement in rice breeding.

Hossain *et al.* (2011) studied the genetic variability, heritability and genetic advance for yield and yield components in 25 medium duration rice genotypes. Observations were recorded for 8 traits namely, plant height, number of panicles per hill, panicle length, number of filled grains per panicle, 1000-grain weight, grain length, grain breadth and yield. High phenotypic and genotypic variances were observed for grain yield, followed by number of filled grains per panicle. Heritability ranged from 50% (grain yield per hill) to 90% (grain breadth). Genetic advance as a percentage of mean was highest for number of filled grain per panicle (70.34), followed by grain yield (68.72). Number of filled grain per panicle, 1000-grain weight, grain length and breadth exhibited less environmental effect and high heritability coupled with moderate to high genetic advance.

Sadeghi (2011) studied to determine variability, heritability and correlations between yield and yield components in 49 landraces of rice (*Oryza sativa L.*) for 2 years. Direct and indirect effects of yield and yield components on seed yield per plant were investigated. Broad sense heritability ranged from 69.21% (Plant height) to 99.53% (Grain width). Grain yield was found to be positively and significantly correlated with grains per panicle, days to maturity, panicle weight, the number of productive tillers, days to flowering, plant height, panicle length, flag leaf width and flag leaf length indicating the importance of these characters for yield improvement in this population. The result of phenotypic path analysis revealed that the number of productive tillers had the highest positive direct effect followed by days to maturity, grains per panicle and 1000 grain weight.

Babu *et al.* (2012) studied the genetic parameters for yield, yield attributing, quality and nutritional characters in twenty one rice hybrids. Analysis of variance revealed significant differences for all the traits. The characters *viz.*, number of filled grains per panicle, number of chaffy grains per panicle and iron content exhibited high Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV). Small differences between GCV and PCV were recorded for all the characters studied which indicated less influence of environment on these characters. The characters *viz.*, number of filled grains per panicle and water uptake exhibited high heritability coupled with high genetic advance indicating that simple selection could be effective for improving these characters.

Bharathi *et al*. (2012) studied the two mutant generations M_2 and M_3 of rice var PY-1. High mean and wide range was observed in the treatment 15Kr + 1% EMS. High heritability coupled with high GA as percent of mean were recorded in M_2 generation compared to M_3 generation. This indicated that both additive as well as dominant gene action might be involved in controlling the above traits in the respective generation.

Bhadru *et al.* (2012) studied a total of 21 rice genotypes (resistant to gall midge biotype 3 and BPH) for their variability and genetic divergence. The highest genotypic and phenotypic coefficient of variation, heritability and genetic advance % of mean corresponded to grains per panicle, seed yield, 1000 grain weight and plant height and direct selection for these traits would be useful for yield improvement in rice. The D^2 values were significant among the 21 genotypes, which were grouped into 6 clusters. Most of the genotypes with same pedigree either male or female parent involved cross combination came under the same cluster and few genotypes in different cluster and genotypes of quite different pedigree may all into the same cluster. Coarse and medium slender high yielding gall midge and BPH resistant varieties genotypes could be utilized in the hybridization programme.

Gangashetty *et al.* (2013) carried out an investigation to know the extent of genetic variability present in the local non-basmati aromatic genotypes of rice. A total of fourty two genetically diverse genotypes were considered for the study. Analysis of variance was found to be significant for all the traits, indicate that there is existence of genetic variability for all the traits varying from lower to higher coefficients of variance. The results found that the higher values of genotypic and phenotypic coefficient of variability was observed for plant height, number of tillers per plant, number of

productive tillers per plant, panicle weight, grain length, test weight, iron and zinc content and grain yield per plant. The moderate genotypic and phenotypic coefficients of variance were recorded for panicle length, grain breadth and L/B ratio. The days to 50 per cent flowering had recorded lower values of genotypic and phenotypic variance. High heritability with high genetic advance was observed for all traits except days to 50 per cent flowering.

Satish *et al.* (2013) assessed variability induced for quantitative characters (days to 50% flowering, days to maturity, plant height, panicle length, panicle bearing tillers per plant and grain yield per plant.) on individual plant basis in M_1 generation. The maximum frequency of chlorophyll mutations per 100 M_2 plants was obtain in M_2 generation in 20 Kr gamma rays + EMS (0.2 %) in Kalanamak following by Badshah Bhog, respectively. The Frequency and spectrum of viable mutations per 100 M_2 plant was maximum in 40 Kr gamma rays + EMS (0.2 %) in Badshah Bhog in M_2 generation. Among chlorophyll mutations, Albino mutant was most frequent in both the genotypes. The different types of chlorophyll mutations observed in M_2 generation were Albino, Xantha, Striata and Viridis.

2.2 Assessing genetic diversity in M_4 mutant lines for yield and yield components.

Baloch *et al*. (2003) studied a high yielding rice mutant IR8-151 selected from gamma rays irradiated population of a coarse (non-aromatic) rice variety IR8. The mutant IR8-151 was significantly better than its mother variety IR8 in all the yield contributing parameters except 1000 grain weight. The mutant showed promising performance for paddy yield in varietal trials. It produced the highest paddy yield per unit area in zonal trials conducted for 3 consecutive years at 10 locations with diverse agro-climatic conditions. It maintained superiority over all the entries by yielding 9196, 7976 and 8021 kg of paddy yield per hectare during the years 1989, 1990 and 1991, respectively. On the basis of overall performance, IR8-151 mutant showed an increase of 10 % and 9 % in paddy yield over its parent IR8 and check variety IR6, respectively.

Mutation techniques are very important tools to study genetic variability, function, action and regulation of genes. Mutation techniques were directly select mutants affecting root morpho physiological traits for mapping and cloning of the genes. After preliminary tests to identify the best radiation dose, 2,500 and 3,500 seeds from cultivar Taim, indica sub species, were irradiated with doses 200 Gy and 250 Gy respectively. After harvesting,

M_2 seeds were dried stored and plantlets were evaluated in hydroponic culture, aiming to identify potential root mutants. The dose of 200 Gy seems to be associated with increases in number of seminal roots. From 9,737 seeds evaluated 623 were putatively root mutants with high and low number of seminal roots by Paulo (2003).

Zeng *et al*. (2003) reported the success of salt tolerance breeding programs employing traditional screening and selection has been limited in the past decades. This study was designed to characterize the genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers. Salt tolerance was then analyzed among molecularly characterized genotypes. Plants of 33 genotypes were grown in sand tanks under greenhouse condition and irrigated with Yoshida nutrient solution. A total of 123 alleles were generated at 25 microsatellite loci among the 33 genotypes. Genotypes of japonica rice grouped into three clusters and those of Indica rice grouped into two clusters based on microsatellite markers. Thirty percent of the alleles detected in 20 breeding lines were not identified in the cultivars analyzed. These alleles may provide favorable allelic combinations if the breeding lines are used for intercrosses. Physiological and morphological characters under salt stress were significantly ($P = 0.05$) different among microsatellite clusters. There was a highly significant correlation ($r = -0.25$; $P = 0.005$) between the matrices of Jaccard genetic similarity based on microsatellite markers and taxonomic distance based on ion data.

Sarkar *et al.* (2006) assessed the genetic divergence using Mahalanobis D2 statistics was carried out among 46 rice genotypes including high yielding local and advanced breeding lines grown in Zn-deficient soil. The genotypes were grouped into seven clusters. Cluster I had the highest number of genotypes (Twenty one) followed by Cluster II (Nineteen) and Cluster V with two genotypes. Other cluster was found to be monogenotypic. Cluster IV showed highest intercluster distance from Cluster VI which was immediately followed by Cluster III and Cluster VII. Highest intracluster distance was observed in Cluster V and lowest in Cluster I. The desirable yield and its contributing traits were distributed mainly in Cluster III followed by Cluster VII and Cluster I. The genotypes within Cluster III, VII and I may be used as parents in hybridization programme to develop high yielding line ideal for Zn-deficient soil.

Singh *et al*. (2006) were studied the genetic divergence of 52 traditional lowland rice (*Oryza sativa* L.) genotypes from five states of North Eastern Region of India using Mahalanobis D^2 statistic investigation. Based on 15 agro-morphological characters, these

genotypes were grouped into six clusters. Out of 52 genotypes, 26 genotypes were grouped in cluster I; cluster VI comprised only one genotype. Genotypes from more than one state were grouped in one cluster, and genotypes from one state were grouped in more than one cluster. Geographical origin was not found to be a good parameter of genetic divergence. Clusters II, III, and IV exhibited high values for most of the characters. Plant height followed by leaf angle and leaf area, highly contributed (32.43%) to the formation of clusters. Clusters II, IV, and V which had maximum inter-cluster distances and high values of plant height, days to 50 per cent flowering, panicle length, grain yield per plant and milling percent, may be used for initiating a hybridization programme.

Bughio *et al.* (2007) studied high yielding rice mutant variety Mehak was developed from a fine aromatic variety Basmati-370, through gamma rays (150 Gy). The mutant variety Mehak was found significantly better than mother variety Basmati-370 with respect to yield and yield contributing traits. On the basis of three years yield it has showed 80% increase in yield over its parent Basmati-370 and 40% yield increase than check variety Super Basmati. The mutant variety is also inherited with excellent aroma and other physico-chemical properties of check varieties Super Basmati and Basmati-2000.

Babaei *et al.* (2009) reported that Mutation induction has become a proven way for creating variation within a crop variety. The molecular behavior of 22 morphological selected M2 generations of Sange-tarom and Taromhashemi rice cultivars (Oryza sativa L.) evaluated using 26 RAPD molecular markers. Sixty one (57%) and 53 (52.5%) polymorphic bands out of 107 and 101 observed band belonged to Sangetarom and Tarom-hashemi respectively. The maximum produced band (15 bonds) related to the OPD11 and the minimum (3 bonds) belonged to the OPH14 RAPD primer, so the average of bands for each primer is almost 6.5. The size of fragments ranged from 300 bp to 1.8 kbp. The result of this experiment is shown that OPA13 and OPH05 primers are most suitable ones due to their high resolution. The highest and least similarity coefficient between Sange-tarom mutant genotypes and control were S78 (87%), and S58 (39%) and also in Tarom-hashemi genotypes were T58 (63%) and T33 (40%) respectively. On the basis of RAPD analysis and dendrogram resulted, genotypes were classified in 3 groups regarding their similarities and distinctness. So the conclusion of this study revealed sufficiently enough variation for rice improvement through gamma ray mutagen.

Lang *et al*. (2009) studied a collection of 200 salt tolerance rice landraces and assessed for genetic diversity using quantitative agro-morphological characters. ANOVA showed highly significant differences (LSD 0.01) among the traits assessed such as grain length, grain width, number of unfilled grains, 1000-grain weight, leaf length and leaf width except panicles per plant and yield. Correlation coefficients showed that all the traits were highly correlated with each other except yield. The diversity indices (H') for quantitative descriptors were high ranging from 0.68 to 0.95. Overall the mean diversity index for all traits was 0.88). Cluster analysis generated by UPGMA grouped the 200 rice landraces into six clusters with similarity coefficient of 20.61. The six clusters were distinct in terms of Culm length, number of filled grains, panicle length, grain length, grain width, yield and biomass.

Zeba (2009) investigated the genetic variation of 31 landraces (LRs) from the saline coastal belt of Bangladesh with salt tolerant Pokkali, Nona Bokra and sensitive IR29 and BRRI 29 were analyzed with 60 evenly distributed rice microsatellite DNA markers. Total of 196 reproducible polymorphic alleles were identified from the band loci. Landraces were divided into 6 distinct groups. Three groups were composed of LRs only from the highly saline southwest. Two groups consisted of LRs from the mild to moderately saline mid-east and northeast coasts. Morphological observations of plant type, such as tiller, leaf and flag leaf angle, height, as well as yield in non-saline soil indicated low variability among the different LRs. The measure of seedling Na and K concentration, Na/K ratios, affected leaf area as well as survival under salinity stress in hydroponics identified 6 LRs from the highly saline southwest as the most tolerant. Landrace DNAs were analyzed with DNA markers linked to the major QTL of Pokkali within a 5cM region of chromosome 1, no similarity was detected between any of the traditional cultivars and Pokkali.

Sasikala *et al.* (2010) studied on germination, root and shoot length variation in six promising rice varieties *viz.,* CO 43, CO 47, CO 48, CO 49, ADT 43 and Improved White Ponni. The germination percentage was decreased after gamma irradiation (100 Gy, 200 Gy, 250 Gy, 300 Gy and 350 Gy). At the dose of 350 Gy compared to ADT 43 (33%) remaining five varieties exhibited the low germination percentage. The gamma ray dose of 300 Gy was causing 42-51% seedling height reductions in CO 43, CO 47, CO 48, CO 49 and ADT43. The seedling height was decreased in decreasing manner with the increase of irradiation dose in the varieties such as CO 47 and Improved White Ponni. At

higher dose of 350 Gy, root length is very much affected in the varieties *viz.*, CO 43 with 76% reduction and 70% reduction in Improved White Ponni. Seed fertility decreased with increase of radiation dose was observed in CO 47, ADT 43 and Improved White Ponni. In ADT 43 seed fertility was reduced approximately 69% at gamma ray dose of 350 Gy.

Banumathy *et al*. (2010) studied the fifty three rice genotypes consisting of high yielding rice varieties/ cultures and IRRI germplasm lines at Rice Research Station, Tirur during Sornavari, 2009 to identify diverse genotypes and evaluated for eight yield and yield attributing characters using D2 analysis, to study the diversity pattern among the genotypes. Based on the analysis, the genotypes were grouped into 11 clusters. Maximum number of 16 and 15 genotypes were grouped under cluster XI and I respectively, while clusters II, IV, V, VI, VIII, IX and X had only two genotypes each and clusters III and VII consisting of 3 and 5 genotypes respectively. Maximum inter cluster D2 value was observed between cluster I and X (32.96) followed by cluster I and IV (32.90). The greater the distance between the two clusters indicates wider the genetic diversity between genotypes. Among the eight traits studied, maximum contribution was made by grain yield (50.87%) followed by days to 50 per cent flowering (15.02%), total grains per panicle (10.52%) and plant height (10.23%). Hence, grain yield, days to 50% flowering, total grains per panicle and plant height together contribute 86.62% towards total divergence. Therefore, these characters may be given importance during hybridization programme.

Pillai *et al*. (2011) conducted studies on variability and correlation to identified salt tolerant mutants from two rice varieties ADT-43 and AST-16. Gama irradiated with (10, 20, 25, 30, 40 and 50 kr), M3 and M4 generation of mutants population were investigated for correlation among yield and its components which can be utilized for selection. Seed yield of mutant lines was higher than parental varieties, the number of tillers, panicle length and plant height were also much higher in some mutants than in parent varieties.

In a study by Das (2012) both the level of genetic diversity and the number of rice genotypes preserved in rice germplasm banks are high but apart from the basmati the rest of the indigenous aromatic genotypes in India have received little attention. Hence, there is an urgent need to catalogue, characterize and conserve the non-basmati indigenous aromatic rice genotypes, which are inextricably integrated with religious and social ceremonies, rituals and traditional knowledge. In addition, these aromatic genotypes are

vital genetic resources for agronomic and quality traits. This study analyses the diversity among 26 indigenous non-basmati aromatic rice genotypes, six basmati and 9 HYV; both morphologically using 12 grain and kernel traits and genetically using 23 previously mapped SSR markers. High genetic diversity was observed for the grain and kernel dimension and quality traits, in the indigenous non-basmati aromatic rice genotypes through D2 analysis. The polymerase chain reaction (PCR) profile obtained from 23 SSR markers generated 172 alleles including 28 rare alleles and 9 null alleles. The ensuing dendrogram obtained from the SSR profiles clustered the basmati rice and the indigenous non-basmati aromatic rice genotypes separately.

Satheeshkumar and Saravanan (2012) investigated the genetic diversity among fifty three genotypes of rice genotypes from various states of south Eastern region of India which were evaluated using Mahalanobis D2 statistic. Based on 15 morphological and quality characters namely, days to first flowering plant height, number of productive tillers per plant, panicle length, filled grains per panicle, total number of grains per panicle spikelet fertility, 100 grain weight, grain length, grain breadth, grain L/B ratio and grain yield per plant, these genotypes were grouped into six Clusters. Geographical origin was not found to be a good parameter of genetic divergence. Hybridization among genotypes from II, IV and VI which had maximum inter Cluster distances and desirable values for quantitative, and quality traits is likely to produce heterotic combinations and wide variability in segregating generations.

Wattoo *et al.* (2012) investigated the mutagenic effects of different concentrations of EMS on germination and yield parameters of two basmati rice cultivars (Super basmati and Basmati 370). EMS was quite effective in genetic variability in Basmati rice. The results revealed significant difference among all the traits studied. The efficiency of EMS was found to depend upon its concentration and it was higher at lower concentration in both genotypes.

Degwy (2013) observed the seeds of the Egyptian rice cultivar Sakha 105 were treated with three gamma radiation doses (15, 20 and 25 Kr) to study the effect of gamma irradiation doses in the process of mutation on the growth, yield and yield-related characters. The dose of 15 kr recorded the highest mean value of heading date and plant height in both generation and number of panicles / plant in M_1 generation. The dose of 20 Kr recorded significantly higher fertility percentage and number of branches/panicle in M_2 generation and number of panicles / plant in M_1 generation. The dose of 25 Kr

produced the highest number of panicles / plant and number of spikelets / panicle in M_2 generation with in-significance difference with the control. While, it produced the lowest mean values for grain yield, number of filled grains/panicle, heading date and plant height in both generations. Among all treatments, the dose of 25 Kr gamma radiation detected the highest variation.

Sudharani *et al*. (2013) studied the rice genotypes with different adaptations to salinity stress and characterized for their genetic diversity using microsatellite markers. A total of 85 primers distributed in all the 12 chromosomes of rice was used to assess the genetic diversity. The eight genotypes were grouped into 4 major clusters. Cluster I consist of RP Bio-226 and CSR-27 and these showed 100 per cent similarity with each other, cluster II consists of CSRC(S)5-2-2-5 and SR26B, which showed 38 per cent of similarity. Cluster III consists of CST 7-1 and CSRC(S) 5-2-2-5 and showed 61 per cent of similarity with each other, they in turn combined with cluster I and II showing 20 per cent of similarity. Cluster IV comprised of Swarna and CSR-30, which are showing 29 per cent of similarity and they combined showing 16 per cent similarity with remaining samples in the cluster. Salt tolerant genotypes found to be mixed with salt sensitive ones. Cluster I and II consisted of salt sensitive and moderately tolerant varieties.

Fakruddin *et al*. (2014) reported genetic diversity among three seventy two lines of rice from two parental varieties *viz.*, BPT-5204 and RP-BIO 226 were irradiated with gamma rays of 30 and 40 Kr was used to evaluated using Mahalanobis D^2 statistic. Based on 17 morphological characters of these mutant lines were grouped in to three clusters. The highest distance was observed between cluster III and I. lowest cluster distance was measured from cluster II and I. percent contribution of character towards divergence was found that number of spikelet per panicle and number of filled grains per panicle contributed for maximum genetic diversity and likely to produce heterotic combination and wide variability in segregating generation.

2.3 Screening of selected M_4 mutant lines for salinity tolerance.

Ali *et al*. (2004) screened ten advanced rice lines were screened for salinity tolerance at seedling stage using a rapid screening technique. Out of the lines tested, five were found tolerant, four were graded as moderately tolerant and one as susceptible. It appeared that tolerant lines had higher root and shoot ratio at seedling stage thus providing a clue about salt tolerance potential of a genotype. Further comparative studies

for salt tolerance in these rice genotypes in artificially saline conditions showed that salinity in general caused a marked reduction in yield and yield components in all the genotypes except in NR-2, where as little negative effect was observed. Thus it seems to possess better potential than Pokkali- a recognized salt tolerant variety, and may boost up the rice production in salt affected areas.

Natarajan *et al*. (2005) studied the screening of rice accessions for growth and yield attributes contributing to salinity tolerance. Fifteen rice accessions from International Rice Soil Stress Tolerance Screening Nursery Trails (IRSSTN) of IRRI were used for the study. Based on the grouping, the rice accessions were grouped in to High Yielding Tolerant (HYT), High Yielding susceptible (HYS), Low Yielding Tolerant (LYT) and Low Yielding susceptible (LYT). Results revealed that the rice accessions of the high yielding and tolerant group recorded a higher value for the yield characters like number of productive tillers per plant, number of filled grains per panicle and thousand grain weight. it clearly indicates the direct effect of growth and yield attributes on higher yield of rice accessions for salinity tolerance.

Thimmegowda (2006) reported the maximum per cent disease incidence of bacterial blight of paddy at Raichur (61.7 %) during *kharif* 2005 and least incidence at Siruguppa (46.78 %). Where as in summer 2006, the maximum per cent disease incidence was recorded at Sindhanur (74.69 %) and least incidence was noticed at Siruguppa (46.78 %).

Sundravadana *et al.* (2007) surveyed for bacterial leaf blight incidence in southern state of India and stated that incidence ranging from 12 to 75 per cent.

Bhowmik *et al*. (2009) opined selection of the salinity tolerance genotypes of rice based on phenotypic performance alone is less reliable and will delay progress in breeding. Recent advent of molecular markers, microsatellites or simple sequence repeats (SSRs) are used to find out salt tolerant rice genotypes. Three selected SSR markers; RM7075, RM336 and RM253 were used to evaluate rice genotypes for salt tolerance. Phenotypic and genotypic evaluation for salinity tolerance was done at the seedling stage. Phenotyping of 11 genotypes was done in hydroponic system using salinized (EC 12 dS/m) nutrient solution. IRRI standard protocol was followed to evaluate salinity tolerance. Large variation in salinity tolerance among the rice germplasms was detected. Plant height and total dry matter of tolerant lines were reduced by 19.0 and 40.6%,

respectively under salt stress (EC 12 dS/m), whereas those of susceptible lines were reduced by 46.0 and 73.5%, respectively. The markers showed polymorphism and were able to discriminate salt tolerant genotypes from susceptible. The genotypes having similar banding pattern with Pokkali were considered as salt tolerant. The SSR markers (RM7075, RM336 and RM253) identified 8, 9 and 7 salt tolerant genotypes, respectively. Through phenotypic and genotypic study, three genotypes viz., Pokkali, TNDB-100 and THDB were identified as salt tolerant rice cultivar. These SSR markers might have sequence homology with salt tolerant rice genotypes and consequently the markers could able to identify salt tolerant rice genotypes from susceptible ones.

Islam and Karim (2010) studied the rice (*Oryza sativa* L.) genotypes for their tolerance to salinity. One hundred rice genotypes and two check cultivars (Pokkali as tolerant and IR29 as susceptible) were exposed to solution of Electrical Conductivity (EC) of 10, 15, and 20 dSm-1 (5:1 molar concentration of NaCl and CaCl2 solution) at germination and early seedling stage. Based on visual salt injury symptoms at 15dSm-1, 13 genotypes were found fairly tolerant to salinity. However, among the 13 genotypes, only Patnai23 showed higher germination index and seedling relative dry weight than the check salt tolerant Pokkali at 15 dSm-1. Beside these, performance of Awned-1, Nonasail and Soloi was also well at this level. The genotypes Patnai23, Awned-1, Nonasail and Soloi showed the best performance under saline condition.

Shivalingaiah and Umesha (2011) took the field survey in major rice growing regions of Karnataka for two consecutive years (2009 and 2010) and reported that the bacterial leaf blight incidence was in the range of 12 to 37 per cent. Highest disease incidence of 37 per cent was noticed in the cultivar Jyothi in Davanagere district and the least disease incidence of 1.6 per cent was recorded in Madikeri district in the cultivar Doddi.

Nakhoda *et al.* (2012) evaluate the 342 synthetic lines of bread wheat with three ones of control (Arg, Bam, Kavir) at salinity and normal conditions was carried out during the 2009-2010 in Iran. Salt tolerance indices including: Stress Tolerance Index (STI), Stress Susceptibility Index (SSI), Tolerance Index (TOL), Mean Productivity (MP) and Geometric Mean Productivity (GMP) were calculated. Result of tolerance indices showed that GMP and STI indices were appropriate indices to identify tolerant lines in salinity stress. On the basis of these indices, 12 lines were identified as the most tolerance lines. There were significant differences among evaluated lines and control lines for seed

yield. The highest values for seed yield were observed in Arg and 22 lines in saline condition. This showed that salinity stress had significant effect on seed yield reduction of some genotypes. Therefore, selection of salt tolerance genotypes with consideration of their indices (e.g. GMP and STI) could be a good strategy for improvement of salt tolerance genotypes in bread wheat. It could be resulted that synthetic lines of 10, 32, 74, 86, 92,98, 114,188, 191, 297,300, 326 that had more seed yield than control varieties of Arg, Bam and Kavir, were identified as the promising lines for wheat breeding programs.

Hosseini *et al.* (2012) investigate the rice improvement for salt tolerance requires reliable assessment of salt tolerance variability among segregation genotypes. Sixty five genotypes of rice (traditional, improved and promising lines grown in north of Iran conditions) were evaluated under salt condition containing 0, 3, 6 and 8 dS/m levels during 2010. Four salt tolerance indices comprising: stress tolerances (TOL), mean productivity (MP), stress tolerance index (STI) and stress susceptibility index (SSI) were used. The indices were adjusted based on yield and yield attributing characters under normal and salt conditions. Results of cluster analysis distinguished tolerance and susceptible genotypes. It was concluded that the potential of these genotypes to tolerate salt stress was found to high MP, STI and low SSI for both on yield and yield attributing characters. Also, by cluster analysis based on tolerant indices, all genotypes were segregated into 4 and 3 groups based on Yp, Ys, MP, GMP and STI and TOL or SSI, respectively. Therefore, genotypes which are tolerant and are susceptible can be used in breeding programs.

Siddiqui *et al*. (2014) studied phenotyping of rice (*Oryza sativa* L.) in salt stress environment using infrared imaging. Results were correlated with the most frequently used physiological parameters such as stomatal conductance, relative water content and photosynthetic parameters. It was observed that stomatal conductance (R2 = –0.618) and relative water content (R2 = –0.852) were significantly negatively correlated with average plant temperature (thermal images), while dark-adapted quantum yield (Fv/Fm, R2 = –0.325) and performance index (R2 = –0.315) were not consistent with plant temperature. Advantages of infrared thermography and utilization of this technology for the selection of stress tolerance physiotypes are discussed.

Farshadfar *et al.* (2014) studied the response of twenty bread wheat (*Triticum aestivum* L.) landraces to drought stress two experiments were conducted in the field experiment was under rainfed and irrigated conditions during 2010-2011 cropping season.

Significant positive correlation was found between grain yield in the stress (Ys) and non-stress (Yp) conditions with mean productivity (MP), stress tolerance index (STI), modified stress tolerance index (MSTI), yield index (YI) and stress non-stress production index (SNPI) indicating that these criteria discriminated drought tolerant landraces with high grain yield under stress and non-stress environments. No significant correlation was observed between Ys with tolerance (TOL), stress susceptibility percentage index (SSPI) and germination stress index (GSI), hence these indicators were not able to identify drought tolerant genotypes. Principal component analysis (PCA) indicated that first and second PCA accounted for 87.72% of variations among the indices. Screening drought tolerant genotypes using mean rank, standard deviation of ranks and biplot analysis, discriminated genotypes Phishtaz , WC-47615 and WC 5050 as the most drought tolerant. Thus, they are recommended for improvement of drought tolerance in common wheat in hybridization programs.

2.4 Molecular diversity studies of salinity tolerant mutant lines.

Seetharam *et al*. (2008) revealed that out of 35 primers of SSR markers, 28 were found to be polymorphic. The PIC value ranged from 0.064 (RM 274) to 0.72 (RM 580) with an average of 0.46. The Jaccard's similarity coefficient ranged from 0.42 to 0.90. The clustering pattern clearly grouped the genotypes based on their response to salinity and clustering was not based on their geographical origin. PCA components explained 38.4percent of variation.

Islam *et al.* (2008) studied one hundred polymorphic SSR markers were used to characterize 21 rice genotypes. These genotypes comprised of nine salt tolerant genotypes including five Pokkali accessions. The materials also included two improved varieties with a major QTL for submergence (*Sub1*) in Swarna background, *i.e.*, IR82810-407 and IR82809-237. The rest were popular or moderately salt tolerant rice varieties of Bangladesh, India, IRRI, or Philippines origin. The highest number of alleles (12) were found for RM418 followed by RM10793 (11), RM3412, RM400, and RM26809 (10). The highest PIC value (0.86) was found for RM10793 followed by RM418 and RM3412 (0.85), RM26809, RM490 and RM287 (0.84), RM16 (0.83), RM493, RM562, and RM253 (0.82). These suggested their greater usefulness for characterizing rice varieties. Pokkali and PSBRc82 were found to be the most genetically distant (78.65%) varieties. The Swarna+*Sub1* genotypes (IR82810-407 and IR82809-237) had the same genetic background; hence, they had highest genetic similarity between them (90%) and also with

Swarna (>70%). Two main distinct clusters/groups were identified from cluster analysis. One cluster consists of mostly improved and adapted genotypes while the second cluster had mostly salt tolerant donors with few exceptions.

Shehata et *al*. (2009) evaluated the morphological and molecular variation among Egyptian Jasmine and its 10 M_5 derived mutants under saline soil conditions. Some mutant lines have the advantage of early maturation nearly one month earlier compared with the original variety Egyptian Jasmine. In addition, other derived mutants significantly surpassed the Egyptian Jasmine in terms of yield and its components. The biomass and grain yield recorded the highest value of expected genetic advance. The values of heritability were high for all yields and yield attributes. Some morphological traits were utilized in order to identify the M_5 morphology. DNA Markers namely Short Sequence Repeats (SSR) and Intron Splice Junction (ISJ) were used to reveal the molecular variations at molecular level among all entries. A significant level of polymorphism based on morphological and molecular levels was observed. The overall evaluation for the newly developed lines revealed that the best line was in Jasmine Sirw Line No. 3 and followed equally by Jasmine Sirw Line Nos. 8 and 9.

Bibi *et al.* (2009) studied rice cultivars IR6 and IR8 were exposed to different doses of gamma radiation and stable mutants along with parents were studied for genetic diversity on the basis of morphological traits and molecular marker (RAPD). Morphological data showed that mutants of IR6 and IR8 performed well as compared to their parents. The genetic variation was determined through RAPD. A total of 74 scorable bands were observed, out of which 47 (63.6%) were polymorphic and 27 (36.5%) were monomorphic. The size of fragments ranged from 201bp – 3.2 kbp. The number of fragments produced by various primers ranged from 1-12 with an average of 4.93 fragments per primer. Maximum 12 bands were amplified with primer A-03 and minimum one band was amplified with primer A-15, A-19 and B-10. Some specific RAPD bands were also identified reflecting the RAPDs application for the identification of rice cultivars/genotypes. Results revealed that variety Sarshar and IR8-178 contain a specific segment of 451bp amplified with primer A-19. The highest similarity was observed between IR8-15A and IR8-178 (96%) and the least similarity was recorded between IR-8 and IR6-15/B (69%). On the basis of RAPD studies, genotypes were grouped on the basis of their similarities and distinctness.

