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Molecular characterization and evaluation of a panel of aromatic mutant-M7 rice (*Oryza sativa L.*) for yield performance

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ABSTRACT

Rice is one of the cultivated crops that have primitive and obsolete cultivars which are extensively conserved in ex-situ condition. Conservation of rice germplasm also helps to preserve the beneficial alleles of rice for future breeding purpose. Moreover, most of the primitive races have not been characterized at molecular level and as such may be lost due to sudden change in environmental factors which may lead to genetic erosion of beneficial alleles. Path analysis revealed that number of panicle per plant had highest direct effect on yield followed by filled grains per panicle whereas indirect effect on individual yield as recorded between number of tillers through number of panicle per plant followed by 1000g weight through filled grain per panicle with significant and positive correlation. From molecular analysis, 22 polymorphic markers revealed that 33 genotypes were clustered as three main cluster and genotypes CT3D12W and CT3D25 was grouped as genetically divergent from 31 lines. In this population, a total of 60 alleles were detected in 22 loci with minimum and maximum of 2 and 4 alleles per locus with average of 2.7 in 33 genotypes, respectively. PIC value ranged from 0.061 (by RM251) to 0.616 (by RM452) with average of 0.390. Markers, RM215 and RM161 were dinucleotide repeated sequence whereas RM452 and RM495 were trinucleotide repeated sequence which was detected more alleles in locus with high PIC value. Dissimilarity coefficient among 31 genotypes was computed by using UPGMA algorithm by using polymorphic markers data.

1. Introduction

Rice is the model crop among the monocot plants which was domesticated from the ancestor of *Oryza rufipogon* during 10,000 years ago in North East part of India (NE India) and southern part of China (Choudhury, *et al.,* 2013). Under the genus of Oryza, there are 21 wild species, out of which 6 species has been identified as tetraploidy and remaining was reported as diploid species. Our present cultivated species of *O. sativa* was diploid with 12 sets of chromosome and a genetic constitution of AA genome of 430 Mbps. Rice still had a unique position among cultivated food crops to meet the food and nutrient requirement of the population. Genus Oryza has more divergence races/landraces in NE India and was classified as aromatic and non-aromatic types of landraces. Among the two aromatic land races have a more diversifications which possess metallothionein and cystine rich protein which are helpful to absorb the Fe in our body. The varieties of basmati were rich in minerals (Zn and Fe) and anti-oxidant (Babu *et al*., 2014). Recently, 50,000 landraces were identified in NE India which emerged by adaptation and cultivation of rice in various climate and season (Khan *et al.,* 2018). Landrace were maintained and grown by farmer as primitive cultivars for different purposes, but they had a lesser yield compared to the recent cultivars. All the landraces have a different peculiar characters, morphologically and biochemically for *e.g.* Chakhao poireiton and Chakhao amubi has high aromatic content, but susceptible to the blast disease. Most of primitive and obsolete cultivars were collected and preserved at ex-situ conservation but those are still characterized and diversified, phenotypically. But, phenotypic characters were influenced by genotype and environments. Classification and

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characterization of germplasm based on phenotypic observation is not an easy technique as those characters were influenced and affected by environmental fluctuation. Even though, most of the primitive and obsolete cultivars had a strong and durable resistance to *Magnaporthe oryzae* that cause the blast disease on leaf and neck region in the panicle, traditional races have sufficient resistance genes in their genome. So far, nearly 102 'R' genes (Kumari *et al.,* 2018) and 350 QTLs were identified in a genome of rice which was used to combat against the initiation and development of pathogen. Recently, only few genes (28) were characterized at molecular level wherein 20 major and 2 minor, to combat blast pathogen which may lead to development of disease as reported by (Alam *et al.,* 2017). Those 102 genes were evenly distributed throughout the chromosomes in the genome of Oryza except chromosome number 3 and most of resistance genes (R) in rice genome followed gene for gene hypothesis. After 3-5 years of releasing highly resistance lines, durability and stability of those genes product of rice was subsequently destroyed by the pathogens which develop the new virulence genes (v) against resistance genes in the genomes. So, the development of durable, stable and heritable resistance genes in new cultivars is a very difficult task for the plant breeders due to development of v genes in a pathogen genome against R gene in a host/rice genome. To overcome these problems breeders have to develop the resistant genotypes with multiple resistance genes. Most of our landraces have more than one R genes to combat against the pathogen in different cultivars (Miah *et al.,* 2017). So, bringing the primitive races into modern cultivars may help development of multi-genic resistance lines which can use as the parents for the development of high yielding hybrids.

