

**Genetic analyses in rice (*Oryza sativa* L.) with
special reference to agro-morphology, quality
and osmotic stress tolerance**

Synopsis

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Rice is one of the major agronomically and nutritionally important cereal food crop and second major global calorie contributor after wheat. At present, there are a large number of rice varieties and more are being developed to enhance yield and grain quality in order to meet the ever increasing consumer demands. In short, it is clear that primarily rice is important to us for its yield (quantity) and grain qualities. Increase of rice yield is depends on a number of factors, of which abiotic stress is the most important one. In this current decade, rice production has been slowed down due to the effect of osmotic stresses by altered climatic conditions. So, the improvement of rice yield and quality are very significant for sustainable consumer demand. For both of these improvemental aspects, utilization of potent rice genetic resources with diverse gene pool is the prime criterion for different types of rice breeding program. It has been shown that different indigenous rice lines and wild-rice species harbour a good number of valuable genes responsible for both quantitative and qualitative traits. The prime aim of the present investigation was collection, characterization and utilization of a subset of rice lines in improvemental breeding program for both yield and quality. Keeping these as aims, the main objectives of this present work were characterization and profiling based informative databank preparation of a subset of promising West Bengal rice genetic resources. The valuable rice lines were collected from diverse agro-climatic conditions of the southern parts of West Bengal which included both wild-rice and traditional rice lines. In addition to these rice lines, one popular rice variety which is the most common improved rice genotype cultivated throughout the upland rice fields of Southern Bengal at present was also included as reference genotype both for phenotyping and genotyping.

A total of 51 West Bengal rice genotypes were collected which consists of 49 indigenous rarely available less popular rice landraces; one non-aromatic (NA) high yielding variety (HYV) and one wild rice. The selected rice genotypes were characterized for agro-morphological and grain quality traits. A subset of 20 lowland rice lines with improved agro-morphology and quality traits were subjected to physio-biochemical screening under varied induced osmotic stresses followed by trait-linked SSR based genotyping.

For the agro-morphological characterization, it was shown that grain-kernel colour (white, creamy white, reddish brown and brown) and their dimensions

(long, short and bold) were varied greatly in the selected rice lines. Kabirraj Sail was recorded as the tallest landrace followed by Balaram Sail; whereas IR36 has shortest plant height. Highest flag leaf area was recorded for Madhumala and lowest in Gobindobhog. Maximum number of tillers and panicles were found in IR36 (a HYV line) and lowest in *Oryza rufipogon* (a wild-rice). Days to flowering and harvest time were also found directly proportional to each other and it confers a variety as early and/or late mature type. From this present study, Satia has been proved as early-mature landrace with shorter life-span; whereas *O. rufipogon* was late mature and perennial in nature. Though IR36 is a high-yielding cultivar, its panicle was too short; whereas Radhatilak, an upland aromatic line has long panicle with lower grain weight. Flag leaf length and breadth were highest in Madhumala and Tangra Sail, respectively; lowest in Lat Sail and Gobindobhog, respectively. Long grain was observed in Malik Sail and short grain was recorded in aromatic Badshahbhog. Raghu Sail has the longest kernel; whereas shortest was found in Gobindobhog followed by Danaguri. Normally aromatic and bold-grained rice have short grain and kernel dimension; whereas lowland varieties possess long grain and kernel. Neizer Sail and Kumrogorh showed highest grain and kernel weight, respectively. Lowest grain weight was found in both Badshahbhog and Laukagorah-N; lowest kernel weight was observed in Danaguri. Aromatic and bold-grained rice varieties have short length and breadth of grains; thus they have lowest grain and kernel weight. IR 36 showed highest length/breadth ratio (shape) of both grain and kernel; whereas this trait is lowest in Marich Sail. IR 36 and Pateni have lowest alkali spreading value (ASV score) and Pakri has its highest score. Neizer Sail showed maximum elongation of its kernel (EKAC) and volume expansion (VEAC) after cooking with highest cooked kernel elongation ratio (CKER). Seeta Sail, a lowland line showed lowest EKAC and CKER. VEAC was very low in Gobindobhog. Dharial was found to have highest amylose and Narayanpurna showed lowest amylose content. Fe and Zn content were found very high in Kabirraj Sail and lowest in IR36. A positive correlation was observed for brown-kerneled rice and its iron (Fe) content; except Kabirraj Sail, where brown-rice is white in colour.