Prabakaran *et al*. (2010) investigated the genetic divergence of 12 rice land races using five SSR markers. A total of 11 alleles were detected in 12 land races and the number of alleles per locus ranged from 2 to 3 with an average of 2.2 per locus. Among the primers used RM 481 indentified more number of alleles and average PIC was 0.43. The dendrogram based on SSR marker analysis grouped the 12 rice accession into six clusters, where cluster VI was the largest with three accessions. The similarity coefficient through Jaccard's revealed that Anna samba and Chetti virippu were ascertained to be the genetically diverse from the other land races. The study also highlighted use of more number of markers for efficient characterizing the land races used for the present study.

Singh *et al.* (2010) studied the two different DNA-based techniques *viz,* simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers were used to estimate genetic diversity among 20 rice genotypes possessing different physiological mechanisms contributing to salt tolerance. A total of 11 clear and repeatable bands were amplified from ten selected SSR primers pairs and 43 fragments were detected from nine ISSR primers. The level of polymorphism was 1.1% with SSR compared to 90.7% with ISSRs. Mean genetic similarity of 0.88 based on SSRs and 0.85 using ISSRs was observed. A total of 43 (39 polymorphic) and 11 bands were detected using 9 ISSR primers and 10 well distributed mapped SSR markers, respectively. Estimates of genetic similarity of ISSRs based on the 39 polymorphic markers between 20 rice cultivars ranged from 0.55 for PR108/CSR19 to 0.94 for Pokkali/CSR20 with an average of 0.81. The estimates revealed by the 11 polymorphic SSR bands showed the average value (0.94) and also the range of genetic similarity (from 0.86 to 1.00 for CSR22/CSR18 and CSR24/CSR20, respectively) reflecting their hyper variability and their high resolution power. The findings are likely to expedite breeding new salt tolerant cultivars by involving parents from diverse molecular clusters.

Senguttuvel *et al.* (2010) revealed that the selection of genetically diverse and resistant genotypes based on association of Na+/K+ ratio with molecular markers is reliable. These markers can also be used to screen large set of germplasm collection to identify and discriminate more salt tolerant rice genotypes from susceptible based on sequence homology with already identified salt tolerant rice genotypes, The cluster and principal component analysis allowed a clear grouping of 25 genotypes grouped into 8 distinct clusters of resistant and susceptible genotypes with high and low level of 52

Na^+ / K^+ ratio. There was a highly significant correlation (R2 = 88.75; p = 5 %) between the SSR markers and physiological trait based on ion analysis.

Nantawan *et al.* (2011) were evaluated thirty rice cultivars for salinity tolerance during the seedling stage. Genetic diversity of all rice cultivars was evaluated using RAPD and SSR markers. The cultivars were evaluated for polymorphisms after amplification with 20 random decamer primers and 20 SSR primer pairs. Mean genetic similarity coefficient was 0.82 for RAPD and 0.70 for SSR. Cluster analysis based on RAPD markers was effective in grouping cultivars based on their salt tolerance ability. Group IA1, IB and IV contained three T, three S and two HS rice cultivars respectively. The MT and MS cultivars which showed similar physiological responses to salinity were resolved into two groups: Group IA2 and Group II comprising ten and eight MT/MS cultivars respectively. Cluster analysis based on SSR markers separated rice cultivars into groups based on genetic relatedness which did not correspond to salinity tolerance level.

Islam *et al.* (2011) reported the detection of QTLs for salinity tolerance at the seedling stage identified in a F2 breeding population derived from the cross between BRRI dhan40, a moderately tolerant female parent with IR61920-3B-22-2-1 (NSIC Rc106), a highly tolerant male parent. Out of total 300 F2 segregating plants, 93 plants with extreme phenotype for salinity stress response were used for selective genotyping based on of visual seedling stage salt tolerance symptom. A total of 260 SSR and two EST markers evenly spread throughout the whole rice genome at 5 Mb intervals were used for parental polymorphism survey. The 90 polymorphic makers were used for QTL mapping for salinity tolerance at seedling stage. QTL analysis using single marker, interval mapping and composite interval mapping detected three major QTLs on chromosome 1, 8 and 10 with phenotypic variances (R2) of 12.50, 29.0 and 20.20%, respectively.

Shehzad (2011) studied five rice varieties viz., Giza 171, Giza 175, Giza 176, Giza 181 and GZ 1368 were the most widely grown Japonica and Indica types in Egypt, possesses at that time many positive agronomic characteristics including wide adaptability, high yield potential, tolerance to stresses and good eating quality. But with the passage of time it has lost its vigor. In Rice Research Program, Egypt, dry seeds of the above mentioned varieties were treated with different doses of gamma rays (100, 200, 300, 400, and 500 Gy) for raising M_1 generation. M_1 plants were established by transplanting in the year 2000 season. One hundred independent lines have been

advanced to M_5 generation enabling evaluation of quantitative traits by replicated trials and promising lines were selected and tested in multi-location trials as M_6, M_7 and M_8 generations. Morphological variations at vegetative and reproductive stages including plant type and various physiological characters were observed in the five populations. The mutant lines characteristics consisted of better resistance to lodging, blast disease, high yield potential, as well as early maturity. Results from yield trials and molecular assessments indicated that the mutants differed genetically from their parents. So, these mutants could be used as a donor parents in rice breeding program and some of them can be recommended as new rice varieties suitable for rice belt in Egypt.

Ashrafuzzaman *et al.* (2012) revealed that six selected SSR markers *viz.*, RM10701, RM304, RM11757, RM336, RM7075, and RM152 were used for identification of salt tolerant genotypes. The band obtain from reaction with different markers were compared to the selected genotypes (BINAdhan-8 and AYT SL-3). Assessment of genetic diversity is an essential component in germplasm characterization and conservation. In DNA profiling, a total of 60 alleles were detected with an average number of alleles of 10 per locus (range 8 to 12 per locus) and the PIC values ranged from a low of the 0.7459 (RM152) to a high of 0.8908 (RM10701) and averaged 0.857. Positive correlations were found between gene diversity, PIC value and number of allele. The Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram constructed from (Nei's, 1972) genetic distance produced two main clusters of 26 rice germplasms. The lowest genetic distance (0.200) was found in CSR-28 vs. CSR90-IR-2 genotype pair indicating that they are genetically much closer among the genotypes. A cluster was consisted with a moderate salt tolerant due to higher similarity, while the mostly susceptible germplasms in the second cluster due to lower genetic distance between germplasms.

Berilus *et al.* (2013) evaluated Twenty four genotypes of rice for polymorphisms after amplification with 30 SSR primer pairs. The results obtained depicted a wide range of variation among the varieties. The diversity parameters confirmed that Bordungsha F1, S5 and Baodhan Bordungsha (deep water rice) were most diverse and were distinctly different from the rests of the genotype and the dissimilarity coefficient ranged from 0.06 to 0.94. This study provides a clear picture of the genetic diversity of rice. Among the set of SSR primers tested, RM44 (0.942), RM495 (0.932), RM125 (0.998) and OSR13 (0.970) were more informative in comparison to other primers.

Rabey *et al.* (2013) while studying the genetic diversity of eight rice cultivars the microsatellite markers were selected to fingerprint the eight rice cultivars under study. These markers were precisely selected in order to represent the entire rice genome. These SSR generated 46 markers; twelve out of them were polymorphic. The number of SSRs alleles per locus varied from 2 for RM133, RM 215 and RM433 to six for RM271, with a mean of 3.83 alleles per locus. Whereas, 245 polymorphic AFLP markers (nearly 87.21 % of the total AFLP markers produced) were generated using five AFLP primer combinations. The polymorphism information content value for the two types of assays was 0.825 and 0.967 for SSR and AFLP markers, respectively. Pair-wise genetic similarity ranged from 0.393 to 0.783 and from 0.103 to 0.682 for AFLP and SSR, respectively. Cluster analysis was achieved based on SSRs and AFLP data and the relationship between the studied rice cultivars were addressed.

Islam *et al.* (2013) investigated the fourteen rice genotypes, composed of six salt tolerant, three submergence tolerant, two drought tolerant genotypes along with three high yielding genotypes, released from Bangladesh Rice Research Institute (BRRI) were used for genetic diversity analysis using 40 simple sequence repeats (SSR) markers. All of the used SSR markers were found polymorphic among the 14 rice genotypes. The amplicon size ranged from 75 bp (RM436) to 330 bp (RM26360). A total of 168 alleles were detected, the number of alleles per locus ranged from 2 (RM252, S03120) to 6 (RM570, S12055, S11033) with an average of 4.2 alleles per locus. Polymorphic information content (PIC) value varied from 0.21 (RM252) to 0.76 (S07024) with an average of 0.57. From genetic distance co-efficient, the highest and lowest genetic distant varieties were found for BRRI dhan28 *viz*., BRRI dhan43 (0.82 %) and BRRI dhan40 vs. BRRI dhan44 (0.37 %) respectively. Unweighted pair group method with arithmetic mean (UPGMA)-cluster analysis divided the rice genotypes into four distinct clusters. The information obtained from this study would be useful for planning the breeding program to develop stress tolerant rice variety with high yielding ability and fine grain quality.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The details of the plant material, the methodology followed, the protocols, statistical tools used for analysis in the present study are presented under the respective experiments.

3.1 Experimental site

Induced mutants were grown in the field during *kharif* 2013-14 at Agricultural Research Station (ARS), Gangavathi. The experiment was carried out in F3 block (normal soil pH condition) as well as in natural saline soil condition having pH 8.5 with Ec 12 dS/m) which is located at the latitude of 15.43° north longitude 76.53° East and altitude of 406 meters above mean sea level (MSL).

3.2 Experimental Methods

3.2.1 Experiment I: To estimate genetic variability, heritability and genetic advance for quantitative characters. (Phenotyping)

The material for the present study consists of two popular rice varieties *viz*., BPT-5204 and RP-BIO-226. The seeds of both the varieties were exposed to 30 Kr and 40 Kr gamma rays treatments from Cobolt 60 source at Bhabha Atomic Research Centre (BARC) Trombay, Mumbai at equilibrium moisture content of eight per cent. Following irradiation treatment of different levels M_4 mutants were grown in the field during *kharif* 2013.This material comprised of 100 mutants, from each 30 Kr and 40 Kr treatments from two varieties which were selected based on yield and yield attributing characters as given below.

Plant Material:-

Sl. No.	Mutant dose	Mutant No.	Characters
1	BPT-5204 (30kr)	1 to 24	Days to 50% Flowering.
2	BPT-5204 (30kr)	25 to 100	Yield characters
3	BPT-5204 (40kr)	1 to 21, 83 to 85.	Days to 50% Flowering.
4	BPT-5204 (40kr	22 to 82, 86 to 100.	Yield characters
5	RP-BIO 226 (30kr)	1 to 37	Days to 50% Flowering.
6	RP-BIO 226 (30kr)	38 to 100	Yield characters
7	RP-BIO 226 (40kr)	1 to 25	Days to 50% Flowering.
8	RP-BIO 226 (40kr)	26 to 100	Yield characters

3.2.2 Experiment II: Molecular diversity of M_4 generation in saline condition

The material for the present study was same as mentioned above but the material comprised of 100 mutants from each 30Kr and 40Kr treatments of two varieties are BPT- 5204 and RP-Bio 226, which were selected based on high yielding characters in M_3 generation.

3.2.3 Experimental layout

During *kharif* 2013, 400 mutants were sown in an Augmented design (Federer, 1977), mutants in three meter row length with spacing of 20 cm between the rows and 10cm between the plants. Each block contained 50 mutants with 2 parental checks (BPT-5204, RP-Bio 226) replicated twice after every 50 entries (Plate 1).

3.2.4 Crop management

The seedlings were raised in wet nursery method followed required agronomic practices and 28 days old seedlings were transplanted in the experimental field at the rate of single seedling per hill with spacing 20 cm between rows and 10 cm between plants. The required cultural operations and plant protection measures as per package of practice recommended by the UAS, Raichur were taken up to ensure uniform and healthy crop. Crop was harvested at its physiological maturity and selected single plant observations were recorded.

3.2.5 Methods of sampling and recording of observations

The observations were recorded by randomly selecting five plants in each mutant line. Mean values were used for statistical analysis. The details of the observation recorded on various traits are presented below.

1. Plant height (cm)

The plant height was measured from five randomly selected tagged plants from base to tip of the panicle and average plant height was taken and expressed in cm.

2. Number of tillers per plant

The total number of tillers per plant including both productive and unproductive tillers were counted and recorded at vegetative and maturity stages.

Plate 1. Overview of mutants showing variation

Plate 2. Early mutant line in mutant population

3. Days to 50 per cent flowering

The total number of days taken by each entry from sowing to opening of first flower in 50 per cent of the plants.

4. Number of panicles (Productive tillers)

The number of panicles per plant was recorded at harvest.

5. Panicle length (cm)

The length of the panicle from its base to the tip was measured and expressed in cm.

6. Number of grains per panicle

Number of grains per panicle were counted using AIDEX waver IC-VA ver.3.0 both filled and chaffy ones in five panicles were recorded.

7. Biological yield/hill (g)

Biological yield of five randomly selected hills was recorded in gram separately and averaged for each replication before threshing.

8. Grain yield per plant (g)

Total weight of all the filled grains from five plants in each entry was measured in grams and recorded at harvest.

9. Harvest index percent

For total biological yield the entire plant above the ground level was observed, sun dried and weighed at maturity. The value of harvest index was calculated from the following formula.

Harvest index = (Economical yield/ Biological yield) X 100

10. 1000 grain weight /Test weight (g)

The weight of one thousand seeds were counted using AIDEX waver IC-VA ver.3.0 and expressed in grams using AFCOSET Weighing Balance.

11. Filled grains per panicle

The number of filled grains per panicle was recorded manually.

12. Per cent of chaffyness

Per cent of chaffyness was calculated from the following formula

$$\text{Per cent of chaffyness} = \frac{\text{Total No. of unfilled grains per panicle}}{\text{Total No. of filled grains per panicle}} \times 100$$

13. Seedling mortality

The per cent of seedling mortality in each entry was calculated from the following formula.

$$\text{Per cent of mortality} = \frac{\text{No. of plants died}}{\text{Total No. of plants planted}} \times 100$$

14. Grain size Classification

Measure the length and breadth by using Grain Shape Tester or Dial Micrometer by Ramaiah (1969)

State	Kernel length (mm)	Length/breadth ratio
Short Slender	< 6.0	< 3.0
Short Bold	< 6.0	< 2.5
Medium Slender	< 6.0	2.5 - 3.0
Long slender	> 6.0	> 3.0
Long Bold	>6.0	< 3.0

3.3 Screening of selected M_4 mutant lines for salinity tolerance

3.3.1 Phenotypic study of salinity tolerance based on yield and yield attributing characters

The plants were screened for the salinity tolerance using Stress Susceptibility Index (Fischer and Maurer, 1978) and mortality percentage (%). In Saline (pH 8.5, EC 12 dS/m) and normal soil setups in augmented design. These mutants were screened based on the yield and yield attributing characters.

Stress susceptibility index (SSI) was calculated by using formula

$$SSI = \frac{1 - \frac{Ys}{Yp}}{SI} \qquad SI = 1 - \frac{\bar{Y}s}{\bar{Y}p}$$

Where Ys is the yield of lines under stress, Yp the yield of lines under normal conditions, $\overline{Ys}$ and $\overline{Y_p}$ are the mean yields of all genotypes in stress and non-stress conditions, respectively. SI is stability index.

3.3.2 Genotypic study of salinity tolerance at seedling stage

The mutants were screened for salt tolerance at seedling stage using IRRI standard protocol (Gregorio *et al*., 1997) in Saline (pH, 8.5, EC 12 dS/m) soil setups in augmented design. The evaluation was done using the modified standard evaluation system (SES) rating the visual symptoms of salt toxicity (IRRI, 1997). This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 13 days and 22 days of rice seedlings after transplanting.

Standard evaluation system at seedling stage

Score	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

3.4 Statistical analysis

The statistical analysis of the data on individual character was carried out on the mean values of each mutants using INDOSTAT package. Different statistical methods employed for the analysis are presented below:

3.4.1 Analysis of variance (ANOVA)

The data recorded on five randomly selected plants in each mutant line. The mean values were subjected to statistical analysis as per Federer (1977) was recorded in order to assess the variability among the mutants.

ANOVA

Source of variation	Degrees of Freedom	Sum of Squares	Mean sum of squares	F-ratio
Blocks	(b-1)	bSS	*bMS*	bMS/EMS
Entries	(e-1)	eSS	*eMS*	eMS/EMS
Checks	(c-1)	cSS	*cMS*	cMS/EMS
Varieties	(v-1)	vSS	*vMS*	vMS/EMS
Checks V/S varieties	1	cvSS	*cvMS*	cvMS/EMS
Error	(c-1)(b-1)	ESS	*EMS*	
Total	N-1	TSS		

Where,

b = number of Blocks,

v = number of Mutants,

e = number of Entries,

c = number of Checks

i) Genetic variability

Mean

Mean value of each character was worked out by dividing the sum total by the corresponding number of observations.

$$\text{Mean } (x) = \frac{\sum x}{N}$$

Where,

$\sum x$ = Sum of all observation for each character in each replication

N = Corresponding number of observations

Range

It was taken as the difference between the highest and lowest mean value for each character.

Range = $X_n - X_1$

Where,

X_n = highest mean value of character

X_1 = lowest mean value of the character

ii) Estimation of genetic variability parameters (Indostat statistical package)

Both genotypic and phenotypic coefficients of variability for all the characters were computed as per the method given by Burton and De Vane (1953).

a) Genotypic Coefficient of Variability (GCV)

$$GCV = \frac{\sigma_g}{\overline{X}} \times 100$$

Where;

σ_g = Genotypic standard deviation

$\overline{X}$ = General mean of the character

b) Phenotypic Co-efficient of Variability (PCV)

$$PCV = \frac{\sigma_p}{\overline{X}} \times 100$$

Where;

σ_p = Phenotypic standard deviation

$\overline{X}$ = General mean of the character

GCV and PCV were classified as follows (Robinson *et al.,* 1949)

Low = 0 – 10 per cent

Moderate = 10-20 per cent

High = > 20 per cent

iii) Estimation of heritability

Broad sense heritability for all the 12 characters was worked out using the formula given by Hanson *et al.,* (1953).

$$h^2 \text{ (per cent)} = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where;

$\sigma^2 g$ - genotypic variance

$\sigma^2 p$ - phenotypic (total) variance

Heritability was classified as (Robinson *et al.*, 1949c)

Low = 0 to 30 per cent

Moderate= 30-60 per cent

High= > 60 per cent

iv) Genetic advance (GA)

Genetic advance as per cent of mean for each character was worked out by adopting the formula given by Johnson *et al.* (1955).

Genetic advance (GA) = $h^2 . \sigma p . K$

Where;

h^2 = Heritability in broad sense

K = Selection differential,

K = 2.06 at 5 per cent intensity of selection

σp = Phenotypic standard deviation

Further, genetic advance as per cent of mean (GAM) was worked out by using the formula given below:

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

Where;

GA = Genetic advance

$\overline{X}$ = General mean of the character

Genetic advance as per cent mean was classified as follows.

Low = 0-10 per cent

Moderate = 10-20 per cent

High = > 20 per cent

iv) Correlation analysis

The correlation coefficients were worked out to determine the degree of association of a character with yield and also among the yield components**.**

To estimate the degree of association between the traits studied, phenotypic correlation was computed by using the formula given by Webber and Moorthy (1952).

$$r_p = \frac{COV(X, Y)}{[V(X).\ V(Y))]^{1/2}}$$

Where,

r_p = Phenotypic correlation co-efficient.

COV (X, Y) = Phenotypic covariance.

V(X) and V(Y) = Phenotypic variances of the traits X and Y

The significance of correlation co-efficient was tested by referring to the table value at n-2 df given by Snedecor (1961).

3.5 Experiment III: Genetic divergence studies based on morphological characters. (Indostat statistical package)

i) Mahalanobis D^2 analysis

The formula given by Mahalanobis (1936) was used to compute the distances between different populations. The square of the Mahalanobis generalized distance between any two populations is given by the formula,

$$\delta^2 = \sum \delta i\ \delta j\ rij$$

Where;

δ^2 = Square of generalized distances

r_{ij} = Reciprocal of the common dispersion matrix

$\delta_I = (U_{i1} - U_{i2})$

$\delta_J = (U_{i1} - U_{j2})$

U = Vector of mean values for all the characters

ii) Clustering of D^2 values

All the [n (n-1)/2] D^2 values were clustered using Tocher's method as described by Rao (1952).

iii) Intra-cluster distance

The intra-cluster distances were calculated by the formula given by Singh and Chaudhary (1977).

Square of intra-cluster distance = $\sum Di^2/n$

Where;

$\sum D_i^2$ = Sum of distances between all possible combination of the entries included in a cluster

n = Number of all possible combinations

iv) Inter - cluster distance

The inter-cluster distances were calculated by the formula given by Singh and Chaudhary (1977b).

Square of inter-cluster distance = $\sum Di^2$ / ninj

Where;

$\sum D_i^2$ = Sum of distances between all possible combination (ninj) of the entries included in the cluster study (i and j)

ni = Number of entries in cluster i

nj = Number of entries in cluster j

3.6 Experiment IV: Diversity studies for salt tolerant mutants using microsatellite markers

3.6.1 Plant Material

Mutants were selected from the four hundred mutant rice plants from the saline M_4 population for the assessment of genetic diversity.

3.6.2 Methods

3.6.2.1 DNA Extraction

The genomic DNA was isolated from fresh leaves following C-TAB (Dellaporta *et al.,* 1983) procedure with minor modifications as explained below.

Preparation of stocks for CTAB method of DNA Extraction (Appendix I)

1. The 2 g of fresh leaf sample was taken and ground with liquid nitrogen to make fine powder
2. Added to prewarmed (65^{o}C) extraction buffer (10 ml) in 50 ml centrifuge tube
3. Kept in water bath at 65^{o}C for 10-15 min with intermittent mixing

4. Samples were cooled to room temperature.

5. Equal volume of chloroform: iso-amyl-alcohol was added and mixed by inverting

6. Spun at 15,000 rpm for 5-10 min.

7. Took supernatant to fresh tube carefully

8. Equal volume of prechilled isoproponal was added and mixed gently

9. Allowed for 1-2 h at -20^{o}C / overnight

10. DNA was spooled or spun down at 15,000 rpm for 5-10 min

11. DNA spool or pellet has re-suspended in Tris-EDTA

3.6.3 Quality-check for DNA

Extracted DNA samples were run through 0.8% agarose gel (1X TAE) and after series of dilutions all samples were brought to uniform concentration of 20-25 ng/µl. Then these samples were tested for amplification using SSR primer and run through 1.4% agarose gel (1X TAE).

3.6.4 Polymerase Chain Reaction (PCR)

3.6.4.1 Requirements for PCR

1. Simple sequence repeats (SSR) Primers: sixteen primers were got synthesized by Xclerius Chemicals Pvt. Ltd., Bangalore. The sequence details of the primers are presented in the Appendix IV.
2. dNTPs : The four dNTPs *viz*., dATP, dGTP, dCTP and dTTP were obtained from Xclerius Chemicals Pvt. Ltd., Bangalore.
3. Taq DNA polymerase: Taq polymerase and 10x Taq assay buffer were obtained from Xclerius Chemicals Pvt. Ltd., Bangalore.

3.6.4.2 PCR reaction and conditions for the amplification of SSRs

The PCR conditions followed were according to information present in manual for microsatellites published by Mahyco Research Foundation, Hyderabad (MRF, 2003). The standard PCR reagents in total volume of 15 µl

3.6.4.3 PCR cycling

PCR reaction was carried out using model Master Cycler Gradient 5331 (Eppendorf, Germany). The cycler was programmed as presented in below,

PCR Cycling Profile

Steps	Temperature (°C)	Duration HH:MM:SS	Cycles
Initial denaturation	94	00:05:00	1
Denaturation	94	00:00:30	35 Cycles
Primer annealing	55	00:00:30	
Primer extension	55	00:01:00	
Complete primer extension	72	00:05:00	1
Dump	15	Until removed	1

Components of PCR master mix

Components	Quantity (µl / tube)
Deionised distilled water	13.55
PCR buffer (10x)	2.0
dNTP (mix) (10 mM each)	1.0
Forward primer (5 pM)	0.6
Reverse primer (5 pM)	0.6
DNA template (20-25 ng/µL)	2.0
Taq polymerase (1U/µL)	0.25
Total	**20**

3.6.4.4 Agarose Gel Electrophoresis

Agarose gel (3%) was prepared using electrophoresis grade agarose (Sigma) in a volume of electrophoresis buffer (1 x TAE) sufficient for constructing a gel (300 ml for 18 x 30 cm gel). Ethidium bromide was added at concentration of 0.5 µg/ml of gel. The

gel was allowed to set fully before removing the comb and loading the sample. Five µl of loading dye was added to 20 µl of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between the wells. A voltage of 1-5 v/cm was given for a time period of three hours for separation of PCR fragments. After electrophoresis, the DNA banding pattern was viewed under UV light and documented.

3.6.4.5 Analysis of amplified profiles

Amplified fragments were scored as '1' for the presence and '0' for the absence of band generating the 0 and 1 matrix and percent polymorphism was calculated by using the following formula

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Experimental Results

IV. EXPERIMENTAL RESULTS

4.1 Experiment I: Genetic variability in M_4 generation

A collection of 400 M_4 mutants of rice (*Oryza sativa* L.) were grown under saline (Ec = 12 dS/m, pH = 8.5) and normal soil condition during *kharif* 2013. Observations on 12 quantitative characters were recorded on selected individuals in each mutant line and the results obtained are presented under different headings as below;

4.1 Genetic variability, heritability and genetic advance

4.2 Correlation coefficient

4.3 Path analysis

4.4 Genetic diversity

4.5 Salinity screening

4.6 Molecular diversity

4.1 Variability, Heritability and Genetic Advance

In the normal soil condition M_4 Mutants exhibited significant amounts of variability for most of characters studied *viz.*, day to 50% flowering, total number of tillers, plant height, panicle length, biological yield, grain yield per panicle, harvest index and in the saline soil plant height, panicle length, biological yield and test weight as evidenced by significance of "F" test at P (0.05) and is presented in Table 1 and Table 2. In both normal and saline soil condition range, mean, phenotypic and genotypic coefficient of variability (Fig. 1 and Fig. 2), heritability estimates in broad sense and genetic advance as per cent mean (Fig. 3 and Fig.4) for these characters is presented in Table 3 and Table 4 respectively.