In thousand years ago, plant breeding started with human selection of plant with high productivity and quality traits. In 17th century, with the discovery of sexual reproduction in plants, people started improvement of desirable traits of crop by interbreeding and crossing of desirable plants. However, with the understanding of effect of mutation in Drosophila and barley in 20th century, plant breeder started to create and increase variability of the traits by mutagenesis in a plant (Batista *et al.,* 2008). Due to the need of increase in yield through crop improvement to feed the growing demands of increase in population, some researcher have done by nutritional method, physiological method and breeding method. But, the easiest way is changing the crop in cellular level *viz.* bases addition and deletion in DNA and chromosomal aberration so that it contribute more variation in particular traits and also have the heritability to next generation (Oladosu *et al.,* 2014).

As compare to transgenic and recombinant breeding, mutation breeding have more contribution to improve the desirable traits in crop creating new variability (Anh *et al.,* 2018). Recently, marker assisted selection (MAS) is an emerging technology in molecular analysis. Molecular analysis is always easy to characterize among huge races/variety at a time and also helpful to analyse the presence of biotic and abiotic stress resistance genes in rice genome by analysing gene based candidate markers. Moreover, genetic constitutions of those races are not responsible for any change in environmental conditions. Characterization and conservation of primitive and obsolete cultivars are most important because farmers cannot maintain huge number of races as sometime those races may be lost by changing environmental conditions, which leads to genetic erosion of beneficial alleles from the primitive races and obsolete cultivars. In the past decade, most of the characterization of germplasm was done by the RAPD (Random Amplified Polymorphic DNA) and SSR (Simple Sequence Repeats) markers. Among these two markers, SSR (also called microsatellite markers) is the best marker for analysing the diversification and for characterization of genotypes at molecular level because of distribution of SSR markers throughout the genome. The microsatellite sequence were present in the rice genome at each intervals of 16 to 20 cM (Ravi *et al.,* 2003) and nearly 20,000 SSR position were tagged and characterized at molecular level (Meti *et al.,* 2013). Thus, SSR is a powerful tool to identify and classify the divergent races at genetic level among the germplasm /genotypes. It will be helpful for the development of superior crosses/ hybrids by crossing between divergent parents at genetic level. Therefore, in view of the above importance, the present study is being proposed with the following broad objectives:

- To evaluate a panel of rice mutants (M7) for yield and its component characters under acidic soils.
- To characterize a panel of rice mutants (M7) using SSR markers.

2. Materials and Methods

The research materials comprise of M_7 rice mutant lines developed from Chakhao poireiton through mutagenesis using EMS. The materials were maintained from previous works done in CPGS-AS and DOR, CAU, Imphal. Name of genotypes and their coding are listed below in table 1. Thirty-three advanced breeding lines including check cultivar and parent were propagated in low land rice field in Randomized Block Design (RBD) with three replications. Each line was allotted randomly in each replication to minimize experimental error.In this study, ten competitive plants at random were taken from each plot in each replication to record the data/observations on 10 phenotypic traits *viz.* plant height at maturity (cm), number of tillers per plant, days to 50% flowering, days to maturity, number of panicles per plant, panicle length, total grain/spikelet, filled grain, 1000 grain weight and grain yield per plant. Days to 50% flowering and days to maturity were recorded on plot basis by visual observations. The data related to phenotype and disease resistance were observed and collected at regular intervals. Genetic diversity of the advanced breeding lines including parent and check cultivars and for blast resistance were analysed utilizing the standard SSR and gene specific markers, respectively. The information about these 50 standard SSR markers were taken from GRAMENE website (https://archive.gramene.org/markers/microsat/). Genomic DNA was extracted by CTAB method which was used the most reliable method for extraction of rice genomic DNA. PCR master mixture has 1XPCR buffer, forward and reverse primer (1.2 μM), 0.12mM dNTPs, 1.75mM $MgCl_2$, nuclease free water.