Statistical analysis for studying the genetic divergence among the studied lines was highly significant [at 5% level ($p < 0.05$) of probability] for agromorphological and grain quality parameters. Grain length (GL) showed significant

(at both 1% and 5% level) positive correlation with kernel length (KL), kernel breadth (KB), grain length-breadth ratio ($G_{L/B}$), kernel length-breadth ratio ($K_{L/B}$), 1000 grain weight (GW) and 1000 kernel weight (KW). Highest positive correlation was found in grain breadth (GB) and kernel breadth (KB). Highest significant negative correlation was recorded in grain breadth (GB) and grain length-breadth ratio ($G_{L/B}$). Weight of 1000 grains (GW) and 1000 kernels (KW) were found positively correlated to each other. Significant positive correlation was also found for flag leaf length (FLL), flag leaf area (FLA), flag leaf angle (LA) and panicle length (PL) with plant height (PH); flag leaf area (FLA) and flag leaf length-breadth ratio ($FL_{L/B}$) with flag leaf length (FLL) and flag leaf breadth (FLB); tiller number (TILL) and panicle number (PN) with flag leaf length-breadth ratio ($FL_{L/B}$); flag leaf area (FLA) and panicle length (PL). Highest positive correlation was present for tiller number (TILL) and panicle number (PN), that is 1 i.e. same in number followed by days to flowering (DFL) and days to harvesting (HARV). Significant positive correlation was found in between Fe and Zn content that is much high; followed by EKAC and CKER. Negative correlation was found in ASV and amylose content with CKER. All traits were showed significant differences in the varietal mean sum of square (MSSv) which indicates that the studied rice genotypes were genetically diverge. Maximum genotypic and phenotypic variations were obtained for PH and LA, moderate in HARV, DFL, PL, TILL and FLL. It indicates that environment can influence these traits as non-stable. Their values are very close in GL, GB, KL, KB, FLB and CKE. Thus it can be considered as stable. Phenotypic coefficient of variation (PCV) was higher in magnitude than genotypic coefficient of variation (GCV) for all the characters studied. High values of GCV suggest better improvement scope for the trait (LA) by selection. Broad sense heritability ($H\%$) and genetic gain (GG) estimates were interpreted as low, medium and high. Combination of high $H\%$ with GG is the main step for trait selection and validation for breeding program. High heritability (%) with high genetic gain was observed in LA. This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance, hence amenable for simple selection. High heritability (%) with moderate genetic gain was found in GW and KW. It indicates that these characters were governed by additive gene interaction. Finally, High heritability (%) with low genetic gain was present in plant height, flowering days and

harvesting days that indicating non-additive gene action; hence heterosis (hybrid vigour) breeding would be recommended for these traits.

Quality trait linked SSR genotyping for the selected rice lines, based on the good and poor performance, revealed a total 19 alleles that were amplified with average of 2.71 per loci. RM264 produces highest number of alleles (i.e. 4) and lowest numbers of alleles (2) were amplified by RM253, RM44 and RM190. RM 44 (97-103 bp long) and RM 190 (96-105 bp long) produce shorter allelic product; whereas RM29 produces higher molecular weight alleles (185-193 bp long). High PIC value was found in RM 264 and low PIC was for RM 253. For RM264 and RM190, Dharial with highest amylose represents lower allelic size (132 bp for RM264 and 95 for RM190); whereas Narayanpurna showing low amylose content followed by Gobindobhog and both are sharing the same allele (105 bp) for RM190. RM190 was performed better than RM253 to trace the marker-trait association. Pateni and IR36, having low ASV, shared the same allele (95 bp) and Pakri, with highest ASV, represents a medium allelic size (98 bp). Lower to moderate ASV was shown by two aromatic lines: Gobindobhog and Narayanpurna. They represent an another allele of 105 bp size. Among the selected rice lines for good and poor performance in quality parameters, only two are observed as aromatic (as per the sensory aromatic test), i.e., Gobindobhog and Narayanpurna, and both share the same allelic size (band size 103 bp) after the fingerprint through RM44, an aroma linked marker. Rest 7 non-aromatic varieties showed another allelic product of 97 bp. RM29, RM224, RM215 and RM44 are reported to be linked with cooked kernel elongation. RM224 can explain it little better than the others regarding the marker-trait association on the basis of correlation between the allelic size and observed qualitative value. Neizer Sail with highest CKE showed high allelic size (146 bp); whereas Seeta Sail with low CKE showed low allelic size of 123 bp. A total of 4 clusters were formed through SSR fingerprinting on nine selected rice varieties. Three normal traditional rice lines (Dharial, Pateni and Pakri) form a single cluster where Dharial and Pateni are closely linked to each other. Two aromatic rices (Gobindobhog and Narayanpurna) form another cluster which is connected with the cluster of normal traditional rice. Three lowland rice (Neizer Sail, Seeta Sail and Kabirraj Sail) forms another cluster where the former two (Neizer Sail and Seeta Sail) are closely connected. To this group of lowland rice, IR36 (an improved rice) forms an