4.1.1 Days to 50% flowering

In normal soil condition, days to 50 % flowering varied from 80 to 123 days with an overall mean of 101.67days. The line No.56 of RP-Bio 226 (30 Kr) was observed as earliest to 50 % flowering is 80days (plate 2). Whereas, the line No.56 of BPT-5204 (30 Kr) was observed late to 50% flowering (123 days) followed by the line No.85 (120 days) of BPT-5204(30 Kr). Similarly in saline soil condition days to 50 % flowering

Table 1. Analysis of variance for yield, yield attributing traits of rice for normal soil

SV	DF	DFF	TNT	NPT	PH	PL	BY	GYP	HI	NGP	NFG	%CF	TW
Block	7	12.99	7.80	8.83	14.00	2.26	801.45*	97.44*	12.81	14549.92	15105.71*	75.69	17.18
Entries	403	42.982**	7.897*	6.803	122.72**	6.778*	4316.157**	58.69*	32.378*	7540.011	8381.322	55.469	14.22
Checks	3	106.53**	6.428	4.485	17.20	4.524	100.00	85.36*	57.694*	16064.78	14894.39	25.038	16.876
Varieties	399	42.58**	7.92*	6.835	118.05**	6.695*	4355.334**	58.34	32.186*	7492.292	8351.973	55.777	14.191
Ch v/s Var	1	12.089	1.27	1.124	2302.08**	46.64**	1333.176*	119.24*	32.8	1005.784	552.64	24.225	17.76
Error	21	15.031	3.726	4.509	14.146	3.25	266.624	25.02	15.115	6285.068	6050.743	33.748	20.522

Table 2. Analysis of variance for yield, yield attributing traits of rice for saline soil

SV	DF	DFF	TNT	NPT	PH	PL	BY	GY	HI	NGP	NFG	%CF	TW
Block	7	40.67	6.83	8.35	36.54	16.02**	59.24	23.53	38.73	22913.17	1860.9	31.22	11.66
Entries	403	38.28	8.04	7.49	126.09**	11.96**	403.97**	68.82	56.65	6277.64	5930.21	21.22	54.53**
Checks	3	76.86	0.34	1.12	612.17**	2.76	54.78	25.32	9.86	3740.46	2885.78	37.27	1.89
Varieties	399	37.056	8.11	7.56	115.40*	12.04**	407.29**	69.27	57.04	6292.95	5950.96	21.11	54.51**
Ch v/s Var	1	405.90**	2.81	0	2931.93**	7.72	127.50	21.89	44.04	7777.68	6782.5	19.29	217.69**
Error	21	33.244	10.95	8.22	54.08	4.19	110.45	76.49	34.13	13014.46	12103.27	32.58	12.37

DFF= Days to 50% flowering.
TNT= total number of tillers per plant.
NPT= Number of productive tillers per plant.
PH = Plant height.
PL = Panicle length.
BY = biological yield.
GYP= grain yield per plant.
HI = Harvest index
NGP = number of grains per panicle.
NFG = Number filled grains per panicle
% CF = percent chaffyness.
TW = Test weight

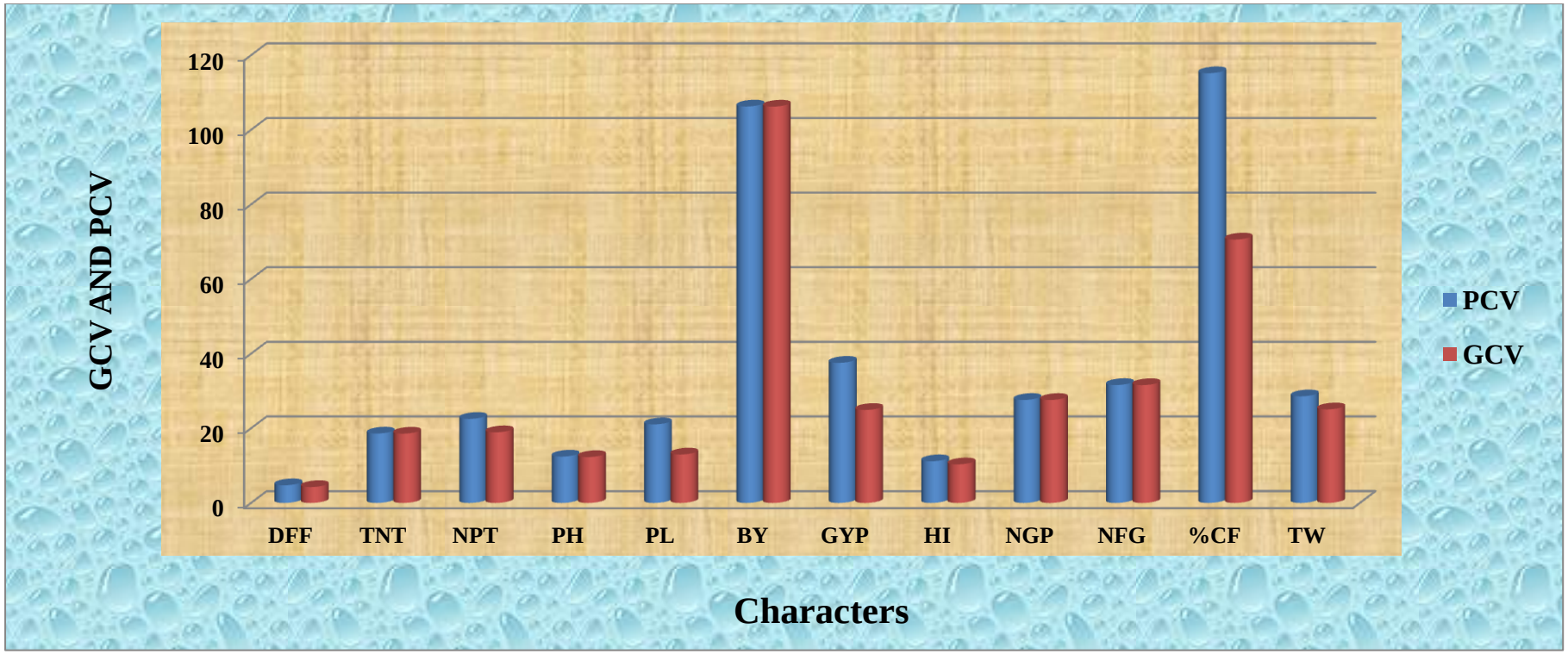


Fig. 1. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for 12 yield and yield attributing characters of normal soil in mutant rice

DFF= Days to 50% flowering.
TNT= total number of tillers per plant.
NPT= Number of productive tillers per plant.
PH = Plant height.
Pl = Panicle length.
BY = biological yield.
GYP= grain yield per plant.
HI = Harvest index
NGP = number of grains per panicle.
NFG = Number filled grains per panicle
% CF = percent chaffyness.
TW = Test weight

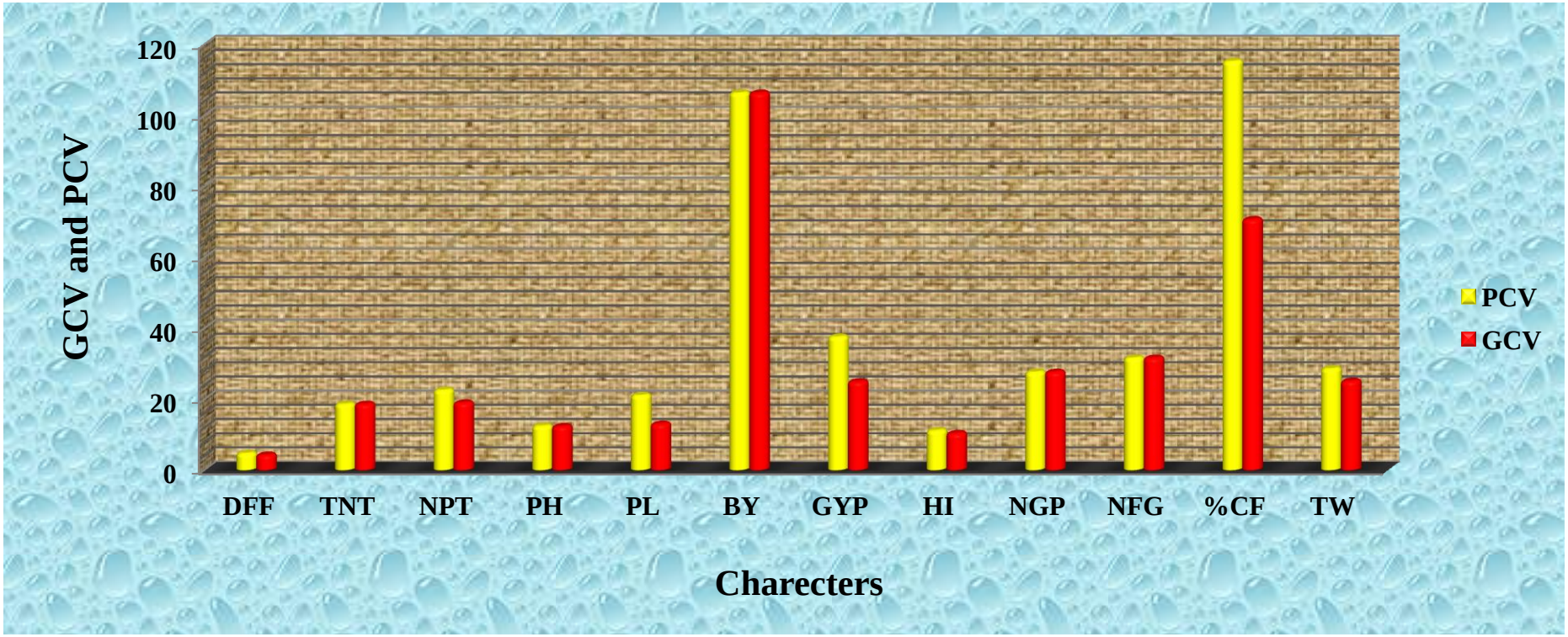


Fig. 2. Genotypic coefficient of variation (GCV) and Phenotypic coefficient of variation (PCV) for 12 yield and yield attributing characters of saline soil in mutant rice

DFF= Days to 50% flowering. TNT= total number of tillers per plant. NPT= Number of productive tillers per plant.
PH = Plant height. Pl = Panicle length. BY = biological yield.
GYP= grain yield per plant. HI = Harvest index NGP = number of grains per panicle.
NFG = Number filled grains per panicle % CF = percent chaffyness. TW = Test weight

Table 3. Estimation of range, mean and different genetic parameters for yield, yield attributing characters of Mutant Rice (M_4 generation/*Kharif*, 2013) for normal soil

Character	Range			Co-efficient of variability		h^2	Expected Genetic advance @ 5%	Genetic advance mean
	Min	Max	Mean	PCV	GCV			
DFF	80	123	101.67	4.74	4.27	81	8.07	-93.73
TNT	8.2	25.5	14.92	18.62	18.59	99	5.71	+38.22
NPT	7.6	22.8	13.61	22.54	18.95	70	4.47	+32.83
PH	62.8	117.8	86.92	12.43	12.31	98	21.67	-25.13
PL	13.6	25.6	19.64	21.07	12.99	38	3.22	+16.51
BY	41.60	89.26	70.61	106.27	106.25	99	133.78	+218.85
GYP	12.20	55.00	29.04	37.57	24.93	44	9.77	+34.08
HI	14.23	67.87	40.23	11.18	10.35	85	10.17	+19.74
NGP	105	610.2	321.51	27.62	27.61	99	180.80	+56.86
NFG	97.6	370	295.85	31.61	31.59	99	190.58	+65.04
%CF	1.72	61.4	10.66	115.19	70.62	37	9.42	-89.19
TW	8.24	31.10	15.45	28.59	25.09	77	7.08	+45.38

DFF= Days to 50% flowering. TNT= total number of tillers per plant. NPT= Number of productive tillers per plant.
PH = Plant height. PL = Panicle length. BY = biological yield.
GYP= grain yield per plant. HI = Harvest index NGP = number of grains per panicle.
NFG = Number filled grains per panicle % CF = percent chaffyness. TW = Test weight

Table 4. Estimate of range, mean and different genetic parameters for yield, yield attributing characters of Mutant Rice (M_4 generation /*Kharif*, 2013) for saline soil

Character	Range			Co-efficient of variability		h^2	Expected Genetic advance @ 5%	Genetic advance mean
	Min	Max	Mean	PCV	GCV			
DFF	87	131	118	4.57	4.59	99	9.82	109.59
TNT	9	23.4	15.03	19.03	19.00	99	5.83	+39.07
NPT	7	21.4	12.56	25.85	22.05	72	4.8	+38.76
PH	63.2	113.2	87.23	12.79	12.66	98	22.41	-28.84
PL	13.6	26.4	20.41	23.38	16.98	52	5.21	+25.40
BY	34.62	88.56	70.61	34.28	34.17	99	41.27	+70.17
GYP	9.20	49.20	30.15	31.26	27.56	76	15.17	+50.05
HI	26.57	58.23	41.21	15.07	14.17	88	14.43	+27.43
NGP	115.4	758	296.64	28.39	28.38	99	171.74	+58.43
NFG	100.6	575.8	279.06	29.24	29.23	99	166.77	+60.17
%CF	0.63	32.71	2.87	59.14	48.14	66	4.76	-80.75
TW	7.98	30.75	16.28	42.81	41.12	95	14.46	+84.58

DFF= Days to 50% flowering. TNT= total number of tillers per plant. NPT= Number of productive tillers per plant.
PH = Plant height. PL = Panicle length. BY = biological yield.
GYP= grain yield per plant. HI = Harvest index NGP = number of grains per panicle.
NFG = Number filled grains per panicle % CF = percent chaffyness. TW = Test weight

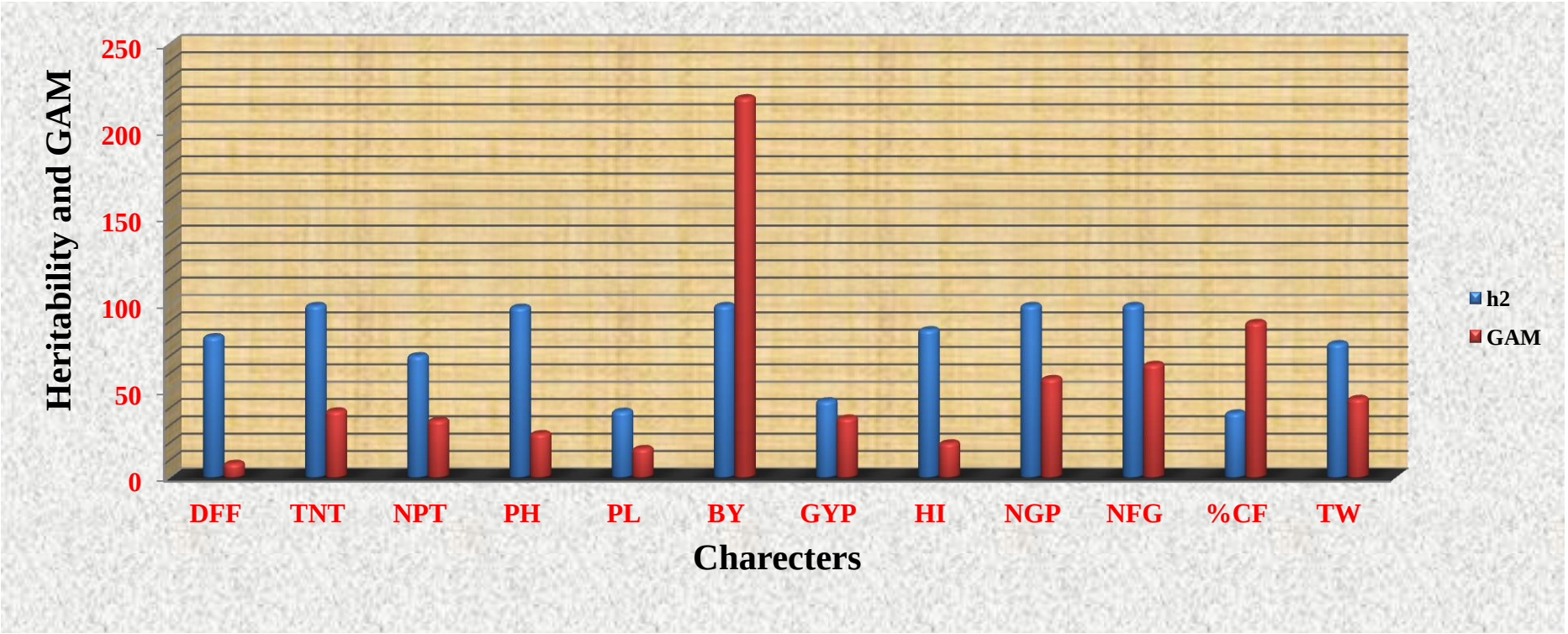


Fig. 3 Heritability (h^2, %) and Genetic advance per cent mean (GAM) for 12 yield and yield attributing characters of normal soil in mutant rice

DFF= Days to 50% flowering.
TNT= total number of tillers per plant.
NPT= Number of productive tillers per plant.
PH = Plant height.
Pl = Panicle length.
BY = biological yield.
GYP= grain yield per plant.
HI = Harvest index
NGP = number of grains per panicle.
NFG = Number filled grains per panicle
% CF = percent chaffyness.
TW = Test weight

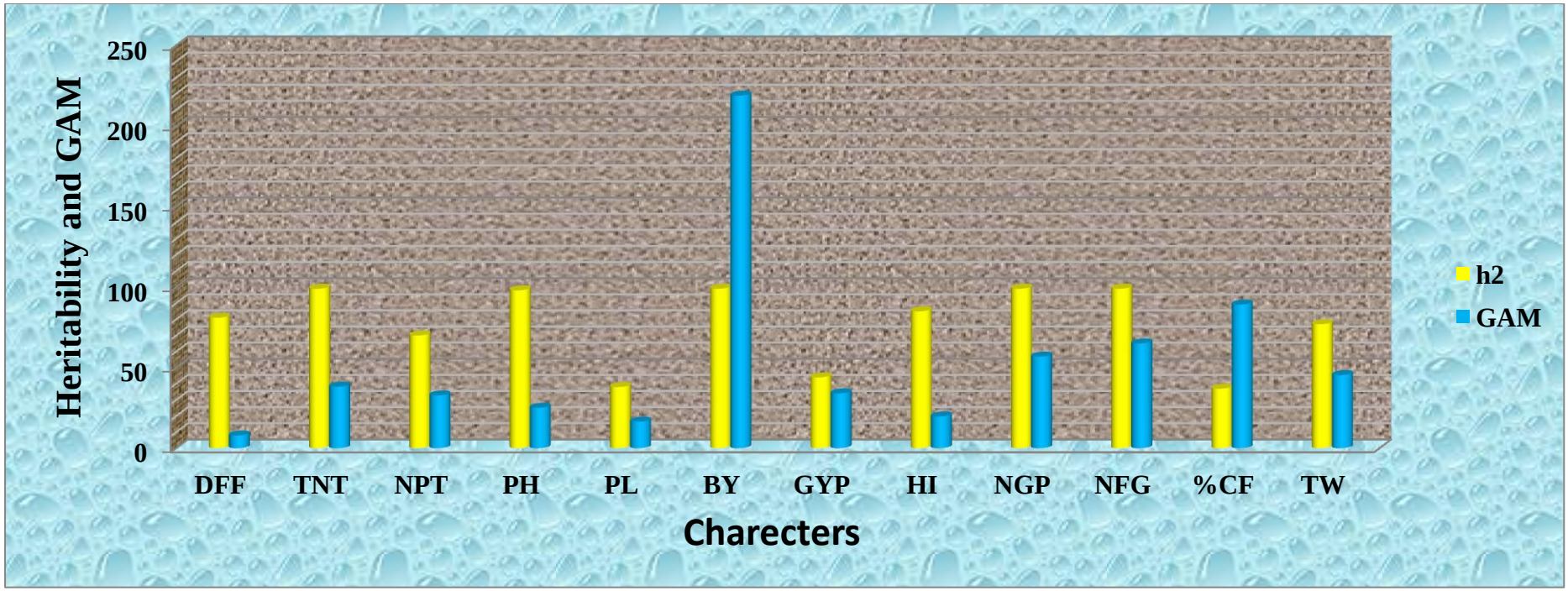


Fig. 4 Heritability (h^2, %) and Genetic advance per cent mean (GAM) for 12 yield and yield attributing characters of saline soil in mutant rice

DFF= Days to 50% flowering.
TNT= total number of tillers per plant.
NPT= Number of productive tillers per plant.
PH = Plant height.
Pl = Panicle length.
BY = biological yield.
GYP= grain yield per plant.
HI = Harvest index
NGP = number of grains per panicle.
NFG = Number filled grains per panicle
% CF = percent chaffyness.
TW = Test weight

varied from 87 days to 131 days with overall mean of 108 days. Line No. 60 of BPT-5204 (30 Kr) recorded as early (87 days) to 50 % flowering. Whereas the line No. 63 of RP-Bio 226 (30 Kr) was recorded the late to 50 per cent flowering (131 days) followed by the line No. 92 (127 days) of BPT-5204 (40 Kr).

In normal soil condition the genotypic and phenotypic coefficients of variation were 4.27 and 4.74 respectively. This trait had high heritability (81) with expected genetic advance (8.07) and per cent mean genetic advance was low (7.94) whereas in the saline soil condition the genotypic and phenotypic coefficients of variation was 4.59 and 4.57 respectively. This trait had high heritability (99) with expected low genetic advance (9.82) and percent mean genetic advance (9.41).

4.1.2 Plant height

In normal soil condition plant height varied from 62.8 to 117.8 cm with an overall mean of 86.92 cm. Line No. 97 of RP-Bio 226 (30 Kr) was dwarfest (62.8 cm) and line No. 50 of BPT-5204 (40 Kr) was the tallest (117.6cm) followed by line No. 17 (116.6 cm) of RP-Bio 226 (40 Kr). Similarly, in saline soil condition the plant height varied from 63.2 to 113.2 cm with an overall mean of 87.23 cm. The line No. 47 of RP-Bio 226 (40 Kr) was dwarfest (63.2 cm) and line No. 46 of BPT-5204 (40 Kr) was tallest (113.2 cm) mutant followed by line No. 29 (112.8cm) of BPT-5204 (30 Kr).

In normal soil condition the genotypic and phenotypic coefficients of variation were 12.43 and 12.31 per cent respectively. This trait exhibited high heritability (98%) both high expected genetic advance (21.67) and per cent mean genetic advance (25.13). Similarly in case of saline soil condition the genotypic and phenotypic coefficients of variation were 12.79 and 12.66 per cent respectively. This trait exhibited high heritability (98%) with high expected genetic advance (22.41) and high per cent mean genetic advance (28.84).

4.1.3 Panicle lengths

In the mutant population observed varied panicle length (plate 3). In normal soil condition panicle length varied from 13.6 to 25.6 cm with an overall mean of 19.64 cm. Line No.70 of RP-Bio 226 (30 Kr) produced largest panicle length (25.6 cm) and line No. 75 of RP-Bio 226 (40 Kr) produced shortest panicle length (13.6 cm). Whereas, in saline soil condition panicle length varied from 13.6 to 26.4 cm with an overall mean of

Plate 3. Mutants showing panicle length variation

Plate 4. Mutants showing variation for grain size

20.41 cm. Line No.11 of BPT-5204(30 Kr) produced largest panicle length (26.4 cm) and line No. 90 of BPT-5204 (30 Kr) produced shortest panicle length (13.6 cm).

In normal soil condition the genotypic and phenotypic coefficients of variation were 21.01 and 12.99 per cent respectively. This trait exhibited moderate heritability (38%) with low genetic advance (3.22). The per cent mean genetic advance was moderate (16.51). Similarly in case of saline soil condition the genotypic and phenotypic coefficients of variation were 23.38 and 16.98 per cent respectively. This trait exhibited moderate heritability (52%) with low expected genetic advance (5.21). The per cent mean genetic advance was high (25.40).

4.1.4 Total number of tillers per plant

In normal soil condition the total number of tillers varied from 8.2 to 25.6 with an overall mean of 13.61. Line No.34 of BPT-5204 (30 Kr) produced the minimum total number of tillers (8.27) and line No.86 of RP-Bio 226 (30 Kr) produced the maximum total number of tillers (25.6). As in case of saline soil condition the total number of tillers varied from 9 to 23.4 with an overall mean of 15.03. Line No.42 of RP-Bio 226 (40 Kr) produced the minimum total number of tillers (9) and line No.86 of BPT-5204 (30 Kr) produced the maximum total number of tillers (23.4).

In normal soil condition the genotypic and phenotypic coefficients of variation were 18.52 and 18.59 per cent respectively. This trait exhibited high heritability (99%) with low expected genetic advance (5.71). The per cent mean genetic advance was high (38.22). Similarly in saline soil condition the genotypic and phenotypic coefficients of variation were 19.03 and 19.00 per cent respectively. This trait also exhibited high heritability (99 %) with expected genetic advance of 5.83. The per cent mean genetic advance was high (39.07).

4.1.5 Number of productive tillers per plant

In normal soil condition number of productive tiller ranged from 7.6 to 22.8 with a mean value of 13.61. Highest number of productive tillers was recorded by line No. 86 of RP-Bio 226 (30 Kr) and line No. 34 of BPT-5204 (30 Kr) recorded the lowest number of productive tillers. In case of saline soil condition number of productive tiller ranged from 7 to 21.4 with a mean value of 12.56. Highest number of productive tillers recorded by

line No.15 of BPT-5204 (30 Kr) and line No. 48 of BPT-5204 (30 Kr) recorded the lowest number of productive tillers.

In normal soil condition the phenotypic and genotypic coefficients of variability observed were 22.54 and 18.59 per cent, respectively. High heritability of 70 percent with low genetic advance of 4.47 was recorded. The per cent mean genetic advance was high (32.83). Similarly in saline soil condition the phenotypic and genotypic coefficients of variability observed were 25.85 and 22.05 per cent, respectively. High heritability of 72 per cent with low expected genetic advance of 4.8 was recorded. The per cent mean genetic advance was high (38.76).

4.1.6 Biological yields per plant

In normal soil the significant difference among the mutants for biological yield per plant was noticed. It ranged from 41.60 to 89.26 grams per plant with a mean value of 70.61 grams per plant. Maximum biological yield was recorded by line No. 97 of RP-Bio 226 (40 Kr) and line No. 18 of BPT-5204 (30 Kr) showed the minimum biological yield per plant. In case of saline soil too significant difference among the mutants for biological yield per plant was noticed. It ranged from 34.62 to 88.56 grams per plant with a mean value of 72.31 grams per plant. Maximum biological yield was recorded by line No.77 of BPT-5204 (40 Kr) and line No. 3 of BPT-5204 (30 Kr) showed the minimum biological yield per plant.

In normal soil condition the phenotypic and genotypic coefficients of variability observed 106.27 and 106.27 per cent, respectively. High heritability of 99 per cent with expected genetic advance of 133.78 was recorded. The per cent mean genetic advance was high (218.85). In case of saline soil the phenotypic and genotypic coefficients of variability observed were 34.28 and 34.17 per cent, respectively. High heritability of 99 per cent with expected genetic advance of 41.79 was recorded. The per cent mean genetic advance was high (70.17).

4.1.7 Grain yield per plant

In the normal soil condition grain yield per plant varied significantly from 12.2 to 55.0 g with a mean of 29.38 g. The lowest grain yield was recorded by line No. 1 of BPT-5204 (40 Kr) and line No.89 of RP-Bio 226 (40 Kr) had the highest grain yield of 55.4 g. In case of saline soil condition grain yield per plant varied significantly from 9.2

to 49.20 g with a mean of 30.15 g. The lowest grain yield was recorded by line No. 3 of BPT-5204 (30 Kr) and line No.12 of RP-Bio 226 (40 Kr) had the highest grain yield of 64.6g.

The phenotypic and genotypic coefficients of variability for this character in the normal soil were 37.57 and 24.93 per cent respectively. Moderate heritability of 44 per cent with an expected genetic advance of 9.77 per cent and the per cent mean genetic advance was high (34.08). In the saline soil condition the phenotypic and genotypic coefficients of variability for this character were 31.26 and 27.56 per cent respectively. High heritability of 76 per cent with a low expected genetic advance of 15.17 per cent and the per cent mean genetic advance was high (50.05).

4.1.8 Harvest Index

Harvest Index of the normal soil ranged from 14.23% to 67.87% with a mean of 40.23%. The maximum harvest index was observed in line No. 89 of RP-Bio 226 (40 Kr) and the minimum harvest index was noticed in line No. 95 of RP-Bio 226 (30 Kr). In saline soil condition the harvest index of the normal soil ranged from 26.57% to 58.23% with a mean of 41.21%. The maximum harvest index was observed in line No. 99 of BPT-5204 (30 Kr) and the minimum harvest index was noticed in Line No. 3 of BPT-5204 (30 Kr).

The phenotypic and genotypic coefficients of variability for this character of the normal soil were 11.18 and 10.35 per cent respectively. High heritability of 85 per cent with an expected moderate genetic advance of 10.19 per cent and moderate (19.74) per cent mean genetic advance was exhibited by this character and the phenotypic and genotypic coefficients of variability for this character of the saline soil were 15.07 and 14.17 per cent respectively. High heritability of 88 per cent with an expected moderate genetic advance of 14.43 per cent and high (27.43) per cent mean genetic advance was exhibited by this character.

4.1.9 Number of grains per panicle

Significant difference among the mutants for number of grains per panicle in the normal soil was noticed. The number of grains per panicle ranged from 105.4 to 610.2 with a mean value of 321.51. Highest number of spikelet per panicle was recorded by line No.12 of BPT-5204 (30 Kr) and line No. 75 of RP-Bio 226 (30 Kr) showed the lowest

number of spikelet per panicle. In the saline soil also significant difference among the mutants for number of grains per panicle was noticed. The number of grains per panicle ranged from 115.4 to 575.8 with a mean value of 296.64. Highest number of spikelet per panicle was recorded by line No. 35 of BPT-5204 (30 Kr) and line No. 78 of RP-Bio 226 (40 Kr) showed the lowest number of spikelet per panicle.

In the normal soil condition the phenotypic and genotypic coefficients of variability observed were 27.62 and 27.61 per cent, respectively. High heritability of 99 per cent with high expected genetic advance of 180.80 and high (56.86) per cent mean genetic advance was exhibited by this character. In the saline soil condition the phenotypic and genotypic coefficients of variability observed were 28.39 and 28.38 per cent, respectively. High heritability of 99 per cent with an expected high genetic advance of 171.74 and high (58.43) per cent mean genetic advance was exhibited by this character.

4.1.10 Number of filled grains per panicle

Significant difference among the mutants for number of filled grains per panicle was noticed in the normal soil condition. The number of filled grains per panicle ranged from 97.60 to 299 with a mean value of 370. Highest number of filled grains per panicle was recorded by line No. 41 of BPT-5204 (40 Kr). In the saline soil condition significant difference among the mutants for number of filled grains per panicle was noticed. The number of filled grains per panicle ranged from 100.6 to 565.5 with a mean value of 279.06 and highest number of filled grains per panicle was recorded by line No.77 of BPT-5204 (40 Kr).

Phenotypic and genotypic coefficients of variability in normal soil condition were observed 31.61 and 31.59 per cent, respectively. High heritability of 99 per cent with high expected genetic advance of 190.58 per cent and high (65.04) per cent mean genetic advance was exhibited by this character. Phenotypic and genotypic coefficients of variability in saline soil condition were observed 29.24 and 29.23 per cent, respectively. High heritability of 99 per cent with an expected genetic advance of 166.77 per cent and high (60.17) per cent mean genetic advance was exhibited by this character.

4.1.11 Percent chaffyness (%)

Percent chaffyness in the normal soil condition ranged from 1.72 to 61.4 with a mean of 10.66. The maximum per cent chaffyness was observed in line No. 72 of

BPT-5204 (30 Kr) and the minimum per cent chaffyness was noticed in line No. 92 of RP-Bio 226 (30 Kr). In saline soil condition per cent chaffyness ranged from 1.72 to 61.4 with a mean of 10.66. The maximum per cent chaffyness was observed in line No. 32 of BPT-5204 (30 Kr) and the minimum per cent chaffyness was noticed in Line No. 79 of BPT-5204 (40 Kr).

In normal soil condition the phenotypic and genotypic coefficients of variability for this character were 115.19 and 70.62 percent respectively. Moderate heritability of 37 per cent with a low genetic advance of 9.42 percent and high (45.38) per cent mean genetic advance was exhibited by this character. In saline soil condition the phenotypic and genotypic coefficients of variability for this character were 59.14 and 48.14 per cent respectively. High heritability of 66 per cent with an expected genetic advance of 4.76 per cent and high (80.75) per cent mean genetic advance was exhibited by this character.

4.1.12 Test Weight (g)

Significant difference among the mutants for test weight in normal soil was ranged from 8.24 g to 31.10 g with a mean of 15.45 g. The maximum and minimum test weight was observed in line No.80 of BPT-5204 (40 Kr) and line No. 71 of RP-BIO 226 (30 Kr) respectively. Significant difference among the mutants for test weight in saline soil ranged from 7.98 g to 30.75 g with a mean of 16.28 g. The maximum and minimum test weight was observed in line No.46 of BPT-5204 (40 Kr) and line No.28 of BPT-5204 (30 Kr) respectively.

The phenotypic and genotypic coefficients of variability for this character in normal soil condition were 28.59 and 25.09 per cent respectively. High heritability of 77 per cent with low genetic advance of 7.08 per cent and (45.38) per cent mean genetic advance was exhibited by this character. In case of saline soil condition the phenotypic and genotypic coefficients of variability for this character were 42.81 and 41.12 per cent respectively. High heritability of 99 per cent with a moderate genetic advance of 14.46 per cent and high (84.58) per cent mean genetic advance was exhibited by this character.

4.1.13 Grain size

Variation observed in grain size was medium bold, medium slender, long bold and long slender. It was depicted in Plate 2. In the M_4 population grain size of 6 mutants are

short slender, 2 mutant lines are short bold, long slender are 375 mutant lines and long bold are 19. This represented in Table 5 and plate 4.

4.2 Correlations studies

4.2.1 Correlation of different characters

The correlation coefficient among different parameters between yield and yield attributing traits and among themselves was worked out in order to determine the extent of association with each component is presented for normal soil in Table 6.

Among 12 characters, the grain yield showed significantly high positive correlation with Harvest index (0.69), Almost similar significant positive magnitude of correlation was recorded by number of filled grain per panicle (0.34) and biological yield (0.19) in the normal soil condition and the grain yield showed significantly high positive correlation with harvest index (0.760) is followed by biological yield (0.45) and number of grains per panicle (0.22) in the saline soil condition.

4.2.2 Phenotypic correlations of morphological characters

4.2.2.1 Days to 50 per cent flowering

In normal soil condition days to 50 per cent flowering showed significant positive correlations with number of grains per panicle (0.17), number of filled grains per panicle (0.14). In saline soil condition, it showed the positive correlation with biological yield (0.098).