3. Result and Discussion

Present day crop improvement is a powerful tool for improving hybrid and hybrid variety but with a drawback that it narrows down the beneficial alleles in a germplasm and cultivars leading to the bottle neck effect on the beneficial alleles. Because of this, characterization of landraces and modern cultivars is necessary to conserve the beneficial alleles in the rice germplasm by analysis of divergent landraces and cultivars at molecular level. So, conservation of old landraces and cultivars were need of the day with morphological and molecular characterization of divergent lines. All the landraces have peculiar genes or characters for adaptation to a kind of adverse biological and non-biological stress condition.

Analysis of allelic status of landraces will help in developing superior crop variety and further development programmes of high yielding hybrid for resistance against biotic and abiotic stress. The old landraces have high heritability of quantitative characters as compare to the modern cultivars. At this juncture of crop improvement where the landraces are depleting due to greater attention to hybrids and high yielding variety, generation of variability through mutation will give genetic enhancement of the crop concerned. Keeping its value of molecular characterization in mind, the analysis of divergence among 33 mutant genotypes including check and also allelic status for blast resistance gene is discussed in this chapter. Phenotype of

Table 1. List of genotypes and respect code

Code	Genotype	Code	Genotype	Code	Genotype
L1	CT3D1	L12	CT3D12	L23	CT3D24
L2	CT3D2	L13	CT3D13	L24	CT3D25
L3	CT3D3	L14	CT3D14	L25	CT3DBS
L4	CT3D4	L15	CT3D15	L26	*Chakhao amubi*
L5	CT3D5	L16	CT3D16	L27	*Chakhao poireiton*
L6	CT3D6	L17	CT3D17	L28	CT3D11W
L7	CT3D7	L18	CT3D18	L29	CT3D12W
L8	CT3D8	L19	CT3D20	L30	CT3D23W
L9	CT3D9	L20	CT3D21	L31	CT3D25W
L10	CT3D10	L21	CT3D22	L32	CT3DBSW
L11	CT3D11	L22	CT3D23	L33	Sabhagidhan

any traits was influenced by genetic potential of genotype and also influenced by environment and interaction of both. For this reason, value of PCV is always higher than the GCV value. Component of genetic variance (ANOVA) was mentioned in table 2. High PCV and GCV were observed in individual yield per plant followed by filled grain per panicle and number panicle per plant whereas low PCV and GCV were measured in days to 80% maturity followed by panicle length. Number of tillers per plant recorded moderate PCV (17.187) and GCV (17.002) value but nearly same value which indicated the less influence of the environmental factor for development of this traits. PCV of individual yield per plant was found to be high then GCV. 1000 grain weight was measured moderate PCV and GCV (%). This same finding was observed by Patel *et al.*, 2014 in the analysis of genetic variance in 44 genotypes of rice. Heritability of trait indicated that how much amount of the trait inherited from parent to their progeny and the same was calculated from the genotypic and phenotypic variation. Selection of the genotype or lines based on heritability alone does not improve any trait; but selection based on both heritability and genetic advance can help to improvement of any quantitative traits in next generation. High h2 and GA were measured in number of tiller per plant followed by plant height and filled grain per panicle whereas low h2 and GA measured in panicle length followed by 1000grain weight. High h2 and GA was observed in plant height. Total grains per panicle had a high h2 and GA in the study.

