GENOTYPE-DEPENDENT RESPONSES OF LOCAL RICE TO EMS MUTAGENESIS: PHENOTYPIC VARIATION, SRAP PROFILES, AND PLOIDY ASSESSMENT

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Abstract

Local rice germplasm can contribute useful variation to crop improvement, yet many Indonesian landraces remain insufficiently characterised at the molecular level. This study assessed phenotypic responses, sequence-related amplified polymorphism (SRAP) profiles, and nuclear DNA ploidy in three local rice varieties—Badigal, Kepiting, and Tsunami—treated with ethyl methanesulfonate (EMS), using IR64 as a reference. Molecular analysis indicated significant differentiation among varieties (ΦPT = 0.432, p < 0.001), whereas grouping by EMS dose was not significant (ΦPT = −0.210, p = 0.974). Five of 16 agronomic traits showed significant differences in the reported analyses; filled-grain percentage was affected by variety and EMS dose, but their interaction was not significant. Genetic and phenotypic distance matrices were not significantly correlated (Mantel r = 0.1438, p = 0.1565). Flow-cytometric measurements showed G1 peak coefficients of variation below 10% in all 39 experimental units. These results indicate genotype-dependent SRAP profile variation after EMS exposure, while providing no evidence of altered ploidy in the tested material. Because SRAP band shifts do not identify causal mutations, and the study concerns the M1 generation, further sequence-based validation and evaluation of M2–M3 progenies are required.

Keyword: *EMS mutagenesis; flow cytometry; local rice; rice genetic resources; SRAP*.

Introduction

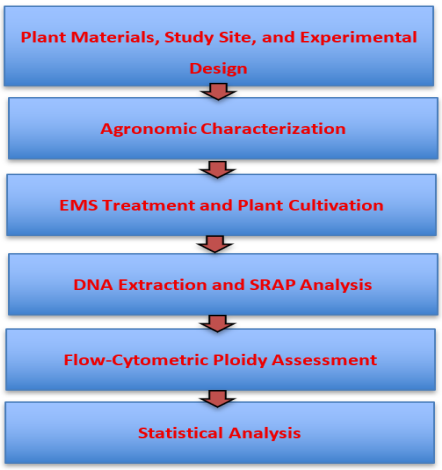
The local varieties Badigal, Kepiting, and Tsunami, originating from Banten and West Java, represent germplasm with distinct reported agronomic attributes. Badigal has been described as having relatively high grain weight but a limited proportion of well-filled grains; Kepiting has a moderate number of productive tillers; and Tsunami has been reported to show high productivity.

Chemical mutagenesis is one approach for generating new variation in self-pollinated crops. Ethyl methanesulfonate (EMS) is an alkylating agent widely used in mutation breeding. A recent large-scale analysis of rice accessions also reported substantial genotype-associated variation in EMS tolerance and mutagenesis efficiency (Xu et al., 2026).

Although EMS mutagenesis has been applied in rice, integrated information combining agronomic observations, multilocus marker profiles, and flow-cytometric assessment remains limited for many locally maintained Indonesian varieties. The present study therefore evaluated (i) phenotypic responses of Badigal, Kepiting, and Tsunami to four EMS concentrations, (ii) SRAP-based genetic profile variation and genetic structure, and (iii) nuclear DNA-content profiles as an indicator of ploidy stability. IR64 was included as an untreated reference cultivar.

The study is intended as an initial characterisation of treated material; it does not claim to identify causal mutations or establish heritability in later generations

Research Method



Picture 1. Research Method

Results & Discussions

Table 1. SRAP Marker Polymorphism

Primer pair	Total bands	Polymorphic bands	PPB (%)	PIC	MI	RP
Me1–Em1	9	8	88.89	0.329	2.63	4.31
Me1–Em2	6	6	100.0	0.387	2.32	3.23
Me1–Em3	6	5	83.33	0.284	1.42	2.46
Me1–Em4	5	5	100.0	0.289	1.44	2.0
Me2–Em1	7	3	42.86	0.156	0.47	1.69
Me2–Em2	5	4	80.0	0.317	1.27	2.62
Me2–Em3	11	7	63.64	0.181	1.27	2.77
Me2–Em4	6	4	66.67	0.095	0.38	0.62
Me3–Em1	2	2	100.0	0.414	0.83	1.23
Me3–Em2	7	5	71.43	0.213	1.07	2.0
Me3–Em3	16	12	75.0	0.234	2.8	5.54
Me3–Em4	8	3	37.5	0.089	0.27	0.92
Me4–Em1	10	6	60.0	0.23	1.38	3.38
Me4–Em2	5	4	80.0	0.369	1.48	3.08
Me4–Em3	9	6	66.67	0.255	1.53	3.38
Me4–Em4	4	2	50.0	0.142	0.28	0.77
Total / mean	116	82	70.69 overall; 72.87 mean	0.249	1.3	40.0

Table 2. EMS-Associated SRAP Band-Pattern

Variety	Stable bands	Band loss	Band gain	Reported change (%)
Badigal	99	6	10	13.9
Kepiting	60	31	24	47.8
Tsunami	68	13	34	40.9

Table 3. Genetic Distance, Clustering, and Dose Response

Variety	Pearson r: dose vs. Jaccard distance	SRAP profile change (%)	Mean testa thickness (µm)	Mean seed-core thickness (µm)
Badigal	0.6464	13.9	193.3	3063
Kepiting	0.9217	47.8	180.0	2500
Tsunami	0.7431	40.9	170.0	2940

Table 4. The AMOVA results

Grouping	Variance source	df	Sum of squares	Mean square	Variance component	Variance (%)	ΦPT	p
Variety	Among varieties	2	0.2599	0.13	0.02445	43.2	0.432	<0.001
Variety	Within varieties	9	0.2893	0.0321	0.03215	56.8	—	—
Variety	Total	11	0.5492	—	0.05661	100.0	—	—
EMS dose	Among doses	3	0.0836	0.0279	-0.01012	0.0	-0.21	0.974
EMS dose	Within doses	8	0.4657	0.0582	0.05821	100.0	—	—
EMS dose	Total	11	0.5492	—	0.04809	100.0	—	—

Conclusion

EMS-treated Badigal, Kepiting, and Tsunami showed variety-dependent changes in SRAP band profiles, with the highest reported profile-change index in Kepiting (47.8%), followed by Tsunami (40.9%) and Badigal (13.9%). AMOVA indicated significant molecular differentiation among varieties but no significant grouping by EMS concentration. Among the 16 agronomic traits, filled-grain percentage showed significant main effects of variety and EMS dose, although the interaction was not significant; the overall non-parametric results for leaf number and panicle length do not isolate EMS-dose effects. The Mantel test detected no significant association between molecular and phenotypic distance matrices. Flow-cytometric measurements showed no detectable change in nuclear DNA-content class, although definitive ploidy assignment requires an internal standard and peak-ratio information. The findings provide an initial characterisation of EMS-treated local rice material, but do not establish the identity or heritability of induced mutations. Evaluation of M2 and M3 generations, replicated molecular profiling, and sequence-based validation are required before selecting stable mutant lines for breeding.