4.2.2.2 Total number of tillers per plant

Total number of tillers showed highly significant positive correlations with number of productive tillers (0.99) but it had significant negative correlation with only plant height (-0.15) in the normal soil condition. In saline soil condition showed highly significant positive correlation with number of productive tillers per plant (0.95).

4.2.2.3 Number of productive tillers per plant

In normal soil condition the total number of productive tillers showed highly significant positive correlations with only per cent chaffyness (0.11) but it has significant negative correlations with plant height(-0.16) and in the saline soil there in no significant correlation with other characters.

Table 5. Grain size classification in m_4 mutant population

Sl. No.	Grain Size	No. of genotypes	Mutant line no.			
			BPT-5204 (30 Kr)	BPT- 5204(40Kr)	Rp-bio 226(30Kr)	Rp-bio 226(40Kr)
1	SS	6	66, 53, 29, 36, 59.			357
2	SB	2	85, 18.			
3	LS	375	40, 65, 82, 100, 21, 64, 2, 44, 84, 22, 14, 15, 92, 51, 52, 73, 20, 77, 45, 11, 95, 55, 43, 41, 98, Bpt-5204, Check, Rp-Bio 226 Check, 27, 48, 16, 60, 68, 33, 34, 47, 67, 17, 62, 13, 23, 58, 1,50, 41, 98, 94, 96, 97, 5,80, 24, 63, 6, 88, 99,12,75,32,4, 61,72,76, 79,10,46, 89,69,93 ,3,71,8,39,70,83,26,86,54, 31,35,56,9,90,49	109, 111, 112, 116, 128, 129, 132, 152, 162, 164, 168, 172, 181, 183, 198, 200. Bpt-5204, Check, Rp-Bio 226 Check, 101 to 108, 113, 114, 115, 117 to 127, 130, 131, 133 to 151, 153 to 161, 163, 165 to 167, 169 to 171, 173 to 180, 182, 184 to 197,199	204, 212, 214, 215, 217 to 220, 228, 230, 235, 236, 328, 239, 240, 244, 247, 250, 252, 260, 261, 266, 276, 281, 288, 299, 300, Bpt-5204, Check, Rp-Bio 226 Check,, 201, 202, 203, 205 to 211, 213, 216,221 to 225, 227, 229, 231-234, 237, 241 to 243, 245, 246, 248 to 249, 251, 253 to 259, 262-65, 267 to275, 277 to 280, 282, 284 to 287, 289 to 298.	306, 308, 310, 311, 323, 328, 3310, 339, 348, 351, 364, 365, 369, 377, 382, 387, 388, 389, 400, Bpt-5204, Check, Rp-Bio 226 Check, 301 to 305, 307, 309, 312 to 322, 324 to 327, 329, 330, 332 to 336, 338, 340 to 347, 349, 350, 352 to 356, 358 to 363, 366, 367, 368, 370 to 374, 376, 378 to 381, 383 to 386, 390 to 392, 394 to 399.
4	LB	19	25, 28, 78, 57, 87, 7, 91, 30, 74, 19, 81, 42, 37, 38		283, 226	337, 393, 375.

Table 6. Comparison of correlation of phenotypic for normal and saline soil

		DFF	NTP	NPT	PH	PL	BY	HI	NGP	NUFG	NFG	%CF	TW	GYP
DFF	**N**	**1**	-0.059	-0.046	0.002	-0.058	0.051	0.039	0.170**	0.063**	0.147**	-0.005	-0.065	0.092
	S		-0.053	-0.0598	-0.029	-0.076	0.098*	0.018	0.035	0.031	0.031	0.022	-0.064	0.067
NTP	N		**1**	0.97**	-0.15**	-0.062	-0.020	0.077	-0.037	0.125*	-0.046	0.102*	-0.079	0.14**
	S			0.953**	0.019	-0.008	-0.021	-0.029	0.018	-0.052	0.025	-0.054	-0.025	0.025
NPT	N			**1**	-0.16**	-0.064	-0.028	0.080	-0.032	0.14**	-0.040	0.11*	-0.078	0.155**
	S				0.081	0.034	-0.014	-0.036	0.037	-0.036	0.043	-0.048	-0.027	0.018
PH	N				**1**	0.574**	-0.040	0.20**	-0.104	-0.065	-0.095	0.043	0.31**	0.172**
	S					0.413**	0.038	0.159**	0.026	-0.109*	0.040	-0.096	0.161**	0.197**
PL	N					**1**	-0.089	0.078	-0.074	-0.087	-0.064	-0.008	0.24**	0.019
	S						-0.045	0.042	0.066	0.145**	0.049	0.089	0.018	0.029
BY	N						**1**	-0.10*	0.117	-0.001	0.112*	-0.045	-0.023	0.198**
	S							0.007	0.083	-0.015	0.088	-0.066	0.040	0.459**
HI	N							**1**	0.21**	0.081	0.20**	-0.024	0.053	0.693**
	S								0.07	-0.04	0.08	-0.04	0.12*	0.76**
NGP	N								**1**	0.19**	0.92**	-0.34**	-0.32**	0.376**
	S									0.29**	0.99**	-0.18**	-0.33**	0.22**
NUFG	N									**1**	0.014	0.76**	-0.12*	0.187**
	S										0.169**	0.826**	-0.19**	-0.012
NFG	N										**1**	-0.45**	-0.31**	0.342**
	S											-0.30**	-0.32**	0.23**
%CF	N											**1**	0.09	0.001
	S												-0.0077	-0.100
TW	N												**1**	0.004
	S													0.075
GYP	N													**1**
	S													

4.2.2.4 Plant height (cm)

Plant height showed highly significant positive correlations with biological yield (0.33), flag leaf length (0.36) and flag leaf width (0.32) but it had significant negative correlation with only number of productive tillers (-0.13).

4.2.2.5 Panicle length (cm)

Panicle length showed highly significant positive correlations with test weight (0.24) in the normal soil condition.

4.2.2.6 Biological yield per plant

Biological yield showed significant positive correlations with number of filled grains per panicle (0.11) but it had significant negative correlation with harvest index (-0.10) and in saline soil there is no significant correlation with other characters.

4.2.2.7 Harvest index

In normal soil condition harvest index showed highly significant positive correlation with number of grains per panicle (0.21) and number of filled grains (0.20) and in saline soil condition harvest index showed highly significant positive correlation with test weight (0.12).

4.2.2.8 Number of grains per panicle

Number of grains per panicle showed highly significant positive correlations with number of filled grains per panicle (0.92) and it has highly significant negative correlations with per cent chaffyness (-0.34) and test weight (-0.32) in the normal soil condition. Number of grains per panicle showed highly significant positive correlations with number of filled grains per panicle (0.99) and it has highly significant negative correlations with test weight (-0.32), per cent chaffyness (-0.18) in the saline soil condition.

4.2.2.9 Number of filled grains per panicle

Number of filled grains per panicle showed highly significant negative correlations with percent chaffyness (-0.45) and test weight (-0.31) in the normal soil condition. Number of filled grains per panicle showed highly significant negative

correlations with percent chaffyness (-0.30) and test weight (-0.32) in saline soil condition.

4.2.2.10 Per cent chaffyness (%)

Per cent chaffyness showed significant positive correlations at phenotypic level with only test weight (0.09) in the normal soil condition but in case of saline soil condition it showed the negative correlation at phenotypic level with only test weight (0.007).

4.3 Path co-efficient analysis

Path co-efficient analysis was carried out both at genotypic and phenotypic levels taking the grain yield as a dependent character and such of the character found significantly correlated with grain yield (days to 50 per cent flowering, plant height, panicle length, test weight, number of tillers per plant, number of productive tiller per plant, number of grains per panicle, per cent chaffyness and filled grains per panicles) independent characters. The results obtained are given in the Table 7 and Table 8 in normal and saline soil respectively. And the direct and indirect effects of different characters on grain yield are presented below

4.3.1 Direct effects of yield contributing characters on grain yield

Ten out of twelve characters had positive and direct effect on grain yield at phenotypic level in normal soil condition as well as in saline soil condition. The characters which had high magnitude positive direct effects in normal soil condition are harvest index (0.6281), biological yield (0.2388), number of productive tillers (0.1950) and number of grains per panicle (0.1866), While the characters *viz.,* total number of tillers (-0.0691) and panicle length (-0.0554) had high magnitude negative direct effects on grain yield.

Similarly, the characters which had high magnitude positive direct effects in saline soil condition are number of filled grains per panicle (1.1717), harvest index (0.7345), biological yield (0.4396) and total number of tillers (0.1378), while the characters *viz.,* number of grains per panicle (-1.0813) and number of productive tillers per plant (-0.0905) had high magnitude negative direct effects on grain yield.

Table 7. Phenotypic path of different yield components on grain yield in rice for normal soil

	DFF	NTP	NPT	PH	PL	BY	HI	NGP	NFG	%CF	TW	GYP
DFF	**0.0155**	-0.0009	-0.0007	0	-0.0009	0.0008	0.0006	0.0026	0.0023	-0.0001	-0.001	0.0923
NTP	0.0041	**-0.0691**	-0.0676	0.0105	0.0043	0.0014	-0.0053	0.0026	0.0031	-0.0071	0.0054	0.147**
NPT	-0.009	0.1908	**0.195**	-0.0312	-0.0126	-0.0055	0.0156	-0.0063	-0.0079	0.0216	-0.0151	0.1546**
PH	0.0002	-0.0179	-0.0188	**0.1177**	0.0676	-0.0047	0.0238	-0.0122	-0.0112	0.005	0.0376	0.1715**
PL	0.0032	0.0034	0.0036	-0.0318	**-0.0554**	0.0049	-0.0043	0.0041	0.0035	0.0004	-0.0134	0.0187
BY	0.012	-0.0048	-0.0067	-0.0096	-0.0213	**0.2388**	-0.0243	0.028	0.0267	-0.0107	-0.0055	0.1979**
HI	0.0243	0.0484	0.0504	0.1268	0.0487	-0.0638	**0.6281**	0.1361	0.1262	-0.0153	0.0334	0.6933**
NGP	0.0317	-0.0069	-0.006	-0.0194	-0.0139	0.0219	0.0404	**0.1866**	0.172	-0.0638	-0.061	0.376**
NFG	0.0114	-0.0035	-0.0031	-0.0074	-0.005	0.0087	0.0156	0.0716	**0.0777**	-0.0354	-0.0242	0.342**
%CF	-0.0004	0.0084	0.0091	0.0035	-0.0007	-0.0037	-0.002	-0.028	-0.0372	**0.0818**	0.0074	0.0007
TW	-0.0029	-0.0034	-0.0034	0.0139	0.0106	-0.001	0.0023	-0.0143	-0.0136	0.0039	**0.0437**	0.004

*= Significant at 5% (0.0976), **= Significant at 1% (0.128)

Residual effect = 0.6118

DFF= Days to 50% flowering.
TNT= total number of tillers per plant.
NPT= Number of productive tillers per plant.
PH = Plant height.
PL = Panicle length.
BY = biological yield.
GYP= grain yield per plant.
HI = Harvest index
NGP = number of grains per panicle.
NFG = Number filled grains per panicle
% CF = percent chaffyness.
TW = Test weight

Table 8. Phenotypic path of different yield components on grain yield in rice for saline soil

	DFF	NTP	NPT	PH	PL	BY	HI	NGP	NFG	%CF	TW	GYP
DFF	**0.0103**	-0.0005	-0.0006	-0.0003	-0.0008	0.001	0.0002	0.0004	0.0003	0.0002	-0.0007	0.067
NTP	-0.0073	**0.1378**	0.1314	0.0026	-0.0011	-0.0029	-0.004	0.0024	0.0034	-0.0074	-0.0034	0.0246
NPT	0.0054	-0.0862	**-0.0905**	-0.0074	-0.0031	0.0013	0.0033	-0.0034	-0.0039	0.0043	0.0025	0.0179
PH	-0.0021	0.0014	0.0059	**0.072**	0.0298	0.0027	0.0114	0.0018	0.0029	-0.0069	0.0116	0.1974**
PL	0.0012	0.0001	-0.0005	-0.0064	**-0.0155**	0.0007	-0.0007	-0.001	-0.0008	-0.0014	-0.0003	0.0292
BY	0.0431	-0.0092	-0.0062	0.0168	-0.0196	**0.4396**	0.0029	0.0366	0.0387	-0.029	0.0177	0.4594**
HI	0.0132	-0.0215	-0.0266	0.1167	0.0309	0.0048	**0.7345**	0.0536	0.0596	-0.0326	0.0862	0.7587**
NGP	-0.0376	-0.0189	-0.0404	-0.0276	-0.0718	-0.0901	-0.0789	**-1.0813**	-1.0727	0.196	0.3593	0.2228**
NFG	0.0367	0.0293	0.0505	0.0468	0.0575	0.1032	0.0951	1.1623	**1.1717**	-0.3459	-0.3712	0.2319**
%CF	-0.0004	0.0011	0.001	0.0019	-0.0018	0.0013	0.0009	0.0036	0.0059	**-0.0199**	0.0002	-0.1004
TW	-0.0004	-0.0002	-0.0002	0.0011	0.0001	0.0003	0.0008	-0.0023	-0.0022	-0.0001	**0.0069**	0.0751

*= Significant at 5% (0.0976), **= Significant at 1% (0.128)

Residual effect: 0.4393

DFF= Days to 50% flowering. TNT= total number of tillers per plant. NPT= Number of productive tillers per plant.
PH = Plant height. PL = Panicle length. BY = biological yield.
GYP= grain yield per plant. HI = Harvest index NGP = number of grains per panicle.
NFG = Number filled grains per panicle % CF = percent chaffyness. TW = Test weight

4.3.2 Indirect effects of yield contributing characters on grain yield

In normal soil condition the indirect effect of days to 50 per cent flowering via other characters was not considerable.

The positive indirect effect of number of productive tillers per plant via total number of tillers (0.1908) and harvest index (0.0156) and percent chaffyness (0.0216) was high.

The indirect effect of plant height via panicle length (0.0676) and test weight (0.0376) was high and positive. The positive indirect effect of harvest index via number of grains per panicle (0.1361), plant height (0.1268), number of productive tiller per plant (0.0504), total number of tillers (0.0489), panicle length (0.0487) and days to 50 per cent flowering (0.0243) was high. The indirect effect of number of grains per panicle via harvest index (0.0404) and days to 50per cent flowering (0.0317) was positive and high and the positive indirect effect of number of filled grains per panicle via only number of grains per panicle (0.0716) was high.

The negative indirect effect of total number of tillers via number of productive tillers (-0.0676) was recorded. The negative indirect effect of number of productive tillers per plant via plant height (-0.0312) and panicle length (-0.0126) was high. While the negative indirect effect of plant height via number productive tillers per plant (-0.0188), total number of tillers per plant (-0.0179) and number of grains per panicle (-0.0122) was high and the negative indirect effect of panicle length via plant height (-0.0318) and test weight (-0.0134) was also high.

The negative indirect effect of biological yield via harvest index (-0.0243), panicle length (-0.0213) and per cent chaffyness (-0.0107) was high. While the negative indirect effect of harvest index via biological yield (-0.0638) and per cent chaffyness (-0.0153) was high. The negative indirect effect of number of grains per panicle via per cent chaffyness (-0.0638), test weight (-0.061) and panicle length (-0.0139) was high. While the negative indirect effect of number of filled grains per panicle via percent chaffyness (-0.0354) and test weight (-0.0242) was high. The negative indirect effect of percent chaffyness via number of filled grains per panicle (-0.0372) and number of filled grains per panicle (-0.028) was high. The negative indirect effect of test weight via only number of grains per panicle (-0.0143) was high.

In saline soil condition the indirect effects of days to 50% flowering, panicle length, per cent chaffyness and test weight via other characters not considerable.

Indirect effect of total number of tillers per plant via number of productive tillers (0.1314) was high and positive. While, the positive indirect effect of biological yield via days to 50% flowering (0.0431), number of filled grains per panicle (0.0387) and number of grains per panicle (0.0366) was high. And the positive indirect effect of harvest index via plant height (0.1167), test weight (0.0862), number of filled grains per panicle (0.0596), number of grains per panicle (0.0366) and panicle length (0.0309) was high. The indirect effect of number of grains per panicle via test weight (0.3593) and percent chaffyness (0.196) was high and positive.

The indirect effect of number of filled grains per panicle via number of grains per panicle (1.1623), biological yield (0.1032), harvest index (0.0951), panicle length (0.0575) and number of productive tillers per plant (0.0505) was positive and high and the negative indirect effect of number of productive tillers per plant via only total number of tillers per plant (-0.0862) was high. While, the indirect effect of biological yield via only percent chaffyness (-0.029) was negative and high.

The negative indirect effect of harvest index via percent chaffyness (-0.0326) and number of productive tillers (-0.0266) was high and the indirect effect of number of grains per panicle via number of filled grains per panicle (-1.0726) and biological yield (-0.091) was negative and high. While the negative the indirect effect of number of filled grains per panicle via test weight (-0.3712) and percent chaffyness (-0.3459) was negative and high.

4.4 Genetic divergence

In the present study, genetic divergence was assessed by Mahalanobis D^2 statistics and Tocher method (Rao, 1952).

4.4.1 Group constellation

The distribution pattern of rice mutants was made into two clusters (Table 9 and Table 10) in normal and saline soil condition respectively. These clusters were formed by grouping all 400 mutant rice lines in such a way that mutant lines within each cluster had smaller D^2 statistics than between cluster.

Table 9. Clustering pattern of 400 mutant lines and checks of rice for normal soil

Clusters	No. of entries	Mutants
I	403	1 To 204 And 206 To 400, BPT-5204 Check, RPBIO-226 check
II	1	205

Table 10. Clustering pattern of 400 mutant lines and checks of rice for saline soil

Clusters	No. of entries	Mutants
I	403	1 to 269, 271 to 400, BPT-5204 Check, RPBIO-226 check
II	1	270

Both normal and saline soil condition cluster pattern revealed that, 1 mutant line were present in cluster II and 399 mutant lines present in cluster I.

4.4.2 Intra and inter relation of clusters

The intra and inter cluster average distances among 2 clusters were variable (Table 11). The highest intra-cluster distance was recorded for cluster I (205.6cm). There were no interactions within cluster I and II in the normal soil condition. Similarly saline soil conditions the intra and inters cluster average distances among 2 clusters were variable (Table 12). The highest intra-cluster distance was recorded for cluster I (170.06 cm). There were no interaction within cluster I and II.

4.4.3 Cluster means

A comparison of the mean values of different cluster for 12 characters has been presented in (Table 13) in normal soil and (Table 14) in saline soil condition. In the normal soil condition cluster I mutant lines exhibited the highest mean to days to 50 per cent flowering (103) followed by cluster II (99). Mutant lines for highest number of tillers per plant were grouped under cluster II (17.60) followed by cluster I with mean value of 14.94 numbers of tillers per plant. 16.60 number of productive tiller per plant mean was recorded by mutant lines belonging to the cluster II followed by cluster I with a mean value of 13.63. With respect to plant height, mutant lines belonging to cluster I showed highest mean value of plant height (86.24 cm), while cluster II showed the least mean value of plant height (82.80cm). When panicle length was considered, cluster I composed of mutant lines showing highest panicle length (21.20 cm), mutant lines in cluster II recorded the lowest mean panicle length (19.55 cm).

As high as 61.16 g biological yield mean was recorded by mutant lines belonging to the cluster I, least mean biological yield of 49.08 g was noticed in cluster II. Grain yield was highest in cluster I with a mean value of 28.69 g and it was least in cluster II (23.20 g). With respect to harvest index was considered, cluster I composed of mutant lines showing highest harvest index value (51.52 %), mutant lines in cluster II recorded the lowest mean harvest index (47.19 %).

Number of grains per panicle was highest in cluster II with a mean value of 330.40 and it was least in cluster I (317.89). Mutant lines for highest filled grains per panicle were grouped under cluster I (299.60) followed by cluster II with mean value of

Table 11. Average inter and intra cluster distance for 12 characters in M4 mutant rice for normal soil

Clusters	I	II
I	**205.26**	1309.31
II		**0.00**

Table 12. Average Inter and Intra cluster distances for 12 characters in M_4 mutant rice for saline soil

Clusters	I	II
I	**170.06**	1307.87
II		**0.00**

293. As high as 10.57 per cent of chaffyness mean was recorded by mutant lines belonging to the cluster II followed by cluster I with a mean value of 10.22 per cent. Highest test weight was recorded by the mutant lines making up cluster I (15.62 g) while cluster II showed the least test weight (13.60 g).

While character wise scoring for cluster means, the most desirable magnitude of the trait is given score 1. Hence, the cluster with least overall score across 12 characters assigned with rank 1 and that cluster with the highest score gets the second rank. The cluster II with overall score of 17 across the 12 characters get I rank followed by cluster I (19) and presented in Table 13.

In saline soil condition Cluster II mutant lines exhibited the highest mean days to 50 per cent flowering (110.45) followed by cluster I (107). Mutant lines for highest number of tillers per plant were grouped under cluster II (17.4) followed by cluster I with mean value of 14.93 numbers of tillers per plant. The number of productive tillers per plant exhibited highest mean in cluster II (14) and least cluster mean was observed in cluster I (12.48). Plant height has highest mean (86.72) in cluster I followed by cluster II (81.8). Panicle length was high in cluster I (20.52) and least in cluster II (8.2).

As high as 62.26 g biological yield mean was recorded by mutant lines belonging to the cluster II, least mean was recorded by cluster I (58.81). Grain yield was highest in cluster II with a mean value of 33.2 g and it was least in cluster I (30.32 g). While the harvest index was considered, cluster II composed of mutant lines showing highest harvest index value (53.05 %) closely followed by cluster I mutant lines (52.64 %). Number of grains per panicle was highest in cluster I with a mean value of 294.01 and it was least in cluster II (250.02).

Mutant lines for highest filled grains per panicle were grouped under cluster I (277.21) followed by cluster II with mean value of 243.4. As high as 6.77 percent of chaffyness mean was recorded by mutant lines belonging to the cluster II. Least mean per cent chaffyness 2.79 per cent was noticed in cluster I. Highest test weight was recorded by the mutant lines making up cluster I (17.13 g) while cluster II showed the least test weight (10.5 g).

While character wise scoring for cluster means, the most desirable magnitude of the trait is given score one. Hence, the cluster with least overall score across 12 characters assigned with rank 1 and that cluster with the highest score gets the second rank. The

cluster I with overall score of 17 across the 12 characters get I rank followed by cluster I (19) was presented in Table 14.

4.4.4 Contribution of different characters towards divergence:

In normal soil condition the number of grains per panicle had maximum contribution (40.75), towards divergence followed by number of filled grain per panicle (39.54), biological yield per plant (5.94), plant height (3.45), days to 50 per cent flowering (3.17), grain yield per panicle (1.66), harvest index (1.65), number of tillers per plant (1.02), number of productive tillers per plant (1.01), panicle length (1.01), test weight (0.76) and percent of chaffyness (0.04) showed in Table 15 and Fig. 5

In saline soil condition the number of grains per panicle had maximum contribution (40.78), towards divergence followed by number of filled grain per panicle (36.89), Biological yield per plant (4.76), plant height (4.07), grain yield per panicle (3.41), number of productive tillers per plant (2.4), harvest index (1.88), days to 50 per cent flowering (1.7), number of tillers per plant (1.22), panicle length (1.02), test weight (0.96) and percent of chaffyness (0.58) is showed in Table 16 and Fig. 6

4.5 Salinity Screening

To evaluate best salinity tolerant mutant lines from 400 mutant lines of rice and effective screening for identification of salt tolerance mutant lines by salt tolerance indices including Stress Susceptibility Index (SSI) and mortality percentage are represented in Table 17.

The line No. 41 of BPT-5204 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-13.06), number of productive tillers per plant (-4.13), plant height (-2.34), grain yield per panicle (-0.60), number of filled grains per panicle (-4.03) and test weight (-4.70) with zero mortality percentage which indicates the salt tolerance. The line No. 209 of RP-Bio 226 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-6.04), number of productive tillers per plant (3.21), plant height (-3.80), grain yield per panicle (-1.40), number of filled grains per panicle (-0.71) and test weight (-0.53) with zero mortality percentage which indicates the salt tolerance. The line No. 4 of BPT-5204 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-0.46), number of productive tillers per plant (-0.28), plant height (2.21), grain yield per panicle (-2.29), number of filled grains per

Table 13. Cluster mean for 12 characters in M_4 mutant rice for normal soil

Clusters	DFF	TNT	NPT	PH	PL	BYP	GYP	HI	NGP	NFG	%CF	TW	Overall Score	RANK
I	103.28 (2)	14.94 (2)	13.63 (2)	86.24 (1)	19.55 (2)	61.16 (1)	28.69 (1)	51.52 (1)	317.89 (2)	293.00 (2)	10.22 (2)	15.62 (1)	**19**	**1**
II	99.00 (1)	17.60 (1)	16.60 (1)	82.80 (2)	21.20 (1)	49.08 (2)	23.20 (2)	47.19 (2)	330.40 (1)	299.60 (1)	10.57 (1)	13.60 (2)	**17**	**2**

Table 14. Cluster mean for 12 characters in M_4 mutant rice for saline soil

Clusters	DFF	TNT	NPT	PH	PL	BYP	GYP	HI	NGP	NFG	%CF	TW	Over all Score	Rank
I	110.45 (2)	14.93 (2)	12.48 (2)	86.72 (1)	20.52 (1)	58.81 (2)	30.32 (2)	52.64 (2)	294.01 (1)	277.21 (1)	2.79 (2)	17.13 (1)	**19**	**1**
II	107 (1)	17.4 (1)	14 (1)	81.8 (2)	18.2 (2)	62.26 (1)	33.2 (1)	53.05 (1)	250.2 (2)	243.4 (2)	6.77 (1)	10.5 (2)	**17**	**2**

DFF= Days to 50% flowering. TNT= total number of tillers per plant. NPT= Number of productive tillers per plant.
PH = Plant height. PL = Panicle length. BY = biological yield.
GYP= grain yield per plant. HI = Harvest index NGP = number of grains per panicle.
NFG = Number filled grains per panicle % CF = percent chaffyness. TW = Test weight

Table 15. Percent contribution of each character towards divergence in characters of mutant rice of normal soil

Sl. No.	Characters	Contribution (%)	Cumulative
1	Number of grains per panicle	40.75	40.75
2	Number of filled grain per panicle	39.54	80.29
3	Biological yield per plant	5.94	86.23
4	Plant height	3.45	89.68
5	Days to 50% flowering	3.17	92.85
6	Grain yield per panicle	1.66	94.51
7	Harvest index	1.65	96.16
8	No. tillers per plant	1.02	97.18
9	No. productive tillers per plant	1.01	98.19
10	Panicle length	1.01	99.20
11	Test weight	0.76	99.96
12	% Chaffyness	0.04	100
	Total	**100**	

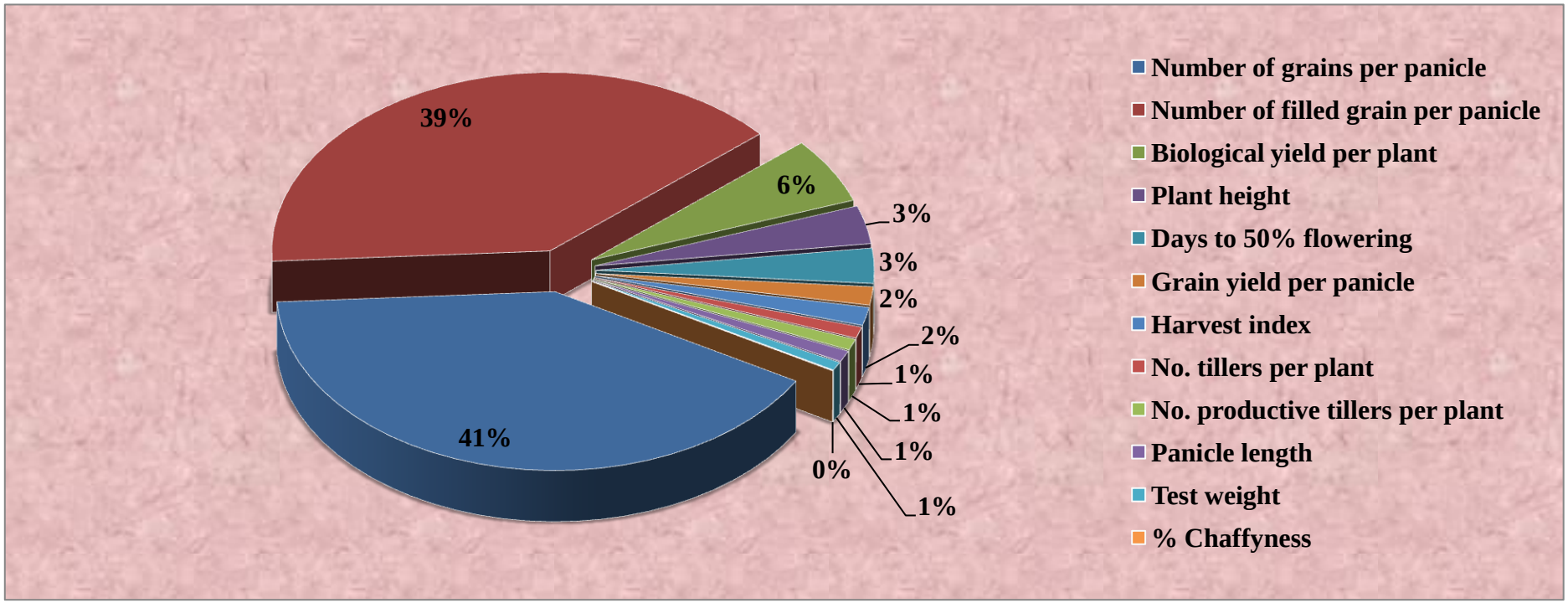


Fig. 5: Contribution of different characters towards genetic divergence for normal soil

Table 16. Percent contribution of each character towards divergence in characters of mutant rice for saline soil

Sl. No.	Characters	Contribution (%)	Cumulative
1	Number of grains per panicle	40.78	40.78
2	Number of filled grain per panicle	36.89	77.67
3	Biological yield per plant	4.76	82.43
4	Plant height	4.07	86.5
5	Grain yield per panicle	3.41	89.91
6	Number of productive tillers per plant	2.4	92.31
7	Harvest index	1.88	94.19
8	Days to 50 percent flowering	1.7	95.89
9	Number of tillers per plant	1.22	97.11
10	Panicle length	1.02	98.13
11	Test weight	1.29	99.42
12	Percent Chaffyness	0.58	100
	Total	**100**	