Similar finding was also observed in total grains per panicle by Patel *et al.,* 2014 in 44 genotypes. In correlation coefficient study among the 10 traits (Table 3), positive correlation between traits indicated positive association of both traits in such manner that if value of one trait increase that of other trait also increase or value of one trait decrease then that of other trait also decrease. Negative correlation coefficient between traits indicated that if there is increasing value of one trait then the other trait value decreases, and vice-versa. Correlation coefficient study among the 10 traits revealed that days to 80% maturity and total days to 50% flowering had high positive significance, whereas path analysis revealed that days to 80% maturity had a negative indirect effect on individual yield through days to 50% flowering. Filled grains per panicle and total grains per panicle had positive and significant correlation but it contributed less and indirect amount on individual yield development through filled grains. Phenotypic correlation of some of the traits had a low positive value with significance but it had a positive indirect effect on individual yield development *viz.* number of tillers per plant and number of panicle per plant, number of tiller per plant and days to 80% maturity, total grains per plant and panicle length, filled grains per panicle and total grains per panicle, 1000 grain and total grains per panicle and 1000 grain and filled grains per panicle. Days to 80% maturity had a negative and indirect effect on yield via days to 50% flowering followed by Days to 50% flowering and filled grains.

Table 2. Analysis of component of variance for 10 characters

Parameter	Mean value	Range		PCV	GCV	ECV	h^2	GA
		Minimum	Maximum					
PH	85.77	59.32	152.14	19.44	18.77	5.07	93.20	32.01
DF	100.10	72.00	118.00	9.08	8.07	4.16	79.00	14.78
DM	131.23	118.00	148.00	6.86	5.13	4.55	55.88	10.36
NTP	15.42	11.00	22.60	17.19	17.00	2.52	97.85	5.34
NPP	9.90	6.00	18.60	21.52	15.37	15.06	51.00	2.24
PL	23.60	19.97	36.71	7.86	3.89	6.83	24.49	0.94
TNGP	134.09	86.40	207.80	19.96	16.96	10.52	72.21	39.81
NFGP	74.00	36.00	139.13	30.33	27.49	12.83	82.12	37.97
1000g	19.88	13.35	24.99	12.85	8.54	9.60	44.20	2.33
IYP	13.18	2.88	25.81	34.89	24.74	24.61	50.27	4.76

Table 3. Path analysis for yield and its attributing character

	PH	DF	DM	NTP	NPP	PL	TNGP	NFGP	1000g	rp (Individual yield)
PH	0.054	0.184	-0.02	0.022	-0.04	0.007	-0.001	0.014	0.019	0.239
DF	-0.02	-0.492	0.056	-0.011	0.029	-0.006	-0.016	-0.068	-0.051	-0.579
DM	-0.016	-0.413	0.067	-0.015	-0.002	-0.008	-0.015	-0.053	-0.038	-0.493
NTP	-0.017	-0.078	0.015	-0.07	0.143	0.004	0.008	-0.004	0.009	0.010
NPP	-0.007	-0.048	0	-0.034	0.3	0.005	0.006	-0.033	-0.009	0.180

PL	0.006	0.05	-0.009	-0.005	0.025	0.059	0.017	0.022	0.011	0.176
TNGP	-0.001	0.193	-0.025	-0.013	0.04	0.024	0.042	0.089	0.025	0.374
NFGP	0.006	0.242	-0.026	0.002	-0.072	0.009	0.027	0.139	0.031	0.358
1000g	0.011	0.261	-0.026	-0.006	-0.027	0.007	0.011	0.044	0.097	0.372
Residual effect = 0.2018										

1000grain weight per plant had a high and positive effect on individual yield through days to 50% flowering followed by filled grains per plant and days to 50% flowering. Number of panicle per plant had a high and direct effect on individual per plant yield. This same finding was observed in seven advanced breeding lines by Rahman *et al.,* 2014. Direct effect of the number of filled grains per panicle contributed towards yield. Mustafa and Elsheikh, 2007 also found positive and direct effect of number of filled grains per panicle in fourteen rice genotypes.