out-group.


Physiological screening revealed that the seed germination (G%) of the studied rice landraces on 7th day was significantly low at the highest concentrations of both PEG (-1.5 MPa) and NaCl (300 mM). With increasing doses of stressors (PEG and NaCl), G% gradually decreased when compared with the control set. The significant exception was found for three genotypes (Lati Sail, Punjab Sail and Rup Sail) showing higher G% than the control for lower doses. Although, both PEG and NaCl inhibit seed germination, PEG showed more inhibitory effects than that of NaCl. Malik Sail, Neizer Sail and Rani Sail showed highest germination (100%) in all osmotic stress conditions. Chapri Sail, Punjab Sail, Rup Sail and Sankar Sail showed 100% germination in all NaCl doses, but not in PEG. Days to seed germination was also delayed with the increasing concentration of stressors. The phenotypic responses in form of shoot and root length in induced osmotic stresses (by PEG and NaCl treatment) were varied greatly. Shoot length in control was maximum (5.7 cm) in Malik Sail, whereas the minimum (3.2 cm) was obtained for Mallik Sail. Overall shoot growth in three different doses of PEG were more than the values found in three different NaCl concentrations. Shoot length in all concentrations of PEG and NaCl were lower than the control ones. Root length was lower in both Mallik Sail and Laxmi Sail (4.5 cm) and highest in Chapri Sail (7.1 cm). Compared to the control, lower concentration of PEG (-0.5 MPa) and NaCl (100 mM) increased the root length. The subsequent higher doses of stressors inhibit the root growth. This reduction in root length was higher in PEG rather than in NaCl doses. Chlorophyll (mg), proline (μg) and total protein (mg) content per gram of mature normal leaf tissue, in control and all stress conditions, showed a considerable variation for the rice landraces. Among the all rice cultivars, Sankar Sail showed the highest and Nekrai Sail showed the lowest quantity of chlorophyll in all the concentrations of PEG and NaCl. Protein content in the lower osmotic stresses, *i.e.* -0.5 MPa PEG and 100 mM NaCl, reveal that Laxmi Sail has gave the highest values (95.2 and 109, respectively) and Chapri Sail has gave the lowest values (56.7 and 58.2, respectively).

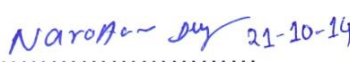
The fingerprint profile revealed that 38 SSR markers were proved to be polymorphic among the total used 45 SSR markers, while the rest seven (RM 8094, RM 10745, RM 315, RM 162, RM 10927, RM 3231 and RM 296) were