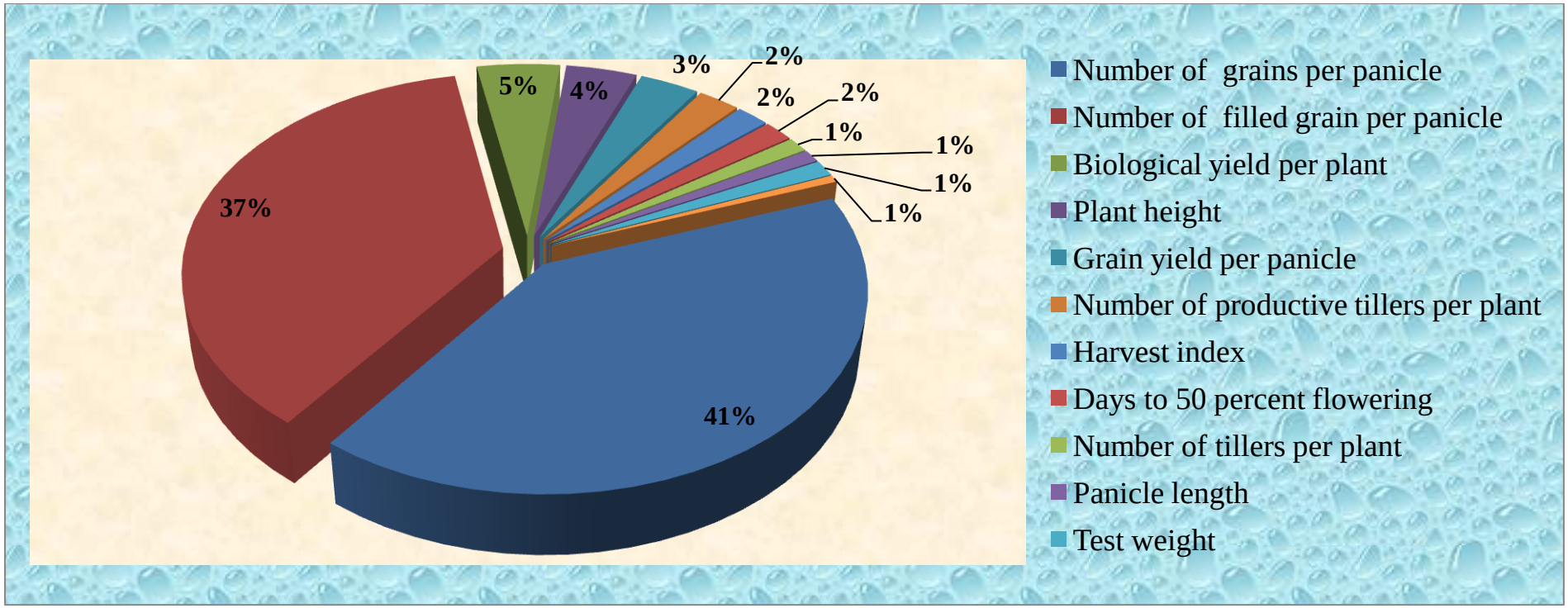


Fig. 6: Contribution of different characters towards genetic divergence for saline soil

Table 17. Stress Susceptibility index (%) of M_4 mutant population

Line no	TNT	NPT	PH	GYP	NFG	TW	MP
41	-13.06	-4.13	-2.34	-0.60	-4.03	-4.70	0.00
209	-6.04	3.21	-3.80	-1.40	-0.71	-0.53	0.00
4	-0.46	-0.28	2.21	-2.29	-6.14	3.20	0.00
114	0.00	1.88	-0.09	3.02	-5.46	-2.96	0.00
186	0.00	0.00	-0.05	-3.80	3.40	0.10	0.00
136	0.00	-0.14	-0.04	2.83	-10.03	-1.81	0.00
138	0.00	0.00	0.00	2.82	-0.59	-0.63	0.00
178	0.00	-1.93	0.08	-1.05	-0.43	2.22	0.00
60	1.01	1.04	-10.62	-0.28	-116.73	-1.28	0.00
353	3.70	-1.56	-3.52	4.09	-4.16	-2.13	0.00
270	-18.60	7.05	6.61	-7.69	3.13	-2.26	0.00
253	-16.85	7.61	1.72	-6.29	0.79	-0.22	0.00
28	-10.08	-3.08	4.55	6.52	-31.11	-15.69	0.00
358	-9.93	13.57	3.29	13.52	1.18	-0.86	0.00
254	-9.89	3.66	-1.09	-4.80	1.23	0.07	0.00
283	-9.35	4.86	1.21	25.00	1.22	6.25	0.00
305	-7.21	9.80	2.57	-5.75	-2.71	2.09	0.00
46	-5.95	-1.03	2.69	2.10	0.08	3.34	0.00
281	-5.42	2.41	2.89	-3.10	0.29	5.15	0.00
398	-0.55	3.64	1.26	7.48	-6.00	1.10	0.00
160	0.00	1.91	-0.05	0.46	-0.46	0.49	0.00
186	0.00	0.00	-0.05	-3.80	3.40	0.10	0.00
199	0.00	1.15	-0.02	8.16	-6.05	-0.44	0.00
183	0.00	2.06	-0.01	6.03	0.64	2.11	0.00
188	0.00	1.07	-0.01	-1.13	3.14	0.85	0.00
103	0.00	0.26	-0.09	9.22	0.75	3.96	0.00
160	0.00	1.91	-0.05	0.46	-0.46	0.49	0.00
172	0.00	1.75	-0.02	-2.38	2.24	0.26	0.00
110	0.00	-5.01	-0.01	3.92	0.38	0.78	0.00
194	0.00	2.24	-0.01	6.65	-0.28	0.50	0.00
BC	1.11	1.02	0.91	3.26	0.73	0.79	5.88
RPC	1.24	-1.32	-1.00	-0.61	6.78	0.96	4.94

TNT= Total number of tillers per plant. NPT = Number of productive tillers per plant.

PH = Plant height. GY = Grain yield per panicle.

TW = Test weight. NFG = Number of filled grains per panicle.

M (%) = Mortality percentage. BLB = Bacterial leaf blight incidence (%)

BC = BPT-5204 Check RPC = RPBIO-226 check

panicle (-6.14) and test weight (3.20) with zero mortality percentage which indicates the salt tolerance.

The line No. 114 of BPT-5204 (40 Kr) showed zero SSI value over check for total tillers per plant and other characters *viz.* number of productive tillers per plant (1.18), plant height (-0.09), grain yield per panicle (3.2), number of filled grains per panicle (-5.46) and test weight (-2.96) are showed lesser value and zero mortality percentage which indicates the salt tolerance. The line No. 186 of BPT-5204(40 Kr) showed zero SSI value over check for total tillers per plant, number of productive tillers per plant and other characters *viz.,* plant height (-0.05), grain yield per panicle (-3.80), number of filled grains per panicle (3.40), test weight (0.10) are showed lesser value and zero mortality percentage which indicates the salt tolerance.

The line No. 136 of BPT-5204(40 Kr) showed zero SSI value over check for total tillers per plant and other characters *viz.* number of productive tillers per plant (-0.14), plant height (-0.09), grain yield per panicle (2.83), number of filled grains per panicle (-10.03) and test weight (-1.81) are showed lesser value and zero mortality percentage which indicates the salt tolerance. The line No. 138 of BPT-5204(40 Kr) showed zero SSI value over check for total tillers per plant, number of productive tillers per plant, plant height and other characters *viz.*, grain yield per panicle (2.82), number of filled grains per panicle (-0.59), test weight (-0.63) are showed lesser value and zero mortality percentage which indicates the salt tolerance.

The line No. 178 of BPT-5204 (40 Kr) showed zero SSI value over check for total tillers per plant and other characters *viz.* number of productive tillers per plant (-1.93), plant height (0.08), grain yield per panicle (-1.05), number of filled grains per panicle (-0.43) and test weight (2.22) are showed lesser value and zero mortality percentage which indicates the salt tolerance. The line No. 6 of BPT-5204 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (1.01), number of productive tillers per plant (1.04), plant height (-10.62), grain yield per panicle (-0.28), number of filled grains per panicle (-116.73) and test weight (-1.28) with zero mortality percentage which indicates the salt tolerance.

Based on the SSI value, the line No. 353 of RP-Bio 226 (40 Kr) total tiller per plant (3.70), number of productive tillers per plant (-1.56), plant height (-3.52), grain

yield per panicle (4.09), number of filled grains per panicle (-4.16) and test weight (-2.13) are shows lesser SSI value and zero mortality percentage it indicate the salt tolerance.

The line No. 270 of RP-Bio 226 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-18.60), number of productive tillers per plant (7.05), plant height (6.61), grain yield per panicle (-7.69), number of filled grains per panicle (3.31) and test weight (-2.26) with zero mortality percentage which indicates the salt tolerance. The line No. 253 of RP-Bio 226 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-16.85), number of productive tillers per plant (7.61), plant height (1.72), grain yield per panicle (-6.21), number of filled grains per panicle (0.79) and test weight (-0.22) with zero mortality percentage which indicates the salt tolerance.

The line No. 28 of BPT-5204 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-10.08), number of productive tillers per plant (-3.08), plant height (4.55), grain yield per panicle (-6.52), number of filled grains per panicle (-31.11) and test weight (-15.69) with zero mortality percentage which indicates the salt tolerance. The line No. 358 of RP-Bio 226 (40 Kr) showed lesser SSI value over check for total number of tiller per plant (-9.93), number of productive tillers per plant (13.57), plant height (3.29), grain yield per panicle (-13.52), number of filled grains per panicle (1.18) and test weight (-0.86) with zero mortality percentage which indicates the salt tolerance.

Based on the SSI value, the line No. 254 of RP-Bio 226 (30 Kr) total tiller per plant (-9.89), number of productive tillers per plant (3.66), plant height (-1.09), grain yield per panicle (-4.80), number of filled grains per panicle (1.23) and test weight (0.07) are shows lesser SSI value and zero mortality percentage it indicate the salt tolerance. The line No. 305 of RP-Bio 226 (40 Kr) had showed lesser SSI value over check for total number of tiller per plant (-7.21), number of productive tillers per plant (9.80), plant height (2.57), grain yield per panicle (-5.75), number of filled grains per panicle (-2.71) and test weight (2.09) with zero mortality percentage which indicates the salt tolerance. The line No. 36 of BPT-5204 (30 Kr) showed lesser SSI value over check for total number of tiller per plant (-5.95), number of productive tillers per plant (-1.03), plant height (2.69), grain yield per panicle (2.10), number of filled grains per panicle (0.08) and test weight (-3.35) with zero mortality percentage which indicates the salt tolerance. The line No. 281 of RP-Bio 226 (30 Kr) had showed lesser SSI value over check for total

number of tiller per plant (-5.42), number of productive tillers per plant (2.41), plant height (2.89), grain yield per panicle (-3.10), number of filled grains per panicle (0.29) and test weight (5.15) with zero mortality percentage which indicates the salt tolerance.

Based on the SSI value, the line No. 398 of RP-Bio 226 (40 Kr) total tiller per plant (-0.55), number of productive tillers per plant (3.64), plant height (1.26), grain yield per panicle (7.48), number of filled grains per panicle (-6.00) and test weight (1.10) with lesser SSI value and zero mortality percentage indicate the salt tolerance. The line No. 160 of BPT-5204(40 Kr) showed zero SSI value over check for total number of tiller per plant and other character *viz.,* number of productive tillers per plant (1.91), plant height (-0.05), grain yield per panicle (-0.46), number of filled grains per panicle (-0.46) and test weight (0.49) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance. The line No. 199 of BPT-5204(40 Kr) showed zero SSI value over check for total number of tiller per plant and other character *viz.,* number of productive tillers per plant (1.15), plant height (-0.02), grain yield per panicle (8.16), number of filled grains per panicle (-6.05) and test weight (-0.44) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance.

The line No. 183 of BPT-5204 (40 Kr) showed zero SSI value over check for total number of tiller per plant and other characters *viz.,* number of productive tillers per plant (2.06), plant height (-0.01), grain yield per panicle (6.23), number of filled grains per panicle (0.64) and test weight (2.11) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance. The line No. 188 of BPT-5204 (40 Kr) showed zero SSI value over check for total number of tiller per plant and other characters *viz.,* number of productive tillers per plant (1.07), plant height (-0.01), grain yield per panicle (-1.13), number of filled grains per panicle (3.14) and test weight (0.85) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance. The line No. 103 of BPT-5204 (40 Kr) showed zero SSI value over check for total number of tiller per plant and other character *viz.,* number of productive tillers per plant (0.26), plant height (-0.09), grain yield per panicle (9.22), number of filled grains per panicle (0.75) and test weight (3.96) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance.

Based on the SSI value, the line No. 172 of BPT-5204 (40 Kr) showed zero for total tillers per plant and other characters like number of productive tillers per plant (1.75), plant height (-0.02), grain yield per panicle (2.38), number of filled grains per

panicle (2.24) and test weight (0.26) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance. The line No. 110 of BPT-5204 (40 Kr) showed zero SSI value over check for total number of tiller per plant and other character *viz.,* number of productive tillers per plant (-5.01), plant height (-0.01), grain yield per panicle (3.92), number of filled grains per panicle (0.38) and test weight (0.78) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance. The line No. 194 of BPT-5204 (40 Kr) showed zero SSI value over check for total number of tiller per plant and other character *viz.,* number of productive tillers per plant (2.24), plant height (0.01), grain yield per panicle (6.65), number of filled grains per panicle (-0.28) and test weight (0.50) are had lesser SSI value with zero mortality percentage which indicates the salt tolerance.

4.5.1 Screening for disease

M_4 mutant lines were screened for the bacterial leaf blight disease incidence which in BPT-5204(30 Kr and 40 Kr) was more than in RP-Bio 226 (30 Kr and 40 Kr) (appendix II). Based on scale of the bacterial leaf blight incidence, maximum disease incidence was observed in the lines *viz*., 58 (59.12 %) of BPT-5204 (30 Kr) and 196 (49 %) of BPT-5204 (40 Kr) respectively. The lines *viz*., 217 (38.00 %) of RP-BIO 226 (30 Kr) and 351 (29.00 %) of RP-Bio 226 (40 Kr) showed maximum disease incidence.

Similarly, minimum incidence was observed in the lines viz., 79 (22.22 %), 7 (24.44 %) of BPT-5204 (30 Kr); 175 (13.33 %), 181 (17.78 %) of BPT-5204 (40 Kr); 293 (11.00 %), 292 (11.00 %) of RP-BIO 226 (30 Kr) and 363 (10.00 %), 341 (11.11 %) of RP-Bio 226 (40 Kr) respectively.

4.6 Molecular diversity studies based using SSR markers

In the present study, 45 rice mutant lines were evaluated for genetic diversity using 16 simple sequence repeat (SSR) markers.

A total of 26 alleles were detected, out of which 24 were polymorphic. Polymorphism percentage was 96. The number of alleles detected per primer pair ranged from 1 to 3 with an average of 2.08 (Table.18). The SSR products size ranged from 110 to 450 bp. The SSR marker profiles of 45 genotypes generated by the primers.

Out of 16 SSR primers used, 11 primers *viz*., RM562, AP3206, RM223, RM459, RM8053, RM510, RM23, RMRM3412, RM7075, RM10772 and RM10793 showed

Table 18. Allelic variation values for SSR markers identified in 45 rice genotypes

Sl. No.	Primer code	Number of alleles	Number of polymorphic alleles
1	AP3406	2	2
2	RM562	2	2
3	RM223	2	2
4	RM459	2	2
5	RM8053	2	2
6	RM510	2	2
7	RM556	2	1
8	RM23	2	2
9	RM3412	2	2
10	RM7075	3	3
11	RM10772	2	2
12	RM10793	3	3

polymorphism, primer RM556 showed monomorphism, whereas, 4 primers *viz.*,RM8094, RM152, RM23 and RM336 failed to amplify. The RM459 primer showed polymorphism at 380 base pairs, while RM10772 and RM10793 showed polymorphism at 450 and 260 base pairs, respectively are showed in Plate 5 and 6.

4.6.1 Cluster analysis

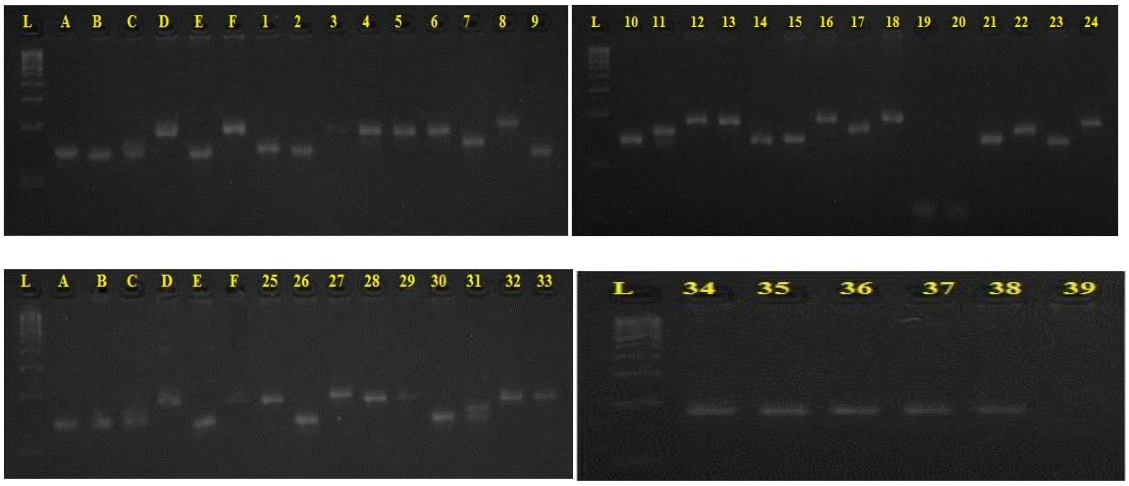
4.6.1.1 Similarity index

The binary data from the polymorphic primers were used for computing simple matching similarity indices. The similarity index values obtained for each pair wise comparison among the 45 genotypes are presented in the Table 20. The similarity coefficients based on 26 alleles of 14 SSR markers ranged from 0.10 and 0.98. Among the 45 genotypes the highest similarity index (0.98) was observed between line No. 54 of BPT- 5204 (40 Kr) and Line No. 1 of BPT- 5204 (40 Kr) and the lowest similarity index (0.10) was observed between RP-Bio 226 check and Pokkali check.

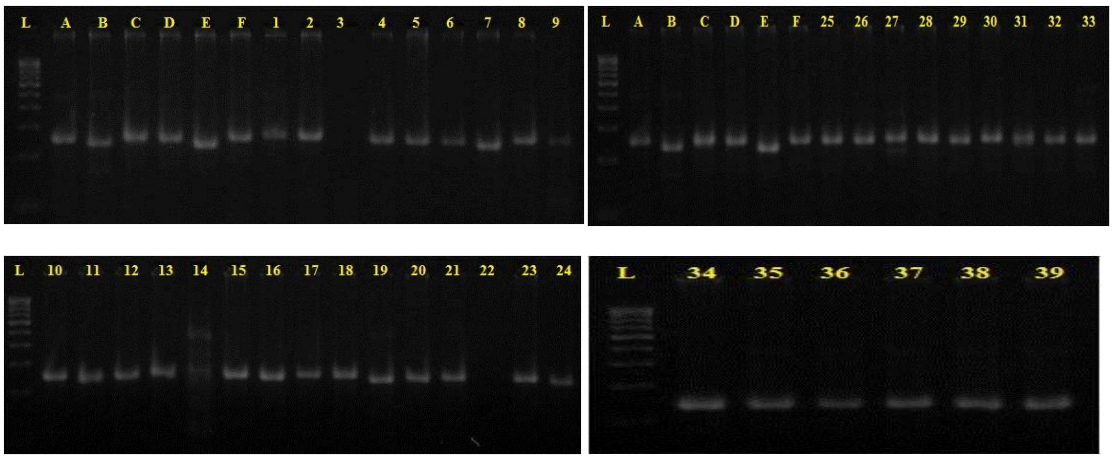
4.6.1.2 Clusters based on dendrogram

The similarity values obtained for each pair wise comparison of SSR markers among the 24 rice genotypes were used to construct dendrogram based on simple matching coefficient indices and the results are presented in Fig. 7. The 45 genotypes formed 12 clusters at nearly 85 percent similarity levels. Among the different clusters, the cluster size varied from 10 (Cluster V, VII) to 1 (Clusters II, VIII and XII).

The list of all the 12 clusters along with the genotypes included and the cluster V and VII were highly heterogeneous representing in Table 19. The cluster I consist of 1, 27, 29. The cluster II consists of 21. The cluster III consists of 16, 17. The cluster IV consists of 2, 3, 25, and 26. The cluster V consists of 7, 8, 20, 24, 23, 13, 34, 36, and 37. The cluster VI consists of 15, 35. The cluster VII consists of 4, 6, 22, 32, 33, 40, 41, 43, 45 and 44. The cluster VIII consists of 30. The cluster IX consists of 14, 18, 19, 39 and 42. The cluster X consists of 5, 10, 12, 31and 38. The cluster XI consists of 11 and 28. The cluster XII consists of 9.



RM10793



RM453

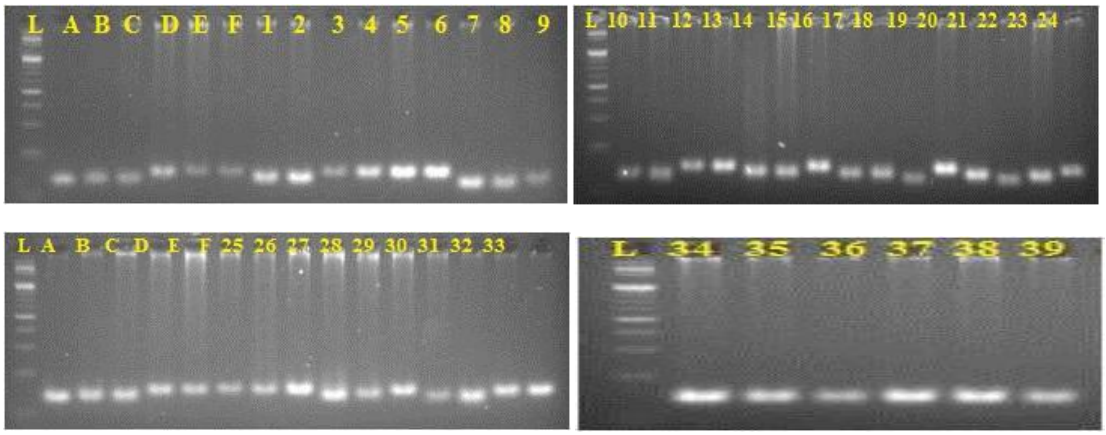
Plate 5. SSR marker profile of rice mutants generated by the primer RM10793 and RM453.

L = ladder (100bp) **A= BPT-5204**

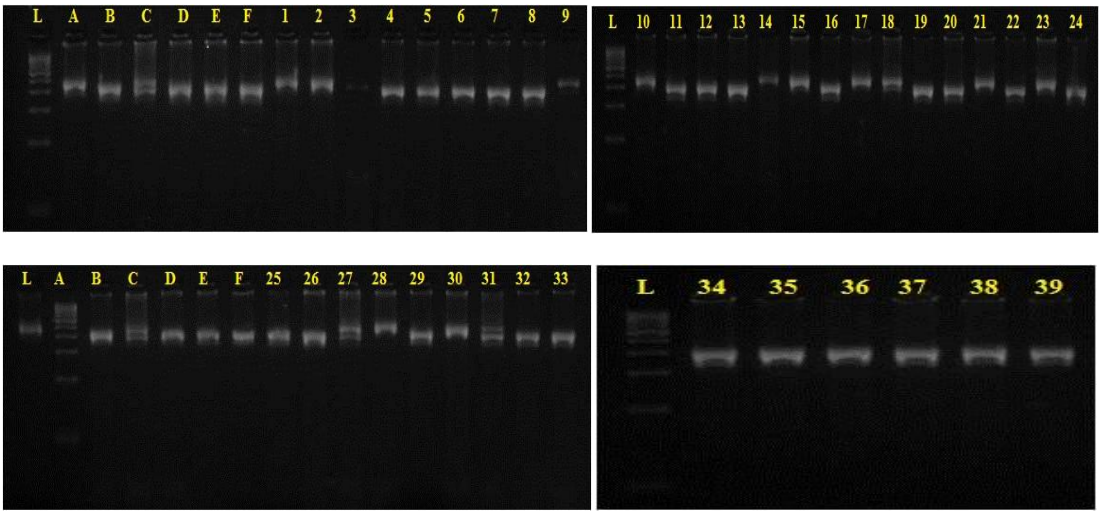
B= RPBIO- 226 **C = Pokkali**

D = IR-29 **E = FL 28**

F = CSR 22 **1 to 39= Mutant lines**



RM7075



RM10772

Plate 6. SSR marker profile of rice mutants generated by the primer RM7075 and RM10772.

L = ladder (100bp) **A= BPT-5204**

B= RPBIO- 226 **C = Pokkali**

D = IR-29 **E = FL 28**

F = CSR 22 **1 to 39= Mutant lines**

Table 18. Allelic variation values for SSR markers identified in 45 rice genotypes

Sl. No.	Primer code	Number of alleles	Number of polymorphic alleles
1	AP3406	2	2
2	RM562	2	2
3	RM223	2	2
4	RM459	2	2
5	RM8053	2	2
6	RM510	2	2
7	RM556	2	1
8	RM23	2	2
9	RM3412	2	2
10	RM7075	3	3
11	RM10772	2	2
12	RM10793	3	3

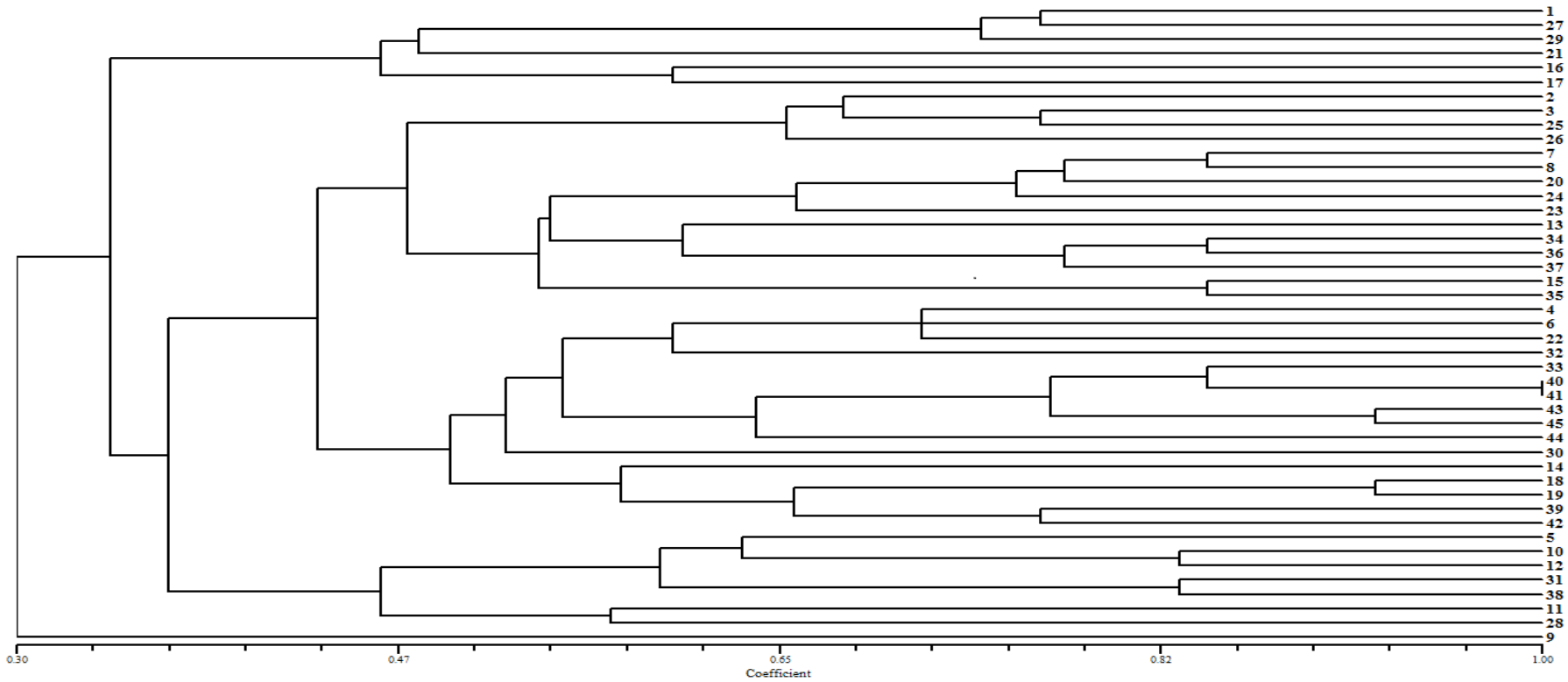


Fig 7. Dendrogram resulting from UPGMA cluster of 45 rice genotypes, based on data derived from 16 SSR markers

Table 19. Cluster compositions of rice genotypes for SSR markers

Cluster no.	No. of genotypes	List of genotypes
I	3	1, 27, 29
II	1	21
III	2	16, 17
IV	4	2, 3, 25, 26
V	9	7, 8, 20, 24, 23, 13, 34, 36, 37
VI	2	15, 35
VII	10	4, 6, 22, 32, 33, 40, 41, 43, 45, 44
VIII	1	30
IX	5	14, 18, 19, 39, 42
X	5	5, 10, 12, 31, 38
XI	2	11, 28
XII	1	9

Table 20. Similarity index computed from SSR data

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	**1**																						
2	0.44	**1**																					
3	0.29	0.64	**1**																				
4	0.44	0.26	0.44	**1**																			
5	0.29	0.28	0.29	0.44	**1**																		
6	0.44	0.41	0.53	0.71	0.28	**1**																	
7	0.53	0.50	0.44	0.26	0.28	0.41	**1**																
8	0.44	0.41	0.53	0.33	0.21	0.50	0.85	**1**															
9	0.29	0.15	0.22	0.28	0.38	0.15	0.44	0.44	**1**														
10	0.22	0.35	0.47	0.44	0.57	0.28	0.35	0.35	0.38	**1**													
11	0.29	0.15	0.22	0.53	0.47	0.35	0.28	0.28	0.47	0.57	**1**												
12	0.29	0.28	0.38	0.53	0.69	0.35	0.28	0.28	0.29	0.83	0.69	**1**											
13	0.35	0.50	0.44	0.41	0.53	0.60	0.50	0.41	0.15	0.44	0.35	0.53	**1**										
14	0.10	0.33	0.44	0.26	0.35	0.41	0.33	0.41	0.35	0.35	0.28	0.28	0.50	**1**									
15	0.28	0.50	0.53	0.20	0.21	0.26	0.50	0.60	0.28	0.35	0.15	0.28	0.33	0.33	**1**								
16	0.53	0.41	0.28	0.26	0.28	0.20	0.26	0.20	0.28	0.21	0.28	0.28	0.26	0.14	0.41	**1**							
17	0.44	0.50	0.28	0.33	0.35	0.33	0.20	0.14	0.15	0.35	0.44	0.44	0.41	0.26	0.20	0.60	**1**						
18	0.21	0.33	0.53	0.60	0.35	0.41	0.20	0.26	0.35	0.53	0.44	0.44	0.33	0.50	0.26	0.33	0.41	1					
19	0.20	0.39	0.60	0.56	0.33	0.47	0.25	0.32	0.33	0.50	0.41	0.41	0.39	0.56	0.32	0.32	0.39	0.92	**1**				
20	0.53	0.50	0.64	0.41	0.15	0.60	0.71	0.85	0.35	0.28	0.21	0.21	0.33	0.33	0.50	0.26	0.20	0.33	0.39	**1**			
21	0.44	0.41	0.53	0.33	0.21	0.50	0.41	0.50	0.28	0.21	0.28	0.28	0.41	0.41	0.41	0.50	0.41	0.41	0.47	0.60	**1**		
22	0.28	0.41	0.64	0.71	0.44	0.71	0.26	0.33	0.15	0.44	0.35	0.53	0.60	0.41	0.33	0.26	0.33	0.60	0.67	0.41	0.50	**1**	
23	0.28	0.41	0.53	0.26	0.28	0.41	0.60	0.71	0.28	0.53	0.28	0.44	0.50	0.50	0.60	0.20	0.26	0.33	0.39	0.60	0.50	0.41	**1**