These above findings were in agreement with the findings of present study. Number of tillers per plant had indirect and positive effect on individual yield per plant through the number of panicle per plant. This finding was observed in analysis of 21 genotypes by Babu *et al.*, 2012. The current study was recorded 0.2018 (20.18%) of residual effect on yield which was clearly indicated that 20% of effect might be from other characters which are not included in this present study. Molecular characterization of 31 genotypes was carried out utilizing 41 SSR markers. Out of the 41 markers, 22 markers showed polymorphic pattern. The remaining 18 markers showed monomorphic pattern. From polymorphic markers studies, a total of 67 alleles were detected in 22 loci in the test population and each locus had a minimum of 2 alleles and maximum of 4 alleles. RM55, RM413, RM1, RM25, RM133, RM259, RM287, RM316, RM277 and RM144 were detected having a minimum of 2 alleles per locus and RM19, RM452, RM495 and RM507 were detected having a maximum of 4 alleles per locus. On an average, the population of 22 loci had 2.7 alleles per locus. Rashmi *et al.,* 2017 also reported an average of 2.7 alleles per locus in 19polymorphic loci in 65 genotypes. Singh *et al.*, 2015 also reported the same result during their study in 20 genotypes with 34 polymorphic markers. In addition to this, the PIC value range was also the same as that measured in this study. PIC value of all the markers had an average of 0.390. The minimum value of PIC (0.061) was calculated for marker RM259 and the maximum value (0.616) was calculated for marker RM452. The average PIC value measured was 0.390 which was the same as analysed by Salgotra *et al.* (2015). The highest PIC value was calculated for marker RM452 (0.616) followed by RM495 (0.604), RM162 (0.574) RM (0.560) and RM161 (0.566). RM452 and RM495 were the best markers revealing the maximum PIC value in the population. Lesser number of repeated sequences (di, tri *etc.*) of markers/primers reveals maximum PIC value and alleles per locus in a population. In this study, markers RM161 and RM215 had di and RM452 and RM495 are tri nucleotide repeated sequence motif revealing high PIC value and alleles per locus which was confirmed by the finding of Sajib *et al.*, 2012. Dendrogram analysis by UPGMA algorithm revealed that all 31 (without Chakhao amubi and Sabhagidhan) genotypes classified into 3 different main cluster groups (main cluster I, main cluster II and main cluster III).

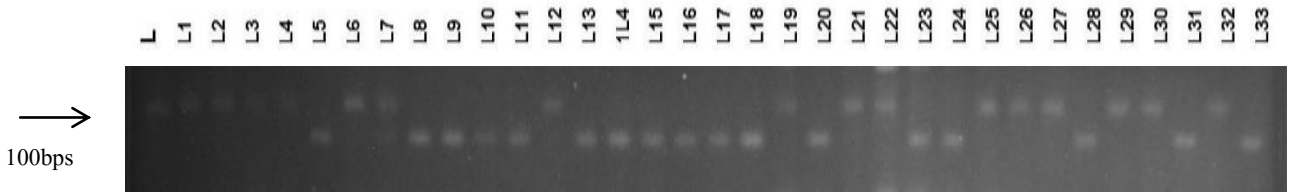
Main cluster I was sub divided into two sub group (sub cluster IA and sub cluster IB) and main cluster II was also sub divided into two category (sub cluster IIA and sub cluster IIB) whereas main cluster III had only one genotype. Main cluster I had two sub clusters; sub cluster IA and sub cluster IB. Sub cluster IA was again separated into two groups *viz.* sub-sub cluster IAI and sub-sub cluster IAII. Sub-sub cluster IAI consists of 6 genotypes namely CT3D11W, CT3D12W, CT3D23W, CT3D25W, CT3DBSW and Chakhao Poireiton whereas sub-sub cluster IAII consisted of only CT3DBS. Sub cluster IB was again grouped into two sub-sub cluster *viz.* sub-sub cluster IBI and sub-sub cluster IBII. Sub-sub cluster IBI consists of 5 genotypes namely CT3D8, CT3D9, CT3D10, CT3D11 and CT3D12 whereas sub-sub cluster IBII consisted of 7 genotypes namely CT3D1, CT3D2, CT3D3, CT3D4, CT3D5, CT3D6 and CT3D7. Main cluster II had two sub clusters; sub clusterIIA and sub cluster IIB. Sub cluster IIA was again split into two groups *viz.* sub-sub cluster IIAI and sub-sub cluster IIAII. Sub-sub cluster IIAI consists of 5 genotypes namely CT3D13, CT3D14, CT3D15, CT3D16 and CT3D17 whereas sub-sub cluster IIAII consists of 4 genotypes namely CT3D18, CT3D20, CT3D21 and CT3D22. Sub cluster IIB was again divided into two groups; sub-sub cluster IIBI (have only CT3D24) and sub-sub cluster IIBII (have only CT3D23). Only one genotype (CT3D25) falls