monomorphic in nature among the studied lines. A total number of 122 alleles were detected, of which 12 were found to be rare (produced only in a single genotype). The number of alleles per locus ranged from 2 (governed by a total of 11 SSRs, i.e., RM212, OSR2, RM287, RM8115, RM10843, RM10890, RM302, RM212, RM316, RM219 and RM288) to 5 (governed by 4 SSRs i.e., RM3412, RM493, RM206 and RM328), with an average of 3.21 alleles across the 45 loci. Based on the identified allelic size differences for the used SSRs, a microsatellite panel was constructed to present the SSR based amplicons' variability across the studied rice genotypes. Twelve rare alleles were identified from RM206 for Lat Sail (152 bp), RM10764 for Mihinagra Sail (156 bp), RM140 for Mallik Sail (210 bp), RM493 for Mallik Sail (130 bp), RM223 for Malik Sail (170 bp), RM562 for Lati Sail (284 bp), OSR28 for Malik Sail (130 bp), RM136 for Lati Sail (113 bp), RM201 for Lati Sail (170 bp), RM271 for Lati Sail (117 bp), RM321 for Mallik Sail (163 bp) and RM212 for Lati Sail (95 bp). The lowest amplicon size belonged to OSR 2 (57-66 bp) and the highest amplicon size belonged to RM 10772 (427-512 bp). The large monomorphic amplicon size was highest (173 bp) for RM 10745 and lowest (105 bp) for RM 162. Highest allelic frequency (90%) were revealed by RM 212 (111 bp) and RM 219 (165 bp) and lowest allelic frequency (35%) were observed for RM 3412 (200 bp), RM 206 (139 bp and 167 bp), RM 328 (270 bp), RM 215 (148 bp) and RM 257 (137 bp). It was noted that out of 5 alleles of RM 206, two alleles (139, 167 bp) having the same (35%) frequency. Similar results were found for RM 8115, RM 10890 and RM 107; where the former two loci showed 2 alleles with same frequency (50%) and the later (RM 107) showed alleles with same frequency (40%). Polymorphism information content or PIC values for 38 polymorphic markers were varied from 0.180 (for RM 212 and RM 219) to 0.765 (for RM 3412 and RM 328) with an average PIC value of 0.524. As the overall observation, the SSR loci RM 3412 followed by RM 328, RM 206 and RM 493, were found to be superior for analysis of allelic diversity in this study. Similarity matrix revealed that the similarity coefficient value ranged from 0.0 (between Balaram Sail - Nekrai Sail and Rup Sail – Sindur Sail) to 0.98 (between Lati Sail and Nagra Sail). The dendrogram showed four major clusters: Cluster I, II, III and IV. Within the first cluster, Chapri Sail and Kabirraj Sail are very much close to each other; whereas in second cluster, Rup Sail and Sindur Sail are closely related. In cluster - III and IV, the pairs of Rani Sail - Ratan Sail and Neizer

Sail - Punjab Sail are closely linked, respectively. The overall degree of polymorphism indicated that the allelic frequency of biallelic markers was higher than the tri-, tetra- and penta- allelic SSRs. The number of SSR amplified alleles and their PIC values were also depend upon the repeat motif and the repeat sequence of the microsatellites. Lower PIC values reflect the highly closeness among the studied rice genotypes showed that (CTT) and (AT) rich repeat motifs amplified with higher efficiency and revealed greater overall polymorphism. In this context, it was observed that SSRs (RM 493, RM 206 and RM 3412) with (CT) - rich di or tri-nucleotide repeats were effective for producing the highest number of alleles as well as highest PIC values than other SSR markers. While variation in the size of SSR amplified alleles result from a change in the number of repeats is the consequence of sequence changes at the primer binding site(s).

The genetic relationship among the studied rice genotypes after SSR based molecular profiling has also been confirmed by the physio-biochemical screening in relation to osmotic stress tolerance. Rup Sail and Sankar Sail showed 100% germination in only NaCl concentration, but not in PEG and were placed in an another cluster. Laxmi Sail, Mihinagra Sail, Ratan Sail and Raghu Sail were proved as stress susceptible cultivars and were placed in one cluster. On the other hand, Rup Sail-Sindur Sail and Punjab Sail-Neizer Sail pairs were found to be closely linked together in other two clusters, respectively. For the selection of the parental rice genotypes for the development of a mapping and/or breeding population, Malik Sail and Rani Sail showed 100% germination with improving other parameters in all stressor (PEG and NaCl) concentrations and were placed in a single cluster and thus we can denote these lines as stress tolerant rice genotypes in this present investigation. Derived from this research work, potential parental landraces can be used in future rice breeding program to generate a hybrid rice variety with overall desired traits after successful crosses with HYVs.


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