24	0.35	0.33	0.44	0.41	0.21	0.60	0.71	0.85	0.35	0.35	0.28	0.28	0.50	0.50	0.50	0.14	0.14	0.33	0.39	0.71	0.41	0.41	0.71
25	0.35	0.71	0.77	0.33	0.35	0.41	0.50	0.41	0.21	0.44	0.21	0.35	0.50	0.33	0.50	0.41	0.33	0.41	0.47	0.50	0.41	0.50	0.41
26	0.44	0.60	0.64	0.50	0.53	0.33	0.41	0.33	0.35	0.64	0.35	0.53	0.41	0.26	0.33	0.41	0.41	0.60	0.56	0.41	0.33	0.50	0.33
27	0.77	0.50	0.44	0.41	0.44	0.41	0.50	0.41	0.28	0.35	0.28	0.44	0.50	0.20	0.33	0.60	0.50	0.33	0.32	0.50	0.60	0.41	0.41
28	0.47	0.28	0.38	0.64	0.38	0.53	0.28	0.28	0.22	0.47	0.57	0.57	0.35	0.15	0.21	0.35	0.44	0.35	0.33	0.35	0.28	0.44	0.28
29	0.77	0.50	0.44	0.41	0.28	0.41	0.71	0.60	0.44	0.35	0.28	0.28	0.33	0.20	0.33	0.41	0.33	0.33	0.32	0.71	0.41	0.26	0.41
30	0.29	0.44	0.47	0.53	0.38	0.53	0.53	0.44	0.29	0.47	0.38	0.38	0.53	0.35	0.28	0.15	0.21	0.44	0.50	0.44	0.21	0.53	0.35
31	0.29	0.28	0.38	0.64	0.57	0.44	0.21	0.28	0.22	0.57	0.47	0.69	0.53	0.35	0.35	0.28	0.35	0.53	0.50	0.21	0.28	0.64	0.35
32	0.35	0.20	0.35	0.60	0.35	0.60	0.50	0.60	0.35	0.35	0.44	0.44	0.50	0.33	0.41	0.20	0.14	0.33	0.39	0.50	0.41	0.60	0.50
33	0.35	0.50	0.64	0.41	0.21	0.60	0.50	0.60	0.21	0.35	0.15	0.28	0.50	0.50	0.50	0.26	0.26	0.50	0.56	0.71	0.60	0.60	0.71
34	0.28	0.60	0.77	0.33	0.35	0.50	0.60	0.71	0.28	0.53	0.28	0.44	0.60	0.60	0.60	0.20	0.26	0.41	0.47	0.60	0.50	0.50	0.71
35	0.28	0.50	0.53	0.26	0.28	0.26	0.50	0.60	0.35	0.44	0.21	0.35	0.33	0.33	0.85	0.33	0.20	0.33	0.39	0.50	0.41	0.41	0.60
36	0.28	0.71	0.64	0.26	0.28	0.41	0.50	0.60	0.21	0.44	0.21	0.35	0.50	0.50	0.71	0.26	0.33	0.33	0.39	0.50	0.41	0.41	0.60
37	0.35	0.50	0.64	0.41	0.44	0.60	0.50	0.60	0.21	0.44	0.35	0.53	0.71	0.50	0.50	0.26	0.33	0.33	0.39	0.50	0.60	0.60	0.60
38	0.22	0.35	0.47	0.53	0.47	0.35	0.28	0.35	0.29	0.69	0.38	0.57	0.44	0.44	0.44	0.21	0.28	0.64	0.60	0.28	0.21	0.53	0.44
39	0.10	0.35	0.47	0.35	0.29	0.35	0.28	0.35	0.29	0.47	0.38	0.38	0.44	0.64	0.44	0.21	0.28	0.64	0.71	0.28	0.35	0.53	0.44
40	0.28	0.41	0.64	0.50	0.28	0.50	0.41	0.50	0.28	0.44	0.21	0.35	0.41	0.41	0.50	0.26	0.20	0.60	0.67	0.60	0.50	0.71	0.60
41	0.28	0.41	0.64	0.50	0.28	0.50	0.41	0.50	0.28	0.44	0.21	0.35	0.41	0.41	0.50	0.26	0.20	0.60	0.67	0.60	0.50	0.71	0.60
42	0.15	0.26	0.44	0.33	0.28	0.33	0.41	0.50	0.44	0.44	0.35	0.35	0.41	0.60	0.50	0.26	0.20	0.60	0.67	0.41	0.50	0.50	0.60
43	0.33	0.32	0.50	0.56	0.20	0.56	0.47	0.56	0.33	0.41	0.33	0.33	0.32	0.32	0.39	0.19	0.19	0.47	0.53	0.67	0.39	0.56	0.56
44	0.15	0.26	0.44	0.33	0.28	0.33	0.26	0.33	0.44	0.28	0.21	0.21	0.26	0.60	0.33	0.26	0.20	0.60	0.67	0.41	0.50	0.50	0.41
45	0.35	0.33	0.53	0.60	0.21	0.60	0.50	0.60	0.35	0.35	0.28	0.28	0.33	0.33	0.41	0.20	0.14	0.50	0.56	0.71	0.41	0.60	0.50

Contd…

	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
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25	0.33	**1**																				
26	0.26	0.71	**1**																			
27	0.33	0.50	0.60	**1**																		
28	0.28	0.44	0.44	0.44	**1**																	

29	0.50	0.50	0.60	0.71	0.44	**1**																
30	0.53	0.53	0.53	0.28	0.38	0.44	**1.0**															
31	0.35	0.28	0.44	0.35	0.38	0.21	0.38	**1**														
32	0.71	0.26	0.26	0.33	0.35	0.33	0.53	0.53	**1**													
33	0.71	0.50	0.41	0.50	0.28	0.50	0.44	0.35	0.50	**1**												
34	0.60	0.60	0.50	0.41	0.28	0.41	0.44	0.44	0.41	0.60	**1**											
35	0.50	0.41	0.41	0.33	0.15	0.33	0.35	0.44	0.50	0.50	0.60	**1**										
36	0.50	0.50	0.41	0.33	0.21	0.33	0.35	0.44	0.33	0.50	0.85	0.71	**1**									
37	0.50	0.50	0.41	0.50	0.35	0.33	0.35	0.53	0.50	0.50	0.85	0.50	0.71	**1**								
38	0.44	0.35	0.53	0.28	0.29	0.28	0.47	0.83	0.44	0.44	0.53	0.53	0.53	0.44	**1**							
39	0.44	0.35	0.35	0.15	0.16	0.15	0.47	0.57	0.44	0.44	0.53	0.53	0.53	0.44	0.69	**1**						
40	0.60	0.50	0.50	0.41	0.28	0.41	0.53	0.44	0.60	0.85	0.50	0.60	0.41	0.41	0.53	0.53	**1**					
41	0.60	0.50	0.50	0.41	0.28	0.41	0.53	0.44	0.60	0.85	0.50	0.60	0.41	0.41	0.53	0.53	0.98	**1**				
42	0.60	0.33	0.33	0.26	0.15	0.26	0.44	0.44	0.60	0.60	0.50	0.60	0.41	0.41	0.53	0.77	0.71	0.71	**1**			
43	0.67	0.39	0.39	0.32	0.41	0.47	0.60	0.33	0.67	0.67	0.39	0.47	0.32	0.32	0.41	0.41	0.79	0.79	0.56	**1**		
44	0.41	0.33	0.33	0.26	0.15	0.26	0.35	0.28	0.41	0.60	0.33	0.41	0.26	0.26	0.35	0.53	0.71	0.71	0.71	0.56	**1**	
45	0.71	0.41	0.41	0.33	0.35	0.50	0.64	0.35	0.71	0.71	0.41	0.50	0.33	0.33	0.44	0.44	0.85	0.85	0.60	0.92	0.60	**1**

Discussion

V. DISCUSSION

For a crop improvement programme, plant breeding is pre–requisite to maintain the genetic variability that allows identification of promising genotypes in the germplasm collection that can be incorporated in the breeding programme to develop promising cultivars. The germplasm could be judged based on the knowledge of extent of variability, character association and direct and indirect effects influencing different characters (path analysis) and genetic divergence in the material.

Taking a clue from this, an attempt has been made at Gangavati, which is a rice bowl of Karnataka where 25 per cent of the Tungabhadra Project command area is reported to be affected by salinity problem interference (Golden jubilee sevenier) which is due to adaption of improper water and soil management practices. To address this problem for effort made through mutation breeding approach for generating increased variability to isolate salt tolerant mutants. Accordingly, BPT-5204 and RP-BIO 226 which are susceptible to salinity were irradiated with gamma rays for further selection of early maturing mutants with BPT-5204 quality background, these varieties were subjected for irradiation 30 Kr and 40 Kr gamma rays and evaluated for variability and salinity tolerance.

The results obtained from the present investigation are discussed under the following headings**:**

5.1 Genetic variability, heritability and genetic advance

5.2 Correlation coefficient

5.3 Path analysis

5.4 Genetic diversity

5.5 Salinity screening

5.6 Molecular diversity

5.1 Genetic variability, heritability and genetic advance

Variability was measured by estimation of mean, coefficient of variation (genotypic and phenotypic), heritability, genetic advance and genetic gain. Environment plays an important role in the expression of phenotype and genotype factors, which are

inferred, from phenotypic observations. Hence, variability can be observed through biometric parameters like genotypic coefficient of variation, heritability (broad sense) and genetic advance. This would be of great help to breeders in evolving a selection programme for suitable gene or genotype. The estimates of variance, coefficient of variation, heritability and genetic advance for all the 12 characters studied discussed here as under.

5.1.1 Genetic variability, heritability and genetic advance

GCV and PCV were moderate both in normal and saline soil condition for total number of tillers per plant, plant height and harvest index which is in conformity with findings of Singh *et al*. (2005), Mamta *et al*. (2007), Jaiswal *et al*. (2007), Sharma and Sharma (2007) and Pillai *et al.* (2011). GCV and PCV were low in both normal and saline soil condition with respect to days to 50 % flowering as also revealed by the findings of Vaithiyalingan and Nadarajan (2006), Gangashetty *et al.* (2012) and Pandey *et al.* (2012) respectively. GCV and PCV were high in normal as well as in saline condition for Biological yield, grain yield per panicle, number of filled grains per panicle and percentage of chaffyness as conformed by Pandey *et al.* (2012), Karim *et al.* (2007) and Babu *et al.* (2012) respectively.

Heritability estimates reveals the heritable portion of variability present in different characters. The knowledge of heritability enables the plant breeder to decide the course of selection procedure to be followed under a given situation. However, heritability values coupled with genetic advance would be more reliable (Johnson *et al*., 1955) and useful in formulating selection procedure. In the present study, heritability estimates in broad sense and genetic advance as per cent of mean were estimated.

Heritability estimates were high for all the characters studied in both saline and normal condition, except for panicle length and number of grains per panicle. So this higher heritability suggested the greater effectiveness of selection and improvement to be expected for these characters in future breeding programme as the genetic variance is mostly is due to additive gene expression and the results are conformed to Siddiqui and Sanjeeva (2010), Selvaraj *et al.* (2011), Singh *et al.* (2011), Pandey *et al.* (2012), Babu *et al.* (2012) and Satish *et al*. (2009).

In the present study, high heritability coupled with high genetic advance as per cent of mean was observed in normal and also in saline soil condition for the characters plant height, biological yield, number of grains per panicle and number of filled grains per panicle. This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance, hence are amenable for simple selection. The high genetic advance over per cent mean coupled with moderate to high heritability suggested the importance of additive gene action for these traits. The moderately high heritability and low genetic advance for remaining characters indicating the presence of non additive gene action and role of environment in expression of these traits.

5.2 Correlation coefficient

5.2.1 Correlation between yield and yield components

The phenotype of a plant is result of interaction of large number of factors. Hence, final yield was the sum total effect of several component factors. Therefore, it was important to know the extent and nature of inter-relationship revealing between grain yield and its component characters and also among themselves. Also it becomes necessary to know the association of grain quality characters among themselves. This would be obtained from simple correlation coefficient which helps a breeder in determining the direction and number of characters to be considered in improving yield and grain quality. In the present investigation, phenotypic correlation coefficient was worked out for plant and grain quality characters.

In the present study correlations among twelve quantitative characters of rice (*Oryza sativa* L.) were computed. The grain yield had significantly high positive correlation with harvest index in both normal and saline soil condition and almost similar significant positive magnitude of correlation was recorded by number of grains per panicle, number of filled grains per panicle, biological yield and plant height in both normal and saline soil condition. But non significant negative association with percentage of chaffyness only in the saline soil condition.

Such highly significant positive correlation with harvest index has been reported by Kole *et al*. (2008) and Idris *et al.* (2012). It has been shown that the mutants which have maximum harvest index results in accommodation of more number of grains per

panicle resulting in high grain yield per plant. Thus, it would be desirable to select rice plant type having maximum harvest index in both saline and normal soil condition

Similarly in normal soil condition the number of grains per panicle, number of filled grains per panicle had highly significant positive correlation with grain yield per plant which is in accordance with findings of Katoch *et al*. (2005), Akinwale *et al.* (2011), Kiani *et al.* (2012) and Selvaraj *et al.* (2011). Similarly, in saline soil condition biological yield, number of filled grains per panicle, number of grains per panicle and plant height had highly positive correlation with grain yield. Similar results were also reported by Krishna *et al*. (2008), Kiani *et al.* (2012), Selvaraj *et al.* (2011), Bhattacharya and Sowbhagya (1980, Akinwale *et al.*, (2011) and Neelima *et al*. (2007).

5.2.2 Correlation among yield components

Study of association of yield components with yield assumes special importance and forms basis for selecting desired strains. Correlation coefficient measures the magnitude and direction of association among the characters. The genotypic correlations between different characters within a plant often arise because of either genetic linkages or pleiotropy (Herald, 1939). It is important to the breeders to establish and understand the existing relationship between the yield and yield contributing characters. It is intended to consider the issues concerning the significant inter relationships among yield character other than grain yield which might aid in conceiving an ideal plant type. In normal soil days to 50% flowering had significant positive correlations with number of grains per panicle, number of filled grains per panicle and similarly in case of saline soil also days to 50% flowering had significant positive correlations with biological yield. This is in accordance with the findings of Padmavathi *et al.* (1996) and Pal *et al.* (2010).

In normal soil condition total number of tillers per plant had significant positive correlations with number of productive tillers per plants and percentage of chaffyness. Similar observation were reported by Akinwale *et al.* (2011), Neelima *et al*. (2007) and Panwar *et al*. (2005) and similarly in saline soil condition total number of tillers per plant had significant positive correlations with number of productive tillers per plants similar observation were reported by Rita *et al.* (2006), but it is negatively significantly correlated with plant height. Similar observations were reported by Sharma and Sharma (2007). The number of productive tillers per plant had significant positive correlations with only percentage of chaffyness in normal soil condition and this is in accordance with

the findings of Borbora *et al*. (2005) and Akinwale *et al.* (2011). In case of saline soil there is significant positive correlation with panicle length, test weight and harvest index. Similar observations were recorded by Akinwale *et al.* (2011), Sivakumar and Kannan, (2005) and Eradasappa *et al*. (2007).

The number of productive tillers had significant negative correlations with plant height which is in accordance with the findings of Rita *et al*. (2006). In both normal and saline soil condition plant height showed highly significant positive correlations with panicle length, harvest index and test weight. Similar observation were recorded by Akinwale *et al.* (2011), Mustafa (2011) and Eradasappa *et al*. (2007) but the plant height had negative correlation with the number of grains per panicle which is in accordance with Shashidhar *et al*. (2005). In normal soil condition panicle length showed highly significant positive correlations with only test weight similar observation were made by Monalisa *et al*. (2006). In both normal and saline soil condition number of grains per panicle showed highly significant positive correlations with only number of filled grains per panicle which is in accordance with Chaudhary and Motiramani (2003) and Sarkar *et al*. (2007).

In both normal and saline soil condition number of filled grains per panicle showed highly significant negative correlations with percentage of chaffyness and test weight which is in accordance with Elayaraja *et al.* (2003), Sharma and Sharma (2007) and Borbora *et al*. (2005).

From the present study of both normal and saline soil condition, it could be understood that, characters *viz,* harvest index, number of grains per panicle, number of filled grains per panicle and plant height need to be given greater importance during selection for improving grain yield per plant as these characters showed positive and highly significant correlation with grain yield per plant.

5.3 Path coefficient analysis

Path analysis was first suggested by Wright (1921). It is standardized partial regression analysis based on cause and effect of relationship and is useful for analysis by sub dividing correlation in a causal scheme.

In normal soil condition direct effect on grain yield reveals that out of twelve characters four characters (Harvest index, biological yield, number of grains per panicle

and plant height) considered for path analysis, harvest index exerted maximum direct effect on grain yield per plant followed by biological yield, number of grains per panicle and plant height. This indicates that, if other factors are held constant, an increase in biological yield individually will reflect in increased yield. The indirect effect of all these characters on grain yield was also high. Many other workers have also considered Harvest index, biological yield, number of grains per panicle and plant height to be most important yield components having greatest direct effect. These results are in conformity with the observations made by Jaiswal *et al*. (2007), Jayasudha S and Deepak Sharma (2010), Mustafa (2011) and Kole *et al*. (2008). While other characters *viz.,* number of productive, tillers per plant and panicle length had high magnitude negative direct effects on grain yield. These results are in conformity with the observations made by Mustafa, (2011) and Krishna *et al*. (2008).

In saline soil condition direct effect on grain yield reveals that number of filled grains per panicle (1.1717), harvest index (0.7345), biological yield (0.4396) and total numbers of tiller (0.1378) had high magnitude positive direct effects, which were in conformity with Siva and Kannan (2005), Eradasappa *et al.* (2007) and Rita *et al*. (2006). While, the negative direct effects of characters *viz.,* number of grains per panicle (-1.0813) and number of productive tillers per plant (-0.0905) are confirmed with Idris *et al.* (2011), Gangashetty *et al.* (2013) and Mustafa, (2011).

In normal soil condition the indirect effect of days to 50% flowering via other characters was not considerable.

In normal soil condition indirect effect on grain yield reveals that harvest index via plant height, number of filled grains per panicle, number productive tillers, total number tillers, test weight and days to 50 percent flowering on grain yield was high and positive, these results are in conformity with the observations made by Monalisa *et al.* (2006), Manna et *al.* (2006), and Neelima *et al.* (2007). Similarly in saline soil condition indirect effect on grain yield reveals that number of filled grains per panicle via number of grains per panicle, biological yield, harvest index, panicle length, number of productive tillers, plant height, 50 percent flowering and total number tillers per plant was high and positive. These results are in conformity with the observations made by Sadeghi, (2011), Neelima *et al*. (2007), Siva and Kannan, (2005) and Rita *et al*. (2006).

5.4 Genetic diversity

The amount of diversity available in the crop decides the success of any crop improvement programme with manifested objectives. Assemblage and assessment of divergence in the germplasm is essential to know the spectrum of diversity. In the present investigation, 400 mutants of rice were studied for their genetic diversity by D^2 analysis as per Mahalanobis (1936) with respect to thirteen important quantitative characters.

5.4.1 Genetic diversity in different groups

Based on D^2 values in saline soil, 400 genotypes were grouped into 2 clusters. 1 mutant line present in cluster II and 399 mutant lines present in cluster I. The formation of cluster with only two accessions may be due to total isolation preventing the gene flow or intensive natural/ human selection for diverse adaptive complexes. The intra cluster distance varied from 00 in cluster II to a maximum distance of 1307.87 in cluster I. This reveals the presence of diverse mutants within different clusters. It is desirable to select mutants from these cluster I showing high inter cluster distance and also with high grain yield as mutants in mutation breeding programmes for obtaining wide variability and desirable segregants.

5.4.2 Analysis of cluster means

All the mutants of normal and saline soil were spread over two clusters and means were scored across the clusters for all the 12 characters.

The highest cluster mean was given the first rank and next cluster possessing next best means were given 2^{nd} for all the traits except for days to 50 % flowering, where lowest mean was given first rank.

Based on the overall score across 12 traits, the clusters were ranked. The lowest scoring cluster was given first rank and next cluster possessing the score above the previous one were 2^{nd} rank. Accordingly, cluster I with overall score of the 12 characters received first rank followed by cluster II in both saline and normal soil condition, this indicating the presence of most promising mutants in them and can be extensively used for further breeding programme to generate new material which results are confirmed with Fakruddin *et al.*(2014).

5.4.5 Contribution of characters towards divergence

Per cent contribution of characters towards divergence in normal soil was analyzed and it was found that number of grains per panicle had maximum contribution (40.75), towards divergence followed by number of filled grain per panicle (39.54) contributed for maximum genetic diversity among the 400 mutant lines. The traits *viz* ., biological yield per plant (5.94), plant height (3.45), days to 50 percent flowering (3.17), grain yield per panicle (1.66), harvest index (1.65), number of tillers per plant (1.02), number of productive tillers per plant (1.01), panicle length (1.01), test weight (0.76) and percent of chaffyness (0.04) contributed very less to the genetic diversity this showed that the mutants possessed unique features for list of the traits studied in normal soil condition.

Similarly, per cent contribution of characters towards divergence in saline soil was analyzed and it was found that number of grains per panicle had maximum contribution (40.78) towards divergence followed by number of filled grain per panicle (36.89) contributed for maximum genetic diversity among the 400 mutant lines. The traits *viz* ., biological yield per plant (4.76), plant height (4.07), grain yield per panicle (3.41), number of productive tillers per plant (2.4), harvest index (1.88), days to 50 % flowering (1.7), number of tillers per plant (1.22), panicle length (1.02), test weight (0.96) and percent of chaffyness (0.58) contributed very less to the genetic diversity this showed that the mutants possessed unique features for list of the traits studied.

5.5 Salinity Screening

Breeding for salinity tolerance of two staple crops in the world including rice and wheat is an important goal, especially for FAO. Evaluations for the effects of salinity stress on rice yield have an important necessity for rice breeding. It is now realized that sustainability as well as productivity could be essential for rice breeding. Stress indices have been used for screening stress-tolerant genotypes. These indices could measure stress intensity based on yield loss under stress conditions in comparison to normal. Such indices are either based on stress resistance or susceptibility of genotypes.

Fischer and Maurer (1978) suggested the stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments, which can be used to identify genotypes that produce high yield under both stressed and non-stressed conditions. Guttieri *et al.* (2001)

suggested that genotypes with larger SSI values had susceptibility to salinity stress in rice genotypes. SSI indices and seed yield were used as stability parameters for identification of salinity resistant genotypes in rice. The present studies were to evaluate best salinity tolerance in 400 rice mutant lines and effective screen for identification of salt tolerance lines in rice.

The line No. 4, 41, 46 and 60 of BPT-5204 (30 Kr) are higher in total number of tiller per plants, number of productive tillers per plant, plant height, grain yield per panicle, number of filled grains per panicle, test weight and zero mortality percentage and also tolerance to the salinity due to the lesser SSI value. These results are lines with the finding of Natarajan *et al.* (2005), Ali *et al*. (2004), Bhowmik *et al*. (2009) and Nakhoda *et al.* (2012)**.**

The mutants lines are higher in total number of tiller per plants, number of productive tillers per plant, plant height, grain yield per panicle, number of filled grains per panicle and test weight in the line no. 103, 110, 114, 136, 138, 160, 172, 178, 183, 186, 194 and 199 of BPT-5204 (40 Kr). These lines are salt tolerant due to the lesser SSI value and zero mortality percentage. This is in accordance with finding Natarajan *et al*. (2005), Siddiqui (2014) and Farshadfar *et al*. (2014).

The line No. 209, 253, 254, 281 and 283 of RP-BIO 226 (30 Kr) are higher in total number of tillers per plants, number of productive tillers per plant, plant height, grain yield per panicle, number of filled grains per panicle, test weight and zero mortality percentage and also tolerance to the salinity due to the lesser SSI value. These results are lines with the finding of Natarajan *et al.* (2005 (1), Ali *et al*. (2004) Bhowmik *et al*. (2009), Nakhoda *et al.* (2012) and Islam and Karim (2010)**.**

The mutants lines are higher in total number of tiller per plants, number of productive tillers per plant, plant height, grain yield per panicle, number of filled grains per panicle and test weight in the line No. 353, 358 and 398 of RP-Bio 226 (40 Kr). These lines are salt tolerant due to the lesser SSI value and zero mortality percentage. This is in accordance with finding Natarajan *et al*. (2005) and Hosseini *et al.* (2012)

5.5.1. Screening for disease.

Bacterial leaf blight (BLB), a major bacterial disease of rice (*Oryza sativa* L.) caused by *Xanthomonas oryzae* pv *oryzae (Xoo)*, is found in most irrigated, rainfed and

deep water temperate and tropical rice growing areas. Among the rice mutant lines maximum disease indence was occurred in line No. 58 (59.12 %) of BPT-5204 (30 Kr), line No.196 (49 %) of BPT-5204 (40 Kr). The line No. 217 (38 %) of RP-Bio 226(30 Kr), line No. 351(29%) of RP-Bio 226(40Kr) similar result observed by Thimmegowda (2006). The minimum disease incidence was observed in the line No. 79 (22.22 %) of BPT-5204(30Kr), line No. 175 (13.33 %), of BPT-5204 (40 Kr). The line No. 293(11 %) of RP-BIO 226(30Kr) and the line No. 363(10.00%) of RP-Bio 226 (40 Kr). The disease incidence level is minimum in RP-Bio 226 (30 and 40 Kr) compared to BPT-5204 (30 and 40 Kr) because of the parent RP-Bio 226 has three resistance genes. Similar result observed in Sundravadana *et al.* (2007) and Shivalingaiah and Umesha (2011).

5.6 Molecular diversity studies based on SSR markers

5.6.1 Genomic DNA Isolation

Leaf samples from 45 rice mutant accessions at 24 days after sowing were collected. DNA was isolated from all 45 genotypes using C-TAB method, at molecular laboratory, college of Agriculture, Raichur, The quantity of DNA ranged from a minimum of 297.4 ngµl^{-1} to 897.9 ngµl^{-1}. The average quantity of DNA obtained was 531.38 ngµl^{-1}. The absorbance ratio of 230/260 depicts the quality of the DNA. The average 230/260 ratio observed was 1.04 ranging from a minimum of 0.92 to a maximum of 1.18 indicating a reasonably good quality of the extracted DNA.

5.6.2 Selection of rice genotypes for diversity analysis

The present study involved a total of 45 rice mutant lines for molecular profiling; These 45 rice mutant lines included check varieties *viz.*, Pokkali, BPT-5204, RP-Bio 226, IR29, FL28 and CSR-22.

5.6.3 Molecular characterization and allelic variability among selected rice genotypes

Diversity study based on morphological characters is time consuming and requires extensive field trials and evaluation while morphological differences may be epigenetic or genetic based characters. During last three decades genetic diversity was studied in plants through morphological markers, isoenzymes and molecular markers. The development of molecular (DNA) marker provides new dimension, accuracy and perfection in the screening of germplasm (Taran *et al*., 2005).

DNA based molecular markers developed during the last two decades of molecular biology research has been utilized for various applications in the area of plant genome analysis. They have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering etc. In the last few years, microsatellites have become one of the most popular molecular markers used with application in many fields. High polymorphism and the relative ease of scoring represent the two major features that make microsatellite of large interest for many genetic studies.

5.6.4 Clustering of rice genotypes

Dendrogram was constructed from the genetic similarity matrix using **N**umerical **T**axonomy **Sys**tem NTSYSpc-2.02j software. Sequential, agglomerative, hierarchical, and nested clustering (SAHN) was used for building the cluster tree. The SSR markers were successful in revealing the presence of four broad groups among the mutant lines. This dendrogram revealed that the genotypes derived from a genetically similar type clustered together (Fig. 7).

Similarity coefficient for similarity index was ranged from 0.10 to 0.99. The lowest genetic diversity was observed RP-BIO 226 and Pokkali checks (0.10) and the highest genetic diversity was observed between line No. 54 of BPT-5204 (40 Kr) and Line No. 1 of BPT-5204 (40 Kr). Highest range of genetic diversity is observed among high dose of gamma radiation. Similar results were obtained by Nantawan *et al*. (2008) and Seetharam *et al*. (2008).

These results indicated there is a narrow genetic base of above rice mutants, which are from same sources except the difference in Xa genes. In BPT-5204 (30 Kr) line No. 91 (Cluster XI) and Cluster II), were from local check showed wider genetic diversity. Similarly (Cluster VIII) and (Cluster XII) showed wider genetic diversity.

The SSR marker data were able to differentiate the mutants into separate clusters; this elucidated the potentiality of SSR markers for the characterization of mutant lines. SSR marker data also revealed that the geographical diversity and genetic diversity are not related.

The twelve clusters are formed through the SSR data revealed that the presence of genetic diversity at molecular level was high among the selected mutant lines which

suggests the genetic diversity more than the morphological diversity giving scope to utilize the material in breeding programme based on their cluster characteristics. Similar results were obtained by Sajida Bibi *et al*. (2009), Muhammad *et al*. (2005) and Nantawan *et al*. (2008).

5.7 Future line of work

- The desired mutant line for yield and earliness selected in M_4 generation from BPT-5204 (30 Kr and 40 Kr) and RP-Bio 226 (30 Kr and 40 Kr) will be advanced to M_5 generation for further evaluation and to be used in further breeding programme.
- The selected saline tolerant M_4 mutant lines also confirmed by trait specific saline tolerant SSR markers indicating phenotypic and genotypic correlation, those lines would be further evaluated with local check like CSR-22 for their performance in next season.
- Identification of salt tolerant genes using SSR markers by QTL mapping.

Summary and Conclusions

VI. SUMMARY AND CONCLUSIONS

Rice is the world's most important food crop and a primary source of food for more than half the world's population. As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is diploid and self-pollinated important and extensively grown food crop ranking at second in area and production in world (Anon., 2013) placed after wheat. India has largest area under paddy in the world and ranks second in the production after China. Genetic modification to some extent paved the way for the success in quality improvement through conventional breeding methods.