under main cluster III. Chakhao amubi and CT3BS. Sub-sub cluster IIAI consists of 5 genotypes namely CT3D13, CT3D14, CT3D15, CT3D16 and CT3D17 whereas sub-sub cluster IIAII consists of 4 genotypes namely CT3D18, CT3D20, CT3D21 and CT3D22. Sub cluster IIB was further divided into two groups; sub-sub cluster IIBI (have only CT3D24) and sub-sub cluster IIBII (have only CT3D23). Main cluster III had only one genotype *i.e.* CT3D25. These clusters were derived from 22 polymorphic markers (for 31 genotypes excluding Chakhao amubi and Sabhagidhan) and 25 polymorphic markers (for 33 genotypes including Chakhao amubi and Sabhagidhan).

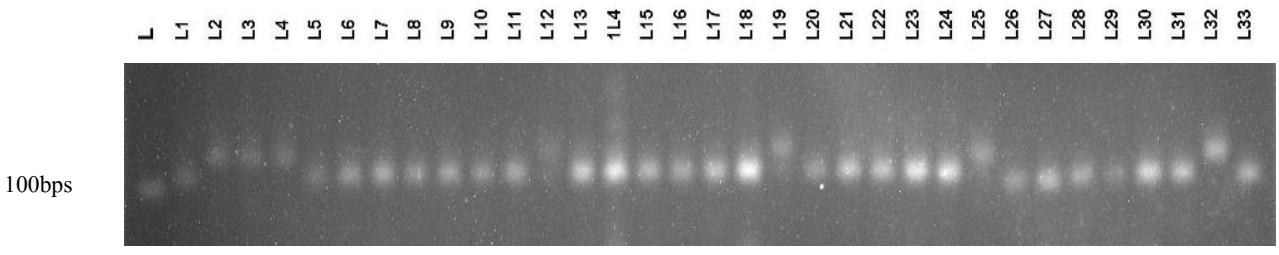
Both the cluster diagrams clearly showed that different sets of genotypes gathered together in a particular cluster group. *e.g.* In both cluster diagrams, major cluster I shared the same genotypes in both IA and IB sub clusters. Major cluster II and major cluster III also had the same genotypes in both cluster diagrams. Babu *et al.,* 2014 and Kibia *et al.,* 2009 reported 3 major clusters groups which were supported the present study. The finding that the components of each cluster in both the grouping showed negligible change with the inclusion of one or two new genotypes/ one or two polymorphic markers clearly indicated that there were strong association between some genotypes and these genotypes always fall under a particular cluster group.

Table 4. List of polymorphic markers and their detected alleles and PIC value

Sl. No.	Markers	No. of allele	PIC	Observed band range	Sl. No.	Markers	No. of allele	PIC	Observed band range
1	**RM277**	2	0.160	110-120	13	**RM271**	3	0.343	100-120
2	**RM19**	4	0.425	200-250	14	**RM287**	2	0.353	80-110
3	**RM1**	2	0.410	100-120	15	**RM316**	2	0.327	190-210
4	**RM25**	2	0.350	140-150	16	**RM413**	2	0.375	80-110
5	**RM55**	2	0.362	220-250	17	**RM431**	3	0.254	250-270
6	**RM124**	3	0.173	260-280	18	**RM452**	4	0.616	190-220
7	**RM125**	3	0.375	110-140	19	**RM495**	4	0.604	140-170
8	**RM133**	2	0.346	230-240	20	**RM507**	4	0.467	240-260
9	**RM161**	3	0.566	180-220	21	**RM144**	2	0.354	90-110
10	**RM162**	3	0.574	210-220	22	**RM536**	3	0.560	230-250
11	**RM215**	3	0.529	150-170	**Average**		**2.58**	**0.361**	
12	**RM259**	2	0.061	160-170					



RM413
Position is chromosome 5



RM271
Position is chromosome 10

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