- In the present investigation, the materials BPT-5204(30Kr and 40Kr) and RP-Bio 226(30Kr and 40Kr) used in this study comprised of M_4 generation of rice with 400 mutants from both in normal soil and saline soil which were collected from Agricultural Research Station, Gangavathi, during *kharif* 2013. The objectives were to assess the relative performance, estimation of genetic variability, heritability and genetic advance, genetic divergence, salinity screening and molecular diversity among the selected rice mutants for grain yield and important yield contributing traits and identify promising mutants with earliness, salinity tolerance for future breeding programme. Results obtained in the present investigation are summarized below.
- In normal soil the analysis of variance showed highly significant differences among the rice mutants for all the parameters except number of productive tillers per plant, number of grain per panicle, number of filled grains per panicle, percent chaffyness and test weight and in saline soil condition analysis of variance showed highly significant differences among the rice mutants for few characters *viz.* Plant height, panicle length, biological yield and test weight in M_4 generation.
- The rice mutants exhibited high variability for the characters like biological yield (106.27 %, 106.25 %), grain yield per panicle (37.57 %, 24.93 %), number of grains per panicle (27.62 %, 27.61 %), number of filled grains per panicle (31.61 %, 31.59 %), percent chaffyness (115.19 %, 70.62 %) and test weight (28.59 %, 25.09 %) in normal soil condition(PCV, GCV), whereas, in the saline soil condition number of productive tiller per plant (25.85 %, 22.05 %), biological yield (34.28%, 34.17%), grain yield per panicle (31.26 %, 27.56 %), number of grains per panicle (28.39 %, 28.38 %), percent chaffyness (59.14 %, 48.14 %) and test weight (42.81 %, 41.1

2%). PCV and GCV were found to be differing very narrowly indicating lesser influence of environment over these characters.

- The characters *viz.*, biological yield (99 %), number of grains per panicle (99 %), number of filled grains per panicle (99 %) and plant height (98 %) in the normal soil and biological yield (99 %), number of grains per panicle (99 %), number of filled grains per panicle (99 %), plant height (98 %) and number of productive tiller per plant (72 %) in saline soil condition exhibited high heritability coupled with high genetic advance indicating that simple selection scheme would be sufficient for these traits to bring genetic improvement in desired direction.
- In normal soil condition grain yield had positive and highly significant association with Harvest index, number of grains per panicle, number of filled grains per panicle and in saline soil grain yield has strong association with biological yield, number of filled grains per panicle and number of grains per panicle which revealed that selection based on these traits would ultimately improve grain yield.
- Path coefficient analysis revealed that harvest index, biological yield, number of productive tillers and number of grains per panicle in normal soil condition similarly in saline soil number of filled grains per panicle, harvest index, biological yield and total number of tiller exhibited direct positive effect on grain yield. Hence, it would be rewarding to lay stress on these characters in selection programme for increasing yield.
- Based on the stress susceptibility index it is indicated that line no. 4, 41, 46 and 60 of BPT-5204 (30Kr), the line No. 103, 110, 114, 136, 138, 160, 172, 178, 183, 186, 194 and 199 of BPT-5204 (40 Kr), line No. 209, 253, 254, 281 and 283 of RP-Bio 226 (30 Kr), line No. 353, 358 and 398 of RP-Bio 226 (40 Kr) were the best lines among all evaluated mutant lines. Stress susceptibility index and Mortality percentage can be used for selecting the salt tolerant rice genotypes. Overall, we can use these lines for improving rice cultivars to salt stress condition in future breeding programs.
- From clustering pattern of Tocher method it is understood that genetic diversity among the mutants are very narrow, characters ranking indicated that number of grains per panicle had high contribution out of 12 characters that are included for analysis, followed by number of filled grains per panicle, biological yield, plant height, days to 50 per cent flowering, grain yield per panicle, harvest index, panicle length, total number of tillers per plant, number of productive tillers per plant, test

weight and percent chaffyness in the normal soil condition. Similarly, in the saline soil, number of grains per panicle had high contribution out of 12 characters that are included for analysis, followed by number of filled grains per panicle, biological yield per plant, plant height, grain yield per panicle, number of productive tillers per plant, harvest index, days to 50 per cent flowering, total number of tillers per plant, panicle length, test weight and percent chaffyness.

- Genetic diversity at molecular level was estimated by using SSR markers. SSR profiles for selected 45 rice mutant lines were generated with 16 random decamer primers. The level of polymorphism generated (96 %) among the mutants was very high. The results obtained based on the analysis of 45 rice mutants using the polymorphic SSR. The number of alleles varied widely among the selected loci. A total of 24 alleles were observed by screening 45 mutants with 16 SSR markers in 26 alleles out of which 25 were polymorphic and used to generate marker profile. The number of alleles ranged from 1 (AP3206) to 3 (RM10793) with an average allele of 2.08.
- SSR markers profile resulted in 12 clusters are VIII cluster count of 10 mutant lines followed by V cluster had 09 mutant lines and IX, X cluster had 05 mutant lines each, the formation of 12 clusters through SSR data revealed that presence of genetic diversity at molecular level was high among selected genotypes. The polymorphism observed in SSR markers among rice mutants showed that effectiveness of this method in determining genetic variations.

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VI. REFERENCES

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APPENDICES

APPENDIX – I

Grain size of M_4 mutant population

BPT 30				BPT 40			RPBIO 30			RPBIO 40		
line no	length	l/b ratio	Remarks	length	l/b ratio	Remarks	length	l/b ratio	Remarks	length	l/b ratio	Remarks
B C	7.52	4.70	LS	7.46	4.91	LS	7.82	3.52	LS	7.4	3.74	LS
R C	7.88	4.78	LS	8.17	4.64	LS	7.41	4.60	LS	6.92	4.17	LS
1	7.95	3.82	LS	7.6	4.61	LS	7.58	4.59	LS	7.6	5.76	LS
2	7.18	4.40	LS	8.81	4.38	LS	7.76	3.86	LS	8.57	3.97	LS
3	8.29	4.88	LS	9.85	4.69	LS	7	3.54	LS	7.83	4.72	LS
4	8.12	4.34	LS	8.1	3.58	LS	7.39	4.77	LS	7.56	4.06	LS
5	7.9	3.67	LS	9	4.23	LS	7.93	3.83	LS	7.59	4.49	LS
6	7.95	3.75	LS	9.41	4.71	LS	8.52	4.79	LS	7.44	3.54	LS
7	8.18	2.85	LB	9.79	4.75	LS	7.65	4.55	LS	7.61	3.57	LS
8	8.4	4.67	LS	8.14	4.47	LS	7.87	4.66	LS	7.34	4.08	LS
9	8.97	5.50	LS	7.31	3.99	LS	7.6	4.66	LS	8.05	3.16	LS
10	8.19	5.06	LS	7.74	4.58	LS	7.74	4.69	LS	7.19	3.38	LS
11	7.5	4.08	LS	7.37	3.22	LS	7.53	4.65	LS	7.26	3.46	LS
12	8.05	3.35	LS	7.41	3.74	LS	7.21	4.74	LS	7.66	3.74	LS
13	7.8	3.71	LS	9.35	3.91	LS	7.83	4.83	LS	8.83	3.81	LS
14	7.3	4.01	LS	7.8	4.73	LS	7.43	4.61	LS	7.85	4.36	LS
15	7.3	4.48	LS	8.6	5.03	LS	7.43	4.73	LS	8.43	5.08	LS
16	7.56	4.45	LS	6.71	4.07	LS	7.54	4.44	LS	8.1	3.95	LS
17	7.7	3.85	LS	8.5	3.65	LS	7.46	4.19	LS	8.39	4.49	LS
18	5.4	2.04	SB	9.24	4.71	LS	7.46	4.58	LS	8.51	5.10	LS
19	7.3	2.39	LB	9.71	4.98	LS	7.36	3.66	LS	8.05	3.64	LS
20	9.47	4.78	LS	8.82	4.57	LS	7.34	3.71	LS	8.18	4.13	LS

21	7.08	3.89	LS	8.8	4.51	LS	7.84	3.84	LS	8.25	4.56	LS
22	7.23	4.28	LS	8.45	3.38	LS	7.65	4.23	LS	8.23	3.92	LS
23	7.8	4.38	LS	8.3	5.53	LS	7.8	4.81	LS	7.26	3.46	LS
24	7.92	4.58	LS	8.15	3.79	LS	7.62	3.56	LS	8.11	4.51	LS
25	8	2.86	LB	8.19	3.04	LS	8.21	4.66	LS	7.81	4.36	LS
26	8.50	4.11	LS	7.9	3.78	LS	7.84	2.81	LB	8.2	4.10	LS
27	7.51	3.66	LS	7.7	5.07	LS	7.94	4.84	LS	7.1	4.44	LS
28	7.7	2.85	LB	7.4	3.30	LS	7.37	4.49	LS	7.35	3.33	LS
29	3.9	3.00	SS	7.49	4.46	LS	7.73	3.09	LS	7.98	4.61	LS
30	7.92	2.97	LB	8.89	4.91	LS	7.38	4.99	LS	8.05	4.38	LS
31	8.7	4.46	LS	7.79	4.50	LS	8.11	5.01	LS	7.39	4.25	LS
32	8.11	4.74	LS	7.86	3.73	LS	7.56	4.91	LS	7.88	3.79	LS
33	7.6	4.66	LS	7.04	4.27	LS	7.81	4.82	LS	8.42	4.63	LS
34	7.6	4.55	LS	7.53	4.80	LS	8.14	5.02	LS	8.5	4.15	LS
35	8.7	4.83	LS	8.09	4.82	LS	7.31	5.30	LS	8.21	4.15	LS
36	4.7	4.70	SS	8.19	4.73	LS	7.03	4.34	LS	7.74	3.29	LS
37	6.5	2.32	LB	7.5	4.57	LS	7.7	4.56	LS	7.94	2.78	LB
38	8.33	1.00	LB	8.13	4.57	LS	7.1	4.47	LS	7.82	4.32	LS
39	8.4	4.40	LS	7.72	4.77	LS	7.35	5.57	LS	7.27	3.30	LS
40	6.4	3.95	LS	9.43	5.27	LS	7.4	4.38	LS	7.98	3.47	LS
41	7.66	4.45	LS	7.9	5.27	LS	7.69	3.59	LS	7.45	3.76	LS
42	6.7	1.46	LB	9.1	4.84	LS	7.59	4.31	LS	8.3	4.88	LS
43	7.65	4.72	LS	8.23	4.40	LS	7.69	4.58	LS	7.6	4.55	LS
44	7.2	3.79	LS	8.04	4.62	LS	7.12	4.45	LS	7.21	3.43	LS
45	7.5	4.69	LS	7.44	3.46	LS	7.92	4.60	LS	7.83	4.63	LS
46	8.2	3.73	LS	7.59	3.91	LS	7.98	4.81	LS	8.74	3.64	LS
47	7.6	3.62	LS	9.2	3.97	LS	7.18	3.26	LS	7.56	4.73	LS
48	7.54	3.34	LS	8.89	4.97	LS	7.95	5.00	LS	7.32	3.85	LS

49	9.2	4.53	LS	9.8	4.56	LS	8.26	4.17	LS	7.61	4.48	LS
50	7.63	4.39	LS	8.52	4.92	LS	6.89	3.43	LS	7.51	5.78	LS
51	7.32	3.18	LS	9.08	4.54	LS	7.74	4.63	LS	7.43	5.67	LS
52	7.4	4.46	LS	6.18	3.79	B’S	7.21	4.65	LS	7.1	3.26	LS
53	3.6	2.40	SS	10.5	5.20	LS	7.5	5.68	LS	8.2	3.90	LS
54	8.68	4.72	LS	7.45	4.26	LS	7.7	3.74	LS	9.18	4.64	LS
55	7.61	3.52	LS	7.5	4.69	LS	8.02	4.20	LS	9.05	3.74	LS
56	8.88	5.62	LS	7.82	3.83	LS	7.86	3.71	LS	9.18	5.34	LS
57	7.4	2.75	LB	7.55	3.39	LS	7.79	4.75	LS	3.63	1.70	SS
58	7.8	4.84	LS	8.23	3.86	LS	7.67	3.67	LS	8.21	4.83	LS
59	5.5	3.06	SS	8	4.62	LS	7.59	3.78	LS	7.93	3.52	LS
60	7.57	3.44	LS	7.9	4.73	LS	7.28	4.73	LS	9.2	5.14	LS
61	8.12	4.78	LS	7.32	3.34	LS	7.08	4.48	LS	6.96	2.86	LB
62	7.79	4.50	LS	7.06	3.31	LS	8.4	4.94	LS	7.61	3.44	LS
63	7.92	4.89	LS	8.03	4.70	LS	8.3	5.19	LS	8.28	4.93	LS
64	7.13	4.60	LS	7.31	4.72	LS	7.56	4.85	LS	7.69	4.69	LS
65	6.9	5.31	LS	8.39	4.69	LS	8.55	3.98	LS	7.48	3.56	LS
66	3.4	3.40	SS	7.57	3.60	LS	7.41	4.72	LS	8.09	4.76	LS
67	7.6	4.69	LS	7.89	3.49	LS	8.5	5.06	LS	7.65	4.30	LS
68	7.58	3.46	LS	7.14	4.43	LS	8.1	4.48	LS	7.98	3.80	LS
69	8.25	4.06	LS	7.89	3.49	LS	7.6	3.42	LS	7.15	3.39	LS
70	8.45	4.83	LS	8.15	4.79	LS	7.43	3.62	LS	8.09	3.61	LS
71	8.35	3.87	LS	7.94	4.64	LS	8.32	4.92	LS	9.13	5.10	LS
72	8.12	4.78	LS	6.6	4.29	LS	8.14	4.96	LS	7.98	3.52	LS
73	7.4	4.30	LS	7.86	3.46	LS	9.19	3.52	LS	7.53	5.02	LS
74	7.9	2.93	LB	8.4	5.06	LS	7.73	3.90	LS	8.7	4.97	LS
75	8.06	3.22	LS	7.61	3.11	LS	8.25	3.82	LS	8.21	3.62	LS
76	8.12	4.83	LS	8.63	5.05	LS	7.07	4.59	LS	8.3	4.80	LS

77	7.4	5.00	LS	7.54	4.51	LS	5.5	3.40	LS	6.91	4.32	LS
78	7.68	2.88	LB	7.92	4.06	LS	7.8	4.73	LS	7.78	2.99	LB
79	8.12	3.69	LS	8.98	5.02	LS	8	3.70	LS	7.89	4.41	LS
80	7.9	3.73	LS	7.69	4.45	LS	7.9	3.02	LS	7.75	3.78	LS
81	7.23	2.49	LB	7.41	3.35	LS	7.32	3.59	LS	8.01	4.47	LS
82	6.9	2.46	LS	8.74	4.91	LS	7.58	4.71	LS	7.56	3.42	LS
83	8.45	4.83	LS	7.3	4.59	LS	7.12	2.55	LB	8.29	4.71	LS
84	7.2	3.13	LS	7.8	4.19	LS	7.73	4.71	LS	8.1	4.50	LS
85	5.1	1.89	SB	7.31	4.06	LS	7.9	4.44	LS	7.9	4.22	LS
86	8.6	3.28	LS	8.35	3.73	LS	8.43	4.66	LS	8.85	4.47	LS
87	7.3	2.58	LB	7.61	4.61	LS	8.5	4.07	LS	7.34	4.22	LS
88	7.95	3.43	LS	8.17	4.89	LS	7.02	3.38	LS	7.29	4.10	LS
89	8.23	3.06	LS	7.68	4.47	LS	7.6	3.74	LS	7.01	4.12	LS
90	9.1	3.27	LS	7.94	4.14	LS	8.42	3.74	LS	9.21	5.12	LS
91	7.95	2.94	LB	8.24	4.20	LS	8.32	4.22	LS	7.81	3.57	LS
92	7.3	3.48	LS	7.66	3.93	LS	9.59	5.24	LS	7.8	5.06	LS
93	8.28	4.31	LS	8.37	3.95	LS	7.9	6.32	LS	7.87	2.81	LB
94	7.8	4.76	LS	8.33	4.17	LS	7.83	3.42	LS	8.29	3.79	LS
95	7.6	4.27	LS	7.23	4.58	LS	8.45	4.38	LS	8.61	3.91	LS
96	7.8	4.04	LS	7.95	3.82	LS	7.9	4.41	LS	7.98	4.69	LS
97	7.89	4.64	LS	7.48	4.65	LS	7.53	4.16	LS	8.16	4.80	LS
98	7.67	4.26	LS	7.49	4.38	LS	9.53	3.81	LS	7.87	4.37	LS
99	7.98	3.66	LS	7.86	5.07	LS	6.99	3.05	LS	7.55	3.68	LS
100	6.9	3.00	LS	6.6	3.88	LS	7.19	2.93	LS	7.29	4.37	LS

LB = Long Bold, SS = Short Slender, SB = Short Bold, LS = Long Slender

APPENDIX – II

Preparation of stocks for CTAB method of DNA Extraction

1 M Tris base (pH 8.0)

Tris base 121.1g/lt. --- 1M

DDW up to 1000 ml

0.5 M EDTA (pH 8.0)

372.24g/lt ------ 1M

4 M NaCl

58.44g/lt ------ 1M

TE Buffer (T10, E1, pH 8.0)

Tris base – 0.121 g/100 ml

EDTA - 0.0372 g/100 ml

50X TAE (for 250 ml)

Tris base – 60.5 g

Acetic acid glacial - 14.25 ml

0.5 M EDTA - 25 ml (pH 8.0)

Volume made up to 250 ml with DDW

PH adjusted to 8.0 with conc. HCl. Autoclaved and stored at room temperature.

Chloroform: Iso amyl alcohol (24:1) (v/v)

70 % Ethanol

Extraction Buffer for CTAB (100 ml)

10% CTAB - 20 ml

1M Tris base - 10 ml

4M NaCl - 35 ml

Sterile Water - make up to 100 ml

Autoclave and add

Mercapto ethanol - 1 ml

PVP - 0.1% (100 mg) 128

 DNA dilution:

N1V1 = N2V2

Volume made up-100 µl

Conc = 10 ng/µl

N 1 = DNA nucleic acid conc (10 ng/µl)

V1 = ?

N 2 = 10 ng/µl

V2 = volume needed.

V1 = N2×V2/N1

 Loading / Tracking dye (10X BPB) - 1 ml

Source 60.7 mg

Bromophenol blue 4.2 mg

ddW up to 1000 ml

Store at 4 °C

 Ethidium Bromide (10 mg/ ml) – 1 ml

Ethidium bromide 10.0 mg

Distilled water 1000 ml

Dissolve, wrap with Aluminium foil and store at 4 °C

Dilute to ~500 ng/L with dW for Staining

Use appropriate molecular weight marker

APPENDIX – III

List of mutants used in Molecular diversity

Sl. No.	Mutant dose	Mutant No.	Particulars
1	BPT-5204 (30 Kr)	1	Tolerant
2	BPT-5204 (30 Kr)	78	Tolerant
3	BPT-5204 (30 Kr)	63	Tolerant
4	BPT-5204 (30 Kr)	71	Susceptible
5	BPT-5204 (30 Kr)	38	Tolerant
6	BPT-5204 (30 Kr)	97	Moderately tolerant
7	BPT-5204 (30 Kr)	4	Tolerant
8	BPT-5204 (30 Kr)	14	Moderately tolerant
9	BPT-5204 (30 Kr)	91	Tolerant
10	BPT-5204 (40 Kr)	173	Tolerant
11	BPT-5204 (40 Kr)	191	Tolerant
12	BPT-5204 (40 Kr)	179	Tolerant
13	BPT-5204 (40 Kr)	165	Susceptible
14	BPT-5204 (40 Kr)	154	Tolerant
15	BPT-5204 (40 Kr)	133	Tolerant
16	BPT-5204 (40 Kr)	131	Moderately tolerant
17	RPBIO-226 (30 Kr)	276	Tolerant
18	RPBIO-226 (30 Kr)	277	Tolerant
19	RPBIO-226 (30 Kr)	280	Susceptible
20	RPBIO-226 (30 Kr)	286	Tolerant
21	RPBIO-226 (30 Kr)	230	Tolerant
22	RPBIO-226 (30 Kr)	233	Moderately tolerant
23	RPBIO-226 (30 Kr)	246	Susceptible
24	RPBIO-226 (30 Kr)	261	Tolerant
25	RPBIO-226 (30 Kr)	265	Moderately tolerant
26	RPBIO-226 (30 Kr)	278	Susceptible
27	RPBIO-226 (30 Kr)	294	Tolerant
28	RPBIO-226 (30 Kr)	283	Susceptible
29	RPBIO-226 (40 Kr)	373	Tolerant
30	RPBIO-226 (40 Kr)	358	Moderately tolerant
31	RPBIO-226 (40 Kr)	303	Tolerant
32	RPBIO-226 (40 Kr)	378	Susceptible
33	RPBIO-226 (40 Kr)	375	Tolerant
34	RPBIO-226 (40 Kr)	345	Tolerant
35	RPBIO-226 (40 Kr)	341	Tolerant
36	RPBIO-226 (40 Kr)	306	Moderately tolerant
37	RPBIO-226 (40 Kr)	387	Tolerant
38	RPBIO-226 (40 Kr)	393	Tolerant
39	RPBIO-226 (40 Kr)	382	Susceptible
40	BPT-5204 Parent	Check	Susceptible
41	RPBIO-226 Parent	Check	Susceptible
42	Pokkali	Check	Tolerant
43	IR- 29	Check	Susceptible
44	FL 28	Check	Moderately tolerant
45	CSR- 22	Check	Moderately tolerant

APPENDIX – IV

List of SSR primers used for molecular diversity analysis

Sl No.	Primer name	Forward sequence	Reverse sequence
1	RM3412	TGATGGATCTCTGAGGTGTAAAGAGC	TGCACTAATCTTTCTGCCACAGC
2	RM8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
3	AP3206	TTCTCATCGCACCATCTCTG	GGAGGAGGAGAGGAAGAAG
4	RM152	CCAAGGGAAAGATGCGACAATTG	GTGGACGCTTTATATTATGGG
5	RM7075	TATGGACTGGAGCAAACCTC	GGCACAGCACCAATGTCTC
6	RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
7	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
8	RM10793	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC
9	RM556	ACTCCAAACCTCACTGCACC	TAGCACACTGAACAGCTGGC
10	RM562	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
11	RM10772	GCACACCATGCAAATCAATGC	CAGAAACCTCATCTCCACCTTCC
12	RM 9	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
13	RM 336	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTTCAGGTTCG
14	RM 8053	AGACATTGCCGATGATAGG	AAGTACCCCACCGAATAGAG
15	RM23	CATTGGAGTGGAGGCTGG	GTCAGGCTTCTGCCATTCTC

APPENDIX – V

Stress Susceptibility Index (%) and Mortality percentage of M_4 mutant population

Line No.	Stress Susceptibility Index (%)						M (%)	BLB
	TNT	NPT	PH	GY	TW	NFG		
1	-6.27	1.79	-11.84	-3.13	19.59	-1.71	8.00	45.00
2	-3.23	0.96	-8.40	-4.49	29.10	-1.01	4.00	35.00
3	11.37	3.59	-2.43	-5.84	5.59	-2.50	0.00	44.44
4	-0.46	-0.28	2.21	-2.29	-6.14	3.20	0.00	35.00
5	5.27	0.87	4.80	0.65	-39.15	-4.86	0.00	44.44
6	3.09	0.80	-1.79	-3.71	42.11	-0.48	12.00	43.00
7	-16.73	-2.71	4.99	-1.11	-1.47	12.25	4.00	24.44
8	-13.99	-4.87	7.98	3.12	-33.38	-2.69	4.00	33.33
9	2.03	0.00	5.31	2.61	-70.25	0.02	8.00	48.88
10	-5.04	-1.52	8.42	3.48	-9.41	1.08	8.00	40.00
11	-5.56	-2.19	4.54	-2.63	27.32	1.23	4.00	49.00
12	2.68	0.96	7.24	6.26	-111.92	4.51	0.00	33.33
13	6.39	1.34	1.35	1.70	-97.46	27.13	4.00	48.00
14	-5.13	-1.28	13.90	-1.42	34.58	14.44	4.00	49.00
15	-2.90	-1.48	3.81	2.21	-2.73	1.47	8.00	33.33
16	11.81	3.17	-0.51	4.35	15.98	6.35	8.00	31.11
17	0.00	-0.49	7.61	7.30	-37.90	-1.32	8.00	44.00
18	-18.74	-4.23	14.06	7.51	0.00	14.35	16.00	40.00
19	0.63	0.70	16.26	-0.17	24.02	-0.72	0.00	44.00
20	5.67	0.13	8.16	0.22	-34.17	-5.83	0.00	41.00
21	5.87	1.42	2.59	1.30	-88.16	-2.45	4.00	37.77
22	-6.16	-1.36	15.57	-0.55	22.70	0.65	44.00	45.00
23	6.27	2.07	-2.52	-2.16	16.66	-3.72	44.00	46.66
24	2.41	0.52	-0.66	-5.12	12.30	-5.38	8.00	49.00
25	-8.62	-2.22	6.26	1.94	-9.82	-0.28	0.00	49.00
26	8.79	2.20	6.84	8.73	-49.03	2.33	0.00	35.55
27	-9.61	-1.30	7.37	-4.16	18.00	-4.50	8.00	49.00
28	-10.08	-3.08	4.55	6.52	-31.11	-15.69	0.00	45.00
29	-4.16	-0.16	8.34	1.01	22.96	1.28	8.00	48.00
30	-10.73	-0.63	5.37	1.20	-18.93	-3.37	8.00	47.00
31	-5.09	0.45	1.04	-0.85	32.11	-8.65	12.00	28.89
32	0.54	0.91	0.00	-3.68	43.83	-3.71	12.00	48.88
33	-24.94	-6.20	4.22	9.71	25.80	1.49	8.00	47.00
34	-22.71	-3.83	1.27	-0.04	50.52	0.40	4.00	55.00
35	-6.09	1.89	5.56	3.33	48.39	11.40	12.00	50.00
36	-7.47	-1.54	-4.30	1.34	46.86	35.32	0.00	45.00

37	-1.85	0.99	-4.03	-3.07	20.66	-0.28	4.00	46.66
38	-4.31	-0.40	9.98	2.13	-26.77	-8.06	8.00	46.66
39	-10.28	-1.28	22.58	0.00	46.54	8.98	0.00	43.00
40	0.48	0.95	5.63	-1.30	39.36	3.07	4.00	49.00
41	-13.06	-4.13	-2.34	-0.60	-4.03	-4.70	0.00	31.11
42	-7.50	-0.41	-0.12	-1.71	-27.59	-6.34	12.00	48.88
43	-0.76	0.63	-2.45	-1.32	29.29	15.07	8.00	41.00
44	9.01	3.23	-7.30	-2.31	-21.32	1.49	4.00	39.00
45	10.19	2.96	6.92	-1.42	-8.78	-3.28	12.00	49.00
46	-5.95	-1.03	2.69	2.10	0.08	3.34	0.00	49.00
47	-7.44	-1.16	-0.72	0.25	-44.37	0.69	8.00	49.00
48	2.83	0.99	-7.19	2.92	-18.55	-1.97	0.00	41.00
49	4.85	1.36	0.92	-3.31	8.03	0.68	0.00	47.00
50	7.52	3.20	1.98	7.03	-81.20	-0.85	8.00	35.00
51	-2.30	-0.36	-1.35	-1.01	5.82	-0.35	4.00	45.00
52	-5.84	-0.77	-7.63	-2.74	19.09	39.76	8.00	45.00
53	5.13	2.37	5.30	1.12	8.02	-1.37	4.00	49.00
54	8.72	2.65	-1.02	1.48	-51.99	3.65	16.00	50.00
55	12.37	3.45	1.24	1.75	7.38	-0.84	0.00	45.00
56	1.23	0.67	-2.17	4.71	-29.38	-0.23	8.00	49.00
57	0.56	1.98	9.62	13.51	-39.39	0.72	4.00	50.00
58	10.61	3.08	-0.26	4.12	-61.50	0.63	8.00	59.00
59	1.62	2.11	9.64	11.04	-48.40	0.99	0.00	49.00
60	1.01	1.04	-10.62	-0.28	-116.73	-1.28	0.00	41.00
61	2.57	1.23	6.50	6.88	11.47	9.16	8.00	44.00
62	1.84	1.60	1.63	-2.00	-81.93	-1.07	12.00	46.00
63	10.46	3.85	4.02	1.87	4.85	-1.73	20.00	46.00
64	0.00	2.31	8.47	2.48	9.06	-0.86	16.00	41.00
65	-3.13	0.45	10.68	3.34	-39.36	8.63	12.00	44.00
66	9.17	3.44	-6.56	-4.37	0.00	1.65	0.00	41.00
67	4.93	2.54	-2.45	-4.11	4.37	-4.45	4.00	48.00
68	6.11	2.91	-14.59	-2.88	18.93	-3.69	0.00	44.00
69	6.90	2.99	-1.65	2.44	-78.30	5.94	8.00	43.00
70	7.52	2.24	26.03	0.84	-13.47	5.59	16.00	43.00
71	5.82	2.06	15.14	6.22	-47.32	2.79	12.00	49.00
72	10.55	3.86	-13.81	-5.02	8.92	-5.21	16.00	48.00
73	-1.30	1.01	3.22	3.08	-7.66	2.48	12.00	42.00
74	3.39	2.04	12.22	12.14	-109.91	-4.11	8.00	37.77
75	5.83	3.08	-15.47	2.57	-41.34	0.10	0.00	45.00
76	7.29	2.52	-18.38	2.31	-18.17	-3.23	0.00	28.89
77	5.07	2.41	-3.59	9.54	-12.27	-1.50	8.00	46.66

78	2.57	1.41	-7.65	2.87	-36.50	-5.12	12.00	45.00
79	-4.86	-0.37	-1.62	8.47	-28.64	0.20	16.00	22.22
80	3.00	1.56	4.40	2.64	-38.62	1.49	8.00	48.88
81	-0.48	1.09	-2.46	-2.16	-25.37	-1.20	12.00	48.88
82	-6.22	-0.32	-0.24	4.16	17.49	0.38	8.00	45.00
83	-3.19	0.74	-3.68	4.47	6.67	-2.99	4.00	51.11
84	6.37	2.19	-2.18	1.53	47.72	17.93	16.00	33.33
85	-3.00	0.65	0.00	9.09	2.37	6.93	4.00	49.00
86	1.38	1.07	-1.46	5.27	35.31	4.30	0.00	35.55
87	6.09	2.30	-1.48	-1.77	23.64	-0.48	12.00	49.00
88	1.87	1.28	-0.16	-2.45	15.28	-0.39	8.00	48.00
89	0.00	2.35	-5.68	-3.75	13.50	0.29	8.00	40.00
90	5.47	1.82	2.26	0.45	-10.40	-4.76	12.00	37.77
91	3.10	2.22	-0.43	2.60	-1.17	-1.01	0.00	42.22
92	-2.11	1.21	-7.97	-0.80	30.77	0.07	8.00	40.00
93	8.87	3.23	-3.50	-0.89	34.91	4.17	4.00	37.77
94	8.81	3.82	-5.12	2.40	21.03	0.72	8.00	44.44
95	-1.25	1.30	-0.55	3.20	-4.45	-2.46	8.00	48.00
96	0.47	1.60	-7.25	-5.26	49.00	0.19	4.00	44.44
97	5.11	3.07	-0.86	9.50	-14.80	2.40	8.00	44.44
98	-2.52	0.35	-1.77	-1.58	23.64	-1.00	4.00	43.00
99	0.88	2.05	-2.32	11.11	4.45	2.34	4.00	40.00
100	4.36	1.60	-5.99	-5.19	40.12	5.32	8.00	37.77
101	0.00	1.08	-0.07	19.85	-0.92	0.73	0.00	40.00
102	0.00	-0.73	0.11	3.35	0.46	7.56	8.00	24.44
103	0.00	0.26	-0.09	9.22	0.75	3.96	0.00	40.00
104	0.00	-3.94	-0.07	3.00	3.38	1.22	4.00	40.00
105	0.00	-6.26	0.03	7.04	-0.39	2.31	8.00	28.89
106	0.00	2.17	-0.13	6.79	4.09	5.79	4.00	44.00
107	0.00	-8.40	-0.11	6.46	5.15	3.40	4.00	28.89
108	0.00	-3.31	-0.06	5.00	-0.73	2.20	16.00	44.44
109	0.00	-4.45	-0.01	4.15	0.24	0.52	4.00	44.44
110	0.00	-5.01	-0.01	3.92	0.38	0.78	0.00	40.00
111	0.00	-0.56	-0.01	2.19	2.18	-0.17	4.00	44.44
112	0.00	1.32	-0.10	-0.62	3.78	-0.61	4.00	31.11
113	0.00	0.70	-0.06	5.25	-2.12	0.17	4.00	35.55
114	0.00	1.88	-0.09	3.02	-5.46	-2.96	0.00	44.44
115	0.00	2.31	0.13	10.49	-0.54	1.47	4.00	48.88
116	0.00	0.18	-0.04	2.83	-0.18	-0.71	4.00	46.66
117	0.00	1.47	0.16	13.40	0.85	3.23	0.00	28.89
118	0.00	1.09	0.04	-0.97	-2.75	13.82	4.00	24.44

119	0.00	2.01	0.05	9.19	-0.71	-0.34	4.00	31.11
120	0.00	-5.49	0.04	2.89	-1.93	2.11	0.00	37.77
121	0.00	3.61	0.14	5.64	-8.66	1.08	4.00	28.89
122	0.00	4.00	0.09	3.85	-1.77	4.00	4.00	35.55
123	0.00	0.78	0.01	5.88	-1.17	-0.27	8.00	28.89
124	0.00	-0.60	0.04	1.22	-7.17	-0.21	4.00	35.55
125	0.00	-1.23	0.13	-2.82	-1.77	1.61	12.00	42.22
126	0.00	3.74	-0.09	-1.55	2.27	-1.24	4.00	40.00
127	0.00	0.92	0.05	-2.49	3.52	4.66	4.00	26.66
128	0.00	2.63	-0.04	-1.18	-10.91	-0.15	8.00	46.66
129	0.00	0.58	0.01	-1.88	-0.13	-1.30	0.00	28.89
130	0.00	2.75	0.08	0.22	3.77	9.01	4.00	22.22
131	0.00	4.37	-0.10	-0.53	-0.05	-1.03	8.00	35.55
132	0.00	0.91	-0.05	0.19	0.65	-1.01	4.00	28.89
133	0.00	-0.51	0.01	5.99	1.63	-1.99	12.00	28.89
134	0.00	-1.89	-0.07	-1.85	-0.07	-2.18	12.00	48.88
135	0.00	2.98	-0.05	-2.26	0.34	-0.32	4.00	40.00
136	0.00	-0.14	-0.04	2.83	-10.03	-1.81	0.00	44.44
137	0.00	0.98	0.10	-0.74	-2.74	-1.55	4.00	43.00
138	0.00	0.00	0.00	2.82	-0.59	-0.63	0.00	40.00
139	0.00	1.84	-0.02	-2.22	1.73	-1.04	16.00	45.00
140	0.00	0.40	-0.01	-2.66	0.20	-0.04	12.00	24.44
141	0.00	3.59	-0.02	-0.92	6.52	0.94	8.00	46.66
142	0.00	3.70	-0.08	1.47	2.47	3.34	8.00	40.00
143	0.00	3.70	0.05	-2.22	3.72	5.61	8.00	26.66
144	0.00	4.64	0.05	-0.29	2.71	-0.67	0.00	28.89
145	0.00	1.01	0.05	2.84	0.54	-0.46	0.00	31.11
146	0.00	1.06	0.15	0.00	2.49	21.87	12.00	37.77
147	0.00	-3.37	-0.06	2.71	1.83	1.46	8.00	28.89
148	0.00	1.07	0.01	-1.12	3.16	-0.31	0.00	31.11
149	0.00	1.75	0.18	8.13	4.04	5.10	4.00	28.89
150	0.00	2.27	-0.09	-4.59	2.91	4.24	8.00	44.44
151	0.00	1.97	-0.07	-4.27	2.61	-0.05	4.00	35.55
152	0.00	2.94	0.01	1.45	4.44	-1.83	8.00	20.00
153	0.00	3.07	0.03	5.19	5.53	9.62	8.00	35.55
154	0.00	-3.60	0.02	9.08	0.91	-0.35	4.00	26.66
155	0.00	1.38	0.06	-2.62	2.42	1.12	8.00	40.00
156	0.00	1.38	0.09	6.40	-1.38	-0.84	4.00	40.00
157	0.00	2.43	0.07	2.47	-2.60	-0.98	8.00	40.00
158	0.00	2.65	0.00	-3.29	1.98	0.71	4.00	43.00
159	0.00	1.50	0.02	-0.69	0.15	-0.46	0.00	31.11

160	0.00	1.91	-0.05	0.46	-0.46	0.49	0.00	31.11
161	0.00	1.42	-0.03	-3.44	0.85	1.07	4.00	40.00
162	0.00	2.84	-0.05	-4.44	0.29	-0.79	4.00	44.44
163	0.00	3.13	0.03	-3.39	1.60	0.42	0.00	28.89
164	0.00	1.01	0.03	-4.07	2.68	-0.38	4.00	35.55
165	0.00	1.84	0.14	-1.43	2.44	0.35	8.00	31.11
166	0.00	2.80	-0.01	-3.39	-1.13	0.71	4.00	35.55
167	0.00	0.00	0.06	6.63	0.90	2.61	4.00	37.77
168	0.00	-0.92	0.00	-3.36	3.84	0.41	4.00	31.11
169	0.00	-2.37	0.00	-2.01	2.71	1.13	0.00	43.00
170	0.00	1.80	-0.02	-1.23	4.16	1.04	4.00	31.11
171	0.00	-8.06	0.11	0.29	2.09	0.38	8.00	24.44
172	0.00	1.75	-0.02	-2.38	2.24	0.26	0.00	31.11
173	0.00	2.85	-0.01	-4.48	-0.21	-0.74	4.00	31.11
174	0.00	0.83	0.04	7.08	1.47	-0.83	24.00	33.33
175	0.00	1.37	0.02	-0.17	2.26	3.12	8.00	13.33
176	0.00	-0.36	0.19	2.72	4.67	0.48	8.00	35.55
177	0.00	0.14	-0.10	3.88	-13.82	-1.99	4.00	40.00
178	0.00	-1.93	0.08	-1.05	-0.43	2.22	0.00	26.66
179	0.00	-6.29	0.03	5.50	-5.27	-0.81	0.00	37.77
180	0.00	-0.97	0.06	-4.64	-8.55	-3.71	12.00	20.00
181	0.00	2.30	-0.03	-3.25	2.28	-0.07	4.00	17.78
182	0.00	3.56	-0.01	3.12	-3.02	1.12	4.00	20.00
183	0.00	2.06	-0.01	6.03	0.64	2.11	0.00	28.89
184	0.00	2.01	0.01	4.80	-2.31	0.56	0.00	24.44
185	0.00	0.97	-0.01	-0.71	2.49	5.07	4.00	46.66
186	0.00	0.00	-0.05	-3.80	3.40	0.10	0.00	24.44
187	0.00	1.36	-0.09	-5.65	-0.19	-0.14	4.00	26.66
188	0.00	1.07	-0.01	-1.13	3.14	0.85	0.00	28.89
189	0.00	1.42	-0.01	4.79	1.56	-1.27	16.00	20.00
190	0.00	3.61	-0.02	-1.34	0.54	1.30	4.00	31.11
191	0.00	0.96	0.00	0.23	-1.41	-0.23	12.00	26.66
192	0.00	-1.21	-0.02	-0.51	-2.51	0.37	16.00	28.89
193	0.00	0.75	-0.04	3.25	2.29	10.60	8.00	35.55
194	0.00	2.24	-0.01	6.65	-0.28	0.50	0.00	24.44
195	0.00	1.64	0.00	6.91	-12.86	-1.25	0.00	44.44
196	0.00	0.15	-0.02	-1.39	2.65	6.23	4.00	49.00
197	0.00	2.73	0.00	4.68	-0.14	-0.88	4.00	24.44
198	0.00	1.82	-0.01	11.16	-4.34	-0.15	0.00	46.66
199	0.00	1.15	-0.02	8.16	-6.05	-0.44	0.00	24.44
200	0.00	1.85	0.01	0.63	3.17	0.54	8.00	26.66

201	5.56	-1.28	-5.89	9.52	0.25	-0.10	0.00	20.00
202	22.22	-5.30	-3.81	16.97	-0.91	0.38	8.00	24.44
203	-4.05	4.59	2.13	19.40	1.75	4.39	4.00	28.89
204	2.31	-1.52	-6.23	0.00	3.00	-1.72	4.00	24.44
205	22.50	-6.73	-4.96	-0.49	1.14	0.88	0.00	22.00
206	31.34	-9.29	-3.99	1.70	2.71	1.44	4.00	25.00
207	-2.67	1.72	-4.67	-2.16	1.49	2.24	24.00	27.00
208	3.79	0.00	-5.28	3.78	1.57	4.48	4.00	25.00
209	-6.04	3.21	-3.80	-1.40	-0.71	-0.53	0.00	18.00
210	2.99	1.72	-5.26	1.38	-3.06	1.49	0.00	15.00
211	-6.41	2.38	2.91	8.85	3.27	2.25	0.00	15.00
212	13.79	-2.20	-7.01	7.83	-0.48	-1.76	4.00	17.00
213	27.86	-9.38	-10.57	-0.55	-2.17	-0.05	0.00	24.44
214	39.06	-10.17	-2.75	1.96	1.76	-0.09	12.00	17.00
215	22.37	-5.25	-8.45	8.07	1.53	0.17	0.00	33.00
216	37.88	-11.67	0.35	6.67	0.10	1.56	8.00	35.00
217	2.46	-0.30	-11.72	5.43	0.71	3.49	4.00	38.00
218	-5.68	3.21	-7.45	12.50	-2.43	0.91	4.00	32.00
219	-2.47	2.22	-7.58	4.80	3.25	26.89	0.00	21.00
220	51.82	-16.67	-7.54	22.11	-8.73	-0.72	0.00	20.00
221	25.45	-6.33	4.44	1.81	-2.29	-2.06	0.00	13.33
222	-3.80	1.72	-6.54	8.09	3.21	2.94	4.00	21.00
223	-16.16	6.91	6.02	-7.21	-0.13	-0.23	8.00	25.00
224	-0.77	1.75	-9.13	10.00	-4.15	-1.06	4.00	21.00
225	5.65	-0.85	7.74	-4.38	-2.94	-3.36	4.00	22.00
226	7.38	-1.13	2.58	2.14	-0.09	2.94	8.00	22.00
227	-9.20	3.75	-1.74	8.66	-0.56	1.94	4.00	20.00
228	15.49	-5.21	-3.43	10.73	1.84	6.09	4.00	27.00
229	-3.85	3.33	13.46	3.96	0.22	-0.80	0.00	20.00
230	9.70	-2.82	0.77	25.16	0.24	-0.73	0.00	20.00
231	13.28	-2.46	-4.07	-12.06	-1.35	-0.95	0.00	21.00
232	3.95	2.78	9.07	16.03	-1.84	0.69	4.00	28.00
233	-4.79	2.94	-6.60	42.01	-4.82	-1.21	12.00	21.00
234	6.58	-1.64	-9.92	-2.60	0.18	0.06	0.00	21.00
235	16.67	-3.23	-2.20	7.55	2.35	2.90	4.00	21.00
236	15.94	-2.34	2.66	-1.63	3.12	0.07	8.00	20.00
237	4.67	-1.96	2.81	-13.86	2.76	0.33	4.00	20.00
238	14.17	-1.52	-0.52	-1.62	1.90	-0.04	4.00	26.00
239	26.92	-7.65	-9.02	-10.89	0.97	3.50	4.00	20.00
240	36.67	-7.18	0.65	-10.31	0.92	1.48	4.00	15.00
241	10.00	-2.68	-5.86	-1.30	1.22	-0.94	4.00	13.00

242	-22.55	9.26	7.92	-7.86	-0.34	-0.09	4.00	28.00
243	-9.44	2.85	2.93	-6.20	-3.02	0.45	16.00	18.00
244	-2.81	1.81	-3.25	-8.38	-0.67	0.12	0.00	23.00
245	-11.57	3.91	0.13	-5.20	1.86	4.32	4.00	11.00
246	-15.26	6.55	15.13	-11.11	-9.54	-4.71	8.00	24.44
247	4.76	1.37	5.11	21.65	1.16	0.52	4.00	11.00
248	7.89	-0.63	-15.29	3.03	2.54	0.42	4.00	19.00
249	12.10	0.82	-11.92	5.53	1.55	-0.29	0.00	18.00
250	1.67	2.34	-6.82	2.30	-1.23	-2.12	0.00	13.00
251	-7.03	3.89	-0.67	7.93	-6.88	-4.01	4.00	16.00
252	4.24	0.30	1.47	-4.92	2.33	-0.09	0.00	18.00
253	-16.85	7.61	1.72	-6.29	0.79	-0.22	0.00	19.00
254	-9.89	3.66	-1.09	-4.80	1.23	0.07	0.00	20.00
255	-12.05	5.06	1.27	6.04	1.65	1.49	4.00	21.00
256	7.05	-1.67	2.65	2.39	1.61	0.43	4.00	22.00
257	18.75	-5.75	-20.47	1.38	2.74	2.37	8.00	20.00
258	-16.25	6.71	-13.96	-9.81	0.43	0.74	4.00	28.89
259	-20.92	8.90	-1.22	2.34	1.51	1.18	4.00	24.44
260	-6.58	2.35	8.97	10.09	1.32	1.42	4.00	24.44
261	22.67	-5.56	6.11	-5.56	-0.94	0.34	0.00	26.66
262	-14.37	4.55	0.72	-1.32	0.97	0.05	0.00	28.89
263	-3.13	2.16	10.67	-16.04	2.31	1.08	4.00	20.00
264	4.39	0.00	9.59	-12.12	0.60	-1.35	16.00	24.00
265	3.01	-0.66	-4.75	20.63	0.97	1.16	4.00	17.78
266	20.18	-5.97	9.83	-17.02	3.40	-0.66	0.00	26.66
267	3.72	-0.98	-0.61	-16.98	0.96	-1.58	4.00	28.89
268	5.05	0.69	-10.94	0.44	1.81	3.01	0.00	29.00
269	6.45	-0.88	1.61	19.71	2.36	2.68	0.00	24.44
270	-18.60	7.05	6.61	-7.69	3.13	-2.26	0.00	22.00
271	-12.78	4.43	-17.30	-0.68	1.46	6.43	4.00	29.00
272	-5.00	3.24	5.83	-14.87	0.29	-5.02	4.00	29.00
273	4.17	-1.00	9.71	-9.77	4.37	5.91	4.00	28.00
274	-13.82	5.24	-16.62	14.14	1.43	1.96	0.00	28.00
275	-15.97	5.90	-3.25	21.80	1.65	2.67	4.00	26.00
276	-13.02	4.84	2.30	-19.33	2.63	-1.04	4.00	20.00
277	10.77	-0.79	-7.86	-4.66	-0.77	-0.62	0.00	26.66
278	12.67	-3.38	-15.30	14.92	0.72	1.33	16.00	28.89
279	1.32	1.58	2.01	-17.93	3.25	-1.08	8.00	11.00
280	17.97	-2.78	-2.65	5.23	0.28	0.88	4.00	19.00
281	-5.42	2.41	2.89	-3.10	0.29	5.15	0.00	28.89
282	-10.47	5.56	1.90	0.70	1.69	4.53	0.00	29.00

283	-9.35	4.86	1.21	25.00	1.22	6.25	0.00	28.00
284	-9.76	3.42	2.07	9.84	1.95	2.22	4.00	20.00
285	-7.07	4.26	2.91	-3.10	3.49	4.23	4.00	27.00
286	-23.05	9.50	2.31	30.39	-2.40	-1.20	0.00	20.00
287	2.42	2.78	1.32	0.97	3.92	6.69	4.00	20.00
288	-5.06	4.33	0.66	1.26	1.24	0.83	16.00	20.00
289	10.00	-1.11	8.45	12.12	-3.04	-2.75	4.00	11.00
290	3.57	0.88	-0.36	9.41	1.56	2.39	8.00	28.00
291	-10.49	4.83	1.90	9.61	3.47	3.57	4.00	11.00
292	-2.08	0.56	6.73	10.08	-4.39	2.90	4.00	11.00
293	-2.50	3.01	4.87	5.47	-2.15	-2.02	8.00	11.00
294	7.81	-0.83	2.83	14.19	-0.38	1.95	0.00	26.66
295	-11.98	4.76	-9.27	14.14	-1.24	2.36	0.00	28.89
296	-3.33	1.65	-21.50	-4.76	2.08	5.69	0.00	29.00
297	8.33	-1.00	-10.51	-2.16	1.16	-1.82	0.00	28.00
298	-1.65	2.24	-3.09	-7.07	3.83	3.43	4.00	28.00
299	1.14	0.23	7.93	-5.75	-0.13	4.20	0.00	27.00
300	-2.50	0.75	7.63	-4.49	1.38	1.05	0.00	20.00
301	0.00	0.67	-2.20	-7.75	11.81	1.02	4.00	24.00
302	-8.23	11.27	-3.68	-1.55	24.07	-1.58	4.00	25.00
303	-7.32	9.78	-7.76	0.41	20.17	1.35	4.00	28.00
304	-8.33	9.95	-1.94	11.25	-10.75	0.56	8.00	20.00
305	-7.21	9.80	2.57	-5.75	-2.71	2.09	0.00	28.00
306	1.41	-0.56	4.57	-6.60	-3.66	0.17	4.00	23.00
307	-6.84	8.33	-1.21	-7.62	-3.20	18.89	0.00	24.00
308	-10.32	11.84	2.06	-16.99	44.10	-0.48	0.00	25.00
309	1.67	-1.23	-5.56	-17.59	-8.47	6.42	4.00	22.00
310	0.87	2.45	1.39	3.33	-16.48	2.34	4.00	26.00
311	-5.28	5.24	-4.33	-12.23	2.08	-0.13	0.00	28.00
312	4.24	-2.61	-6.31	-5.16	-4.00	2.19	4.00	20.00
313	-8.41	10.00	-9.09	-20.77	-4.79	1.07	4.00	25.00
314	6.79	-3.92	-5.53	-13.37	68.66	-1.30	4.00	22.00
315	-2.42	5.56	6.53	11.68	-16.38	0.19	4.00	25.00
316	-10.97	12.38	8.91	0.32	19.09	-4.33	4.00	15.00
317	-5.70	8.70	13.04	-9.64	-4.00	0.62	4.00	28.00
318	30.36	-30.07	4.99	-4.48	-15.51	2.39	4.00	21.00
319	6.85	-3.43	6.18	-4.19	-0.27	0.09	8.00	28.00
320	12.24	-8.68	2.13	-32.32	-8.19	0.62	4.00	20.00
321	-6.06	7.84	-1.77	2.25	1.97	0.10	4.00	29.00
322	-2.53	4.17	-14.05	-4.69	5.72	2.76	8.00	21.00
323	-10.61	13.33	-3.72	4.49	10.64	2.91	4.00	20.00

324	-3.79	5.83	0.58	-0.97	-1.21	27.58	8.00	29.00
325	-7.33	6.67	-1.72	-4.94	15.33	1.80	4.00	27.00
326	-0.39	1.71	-6.00	7.21	37.72	-3.55	4.00	25.00
327	7.22	-2.38	-0.97	-0.23	8.36	1.24	8.00	28.00
328	-1.83	3.98	-8.62	3.38	-13.34	0.50	4.00	24.00
329	5.38	-1.15	11.14	-2.62	-5.36	-1.32	4.00	18.00
330	-6.67	8.12	12.94	-5.67	26.04	-2.63	4.00	28.00
331	9.90	-8.19	8.30	-20.54	18.18	-3.24	4.00	20.00
332	-2.14	5.09	-3.97	-30.89	17.09	-3.84	4.00	22.00
333	11.86	-9.09	1.19	3.66	-10.55	3.88	4.00	18.00
334	-3.38	5.56	8.41	7.73	6.59	-2.03	8.00	25.00
335	-6.23	7.32	4.86	-25.56	26.18	-0.50	0.00	29.00
336	-0.78	2.19	-5.31	6.71	-6.76	-1.38	4.00	14.00
337	9.95	-5.91	0.22	13.36	-8.42	-0.69	8.00	25.00
338	0.84	3.15	2.16	-1.20	8.14	-0.26	16.00	14.00
339	11.62	-10.00	7.47	1.48	4.99	-1.68	0.00	25.00
340	2.03	2.16	-3.18	-14.44	-6.16	2.29	8.00	13.00
341	-2.78	4.49	-6.20	-6.31	5.37	0.84	0.00	11.00
342	8.60	-5.04	0.71	-5.08	22.39	-0.27	4.00	22.00
343	-1.22	3.24	2.82	-10.63	-3.44	0.58	16.00	25.00
344	-1.85	2.08	8.32	-2.07	14.94	-1.18	4.00	22.00
345	0.87	-2.45	-25.45	-10.90	3.55	15.21	4.00	25.00
346	-3.40	4.55	-22.57	-1.52	16.63	0.70	4.00	27.00
347	-8.24	6.85	10.52	12.16	25.65	-5.61	12.00	26.00
348	-5.13	7.21	0.51	8.77	-4.45	-0.17	0.00	11.00
349	-3.75	3.90	-7.69	-11.36	8.55	-3.05	16.00	24.00
350	7.65	-11.59	11.26	10.84	-18.68	17.57	4.00	15.00
351	-3.66	6.85	-0.14	2.99	1.84	-4.26	12.00	29.00
352	13.89	-11.73	1.53	9.44	-4.99	-1.46	4.00	21.00
353	3.70	-1.56	-3.52	4.09	-4.16	-2.13	0.00	16.00
354	-14.66	14.42	-2.04	5.75	-8.74	3.95	4.00	20.00
355	-7.04	9.47	-14.09	-6.60	0.13	9.88	4.00	17.78
356	4.62	-5.08	-21.53	1.74	16.81	-1.08	8.00	24.00
357	-1.59	4.33	7.77	3.29	46.53	-2.65	4.00	25.00
358	-9.93	13.57	3.29	13.52	1.18	-0.86	0.00	26.00
359	12.12	-9.84	3.18	-4.11	4.07	6.89	12.00	26.00
360	21.21	-17.49	1.45	6.39	24.78	-3.11	4.00	21.00
361	4.50	-4.62	1.41	17.53	-10.65	5.35	4.00	15.00
362	-8.17	8.52	9.19	9.19	3.29	-0.57	0.00	28.89
363	1.39	2.08	4.84	5.81	16.99	-2.88	4.00	10.00
364	-7.50	10.36	9.53	-16.54	12.43	-0.53	8.00	12.00

365	15.38	-14.97	9.13	-1.44	9.08	-0.12	8.00	24.44
366	7.96	-6.78	10.02	16.09	-13.65	1.28	4.00	20.00
367	16.37	-17.95	-3.96	0.00	33.52	0.03	4.00	15.00
368	-2.35	4.76	-2.74	12.16	10.47	-0.11	4.00	24.00
369	6.67	-3.70	-3.30	13.54	9.21	-0.92	4.00	18.00
370	4.47	-3.60	2.38	0.00	-2.00	1.33	12.00	20.00
371	11.98	-9.60	3.13	12.57	-12.12	0.77	8.00	11.00
372	-2.70	2.05	0.21	4.56	-1.21	-2.06	4.00	13.33
373	-9.32	12.79	-5.39	4.26	-13.86	-1.29	4.00	13.33
374	12.00	-13.82	0.41	11.01	-14.62	21.10	0.00	16.00
375	2.16	-1.45	-5.77	2.33	16.48	0.25	0.00	15.00
376	13.33	-17.58	-13.30	-11.76	-4.50	1.87	0.00	18.00
377	9.84	-10.46	0.31	15.82	-10.02	-2.78	4.00	19.00
378	12.38	-15.30	4.67	-5.64	-3.23	0.19	4.00	20.00
379	9.90	-12.93	1.40	2.12	-2.18	-0.69	4.00	24.00
380	6.79	-7.09	6.53	7.20	-14.50	0.73	0.00	28.00
381	-4.17	8.77	1.45	6.64	14.38	-0.05	4.00	28.00
382	-1.30	3.70	2.68	4.42	3.28	-0.16	8.00	25.00
383	15.15	-8.21	3.07	2.75	8.86	-1.58	4.00	21.00
384	11.29	-6.55	0.74	3.51	5.73	1.55	4.00	22.00
385	-0.75	4.02	1.38	7.77	-8.43	1.05	4.00	21.00
386	12.22	-6.43	5.57	-5.64	1.59	4.78	0.00	19.00
387	27.32	-22.01	-6.42	7.08	-5.80	0.96	4.00	11.00
388	3.07	0.98	4.40	-0.84	-3.92	1.48	4.00	11.00
389	-3.17	6.32	1.01	12.00	2.55	-0.82	4.00	20.00
390	-9.48	11.23	4.14	-3.05	6.62	1.08	4.00	20.00
391	1.36	2.25	4.91	4.69	3.20	-0.24	4.00	11.00
392	0.74	3.29	3.46	4.74	14.62	0.48	0.00	16.00
393	12.75	-14.69	5.43	7.63	0.70	0.16	4.00	20.00
394	2.95	-0.45	2.98	0.18	-1.27	-0.23	4.00	15.00
395	1.30	0.95	-1.51	2.03	24.14	-3.89	0.00	28.00
396	1.22	2.99	1.89	-1.67	-5.80	-0.53	4.00	22.00
397	0.51	1.67	-2.05	-0.21	-11.61	2.12	4.00	11.00
398	-0.55	3.64	1.26	7.48	-6.00	1.10	0.00	20.00
399	9.33	-6.97	2.09	6.39	-10.74	5.75	0.00	11.00
400	1.39	1.03	19.57	-4.63	-0.46	22.95	4.00	11.00
BC	1.11	1.02	0.91	3.26	0.73	0.79	5.88	38.41
RPC	5.54	-1.32	-1.00	-0.61	6.78	0.96	4.94	17.77

TNT= Total number of tillers per plant. NPT = Number of productive tillers per plant.
PH = Plant height. GY = Grain yield per panicle.
TW = Test weight. NFG = Number of filled grains per panicle.
M (%) = Mortality percentage. BLB = Bacterial leaf blight incidence (%)
BC = BPT-5204 Check RPC = RPBIO-226 check

APPENDIX – VI

Mean monthly meteorological data for the year 2013 recorded at Agricultural Research Station, Gangavathi

Month	Rainfall (mm)	Temperature (^{o}C)		Relative Humidity (%)
		Maximum	Minimum	
January	0.00	31.23	16.95	65.71
February	0.00	31.79	16.71	60.46
March	0.00	34.71	15.92	54.39
April	5.50	36.92	17.70	59.17
May	102.00	38.10	21.42	73.71
June	96.50	32.53	20.30	80.60
July	34.50	29.29	18.87	76.52
August	22.50	29.71	18.32	74.00
September	118.50	23.18	13.85	61.10
October	108.50	29.97	17.18	72.81
November	2.00	29.35	14.75	72.81
December	0.00	29.07	12.25	72.68
Total	**490.00**			

EVALUATION AND MOLECULAR CHARACTERIZATION OF ADVANCED MUTANT LINES IN RICE (*Oryza sativa* L.)

MAHENDRAKUMAR, V. B. **Prof. MOHAMMED IBRAHIM**
Major advisor

ABSTRACT

The present study was undertaken to evaluate 400 mutant lines comprising of 100 mutants from each 30Kr and 40Kr treatments of two varieties *viz.,* BPT-5204, RP Bio-226, during *Kharif* 2013, at Agriculture research station, Gangavathi, University of Agricultural Sciences, Raichur were evaluated in augmented design with 2 parental checks for genetic divergence in both normal and saline soil, salinity screening and molecular diversity in saline soil condition. Genotypes were also evaluated for extent of variability, correlation, and path analysis, genetic divergence based on both morphological character for 12 traits and a set of 45 genotypes evaluated for molecular diversity using 16 SSR primers. ANOVA revealed highly significant difference among the genotypes for quantitative traits *viz*., plant height, panicle length, biological yield. High PCV and GCV estimates were recorded biological yield, grain yield per panicle, number of grains per panicle, number of filled grains per panicle and test weight, high heritability were recorded except panicle length and grain yield per panicle and high genetic advances were recorded except days to 50% flowering, yield component characters harvest index, biological yield, number of filled grains per panicle and number of grains per panicle exhibited highly significant association with grain yield. Genotypic path coefficient analysis revealed that harvest index in normal soil and number of filled grains per panicle in saline soil had the highest positive direct on grain yield, using Mahalanobis D^2 analysis revealed that number of grains per panicle and number of filled grains per panicle contributed greatly towards divergence. Two different clusters were formed with maximum number of mutants (399) in cluster (I) in both saline and normal soil condition. Line no. 4, 41, 103, 110, 209, 253, 353, 358 are tolerant for salinity based on stress susceptibility index and Mortality percentage. Based on SSR analysis revealed that simple matching indices shows highest similarity index between 40 and 41, based on dendrogram 12 different clusters at nearly 96% similarity levels, it was also found that among all the clustering VII was highly heterogeneous, In conclusion, allelic diversity revealed by 16 SSR primers was not sufficient enough to distinguish between mutant lines. The alleic variation was lower within mutant population.