



ENHANCED YIELD AND YIELD COMPONENT TRAITS OF THE MUTANTS DERIVED FROM RICE CV. SAMBA MAHSURI-SUB1 AND POKKALI THROUGH INDUCED MUTATION

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KeyWords

Abiotic stress, climate change, drought, genetic variability enhancement, induced mutation tissue culture, mutants, submergence, wildtype

ABSTRACT

Climate change resilient varieties are considered to be a long term solution to the yield loss brought about by certain phenomena caused by the changing climate. Abiotic stresses, such as drought, submergence and saline intrusions to rice field, are the most common climate-change related phenomena that cause yield losses in rice. Modern biotechnology tools, such as tissue culture and induced mutation by gamma radiation, are used in rice breeding to address this threat by enhancing genetic variability to improve plant architecture and phenotypic acceptability of rice cultivars with known tolerance to abiotic stresses but of poor phenotype. Tissue culture and gamma irradiation of submergence tolerant rice cultivar, Samba Mahsuri-Sub1 and saline tolerant, Pokkali, generated elite lines with improved phenotype and with retained abiotic stress tolerance. The study evaluated the field performance of the mutant lines in comparison with their respective wildtypes. The lines were evaluated for agronomic traits viz., panicle length, productive tiller, grain yield and yield components. Comparing Samba Mahsuri-Sub1 and its mutant, the mutant line had longer panicle length (23.1 cm) than the wildtype (21.9 cm). The grain yield of the mutant (9.290 tha⁻¹), though numerically greater than the wildtype (8.360 tha⁻¹), was statistically not significantly different. In terms of yield components, though the wildtype had greater no. of spikelets per panicle compared to the mutant, the grains were significantly longer by 5.5 % than the wildtype which contributed to a higher grain yield. Pokkali-derived mutant was improved in productive tiller by 4.22%. Grain yield of the Pokkali-mutant (9.750 tha⁻¹) was also significantly higher than the wildtype (2.740 tha⁻¹) by 7.0%. Yield component parameters of the mutants were also significantly higher, but grain size and shape was not significantly different to the original Pokkali. The improved breeding lines generated from induced mutation of Samba Mahsuri-Sub1 and Pokkali can be used as novel sources of genes for abiotic tolerance with acceptable phenotype and acceptable grain yield.

INTRODUCTION

Increasing rice production to meet the growing demands for food source due to increasing population has always been one of the main goals of the Department of Agriculture of the Philippine government. From 2015 to 2018, a 5% growth in Philippine population was incurred [1], whereas rice production was reduced by 1.4% from April to June of 2018 [2]. One of the long-term solution being exploited is the development of new high yielding rice genotypes through rice R&D adapted to changing climate affecting grain yield of the crops. Increasing yield has always been the primary objective of any rice breeding programs for both favorable and marginal rice environments. Utilization of various breeding technologies remains the main strategy of these programs in developing rice genotypes with superior grain yields. One of the most common and probably the most rapid technology is through induced mutation techniques such as gamma irradiation and *in vitro* culture (IVC). Since the introduction of these technologies and their efficiency in enhancing genetic variation for crop improvement, numerous crops with superior traits have already been developed through the technology and are currently being utilized worldwide [3]. From 2004 to 2017, more than 20 rice varieties adapted to fragile rice environments with higher yield were developed by Philippine Rice Research Institute (PhilRice) and are now commercially available for cultivation and consumption [4].

Through induced mutation rice genotypes *viz.*, traditional varieties, cultivars, accession with low yield and other non-desirable phenotypes, but contains good genes for abiotic tolerance, disease resistance and good grain quality are improved. *Samba Mahsuri-Sub1* and *Pokkali* are among these genotypes with poor phenotypes but of robust tolerance to submergence and salinity, respectively. In this study, these genotypes were subjected to tissue culture and gamma irradiation to improve their non-acceptable traits, but retaining their abiotic stress tolerance, and at the same time enhance their yield contributing traits to increase grain yield.

MATERIALS AND METHOD

IVC of submergence tolerant rice cultivar, *Samba Mahsuri-Sub-1*, and seed irradiation of 250 Gy of ^{60}Co gamma radiation of saline tolerant traditional rice variety, *Pokkali*, generated promising lines *viz.*, PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10 and PR34358-5-Pokkali-AC37 (DrS93), respectively. These lines, together with their corresponding wildtypes, were evaluated for field performance in 2018 dry season (DS).

Field performance evaluation of the mutant lines

The evaluation was laid out in an RCBD experimental design, with three replications and a plot area of 12 rows x 22 hills, with 25 cm distance in between rows and hills. The lines and the wildtypes were evaluated for agronomic traits, grain yield, yield component and grain size and shape. The evaluation was conducted based on the standard protocol for field evaluation published in the Field Operations Manual (2007) of the Philippine Rice Research Institute (PhilRice) [5].

Gathered Data and Statistical Analysis

The mutant lines and the wildtypes were evaluated for agronomic traits, which included panicle length- measured from the neck to the tip of the last spikelet, and the no. of productive tiller- obtained from a 2 x 2 hill sampling area. Grain yield was obtained by weighing the harvested dried grains from the harvested plots. Moisture content was corrected based on the moisture correction factor published in the PhilRice Operations Manual (2007), and yield was adjusted using the formula published in the same manual. For yield components, four 3 hill x 3 hill sampling units were randomly collected. Grains of the harvested hills were bulked and were counted for filled and unfilled grains per m^2 . The grain weight of 1000 seeds was also determined. Grain size and shape was determined by measuring the grain length (GL) and grain width (GW) of 10 decorticated grains using a digital caliper (Chicago Brand, Model no. 5001). Grain shape (GS) was determined by getting the ratio of the grain length and width. GL, GW and GS of the grains were classified based on the published classification (PPV and FRA, 2007).

Analysis of variance (ANOVA) was used to analyze the variation in responses using PB Tools Version 1.3 (2013-2020) and STAR Version 2.01.1 (2013). Means were compared using Tukey's Comparison of means, and mutant lines were compared to the wildtype by Dunnett's comparison of means, at Alpha =0.05.

RESULTS AND DISCUSSION

Evaluation for Agronomic Traits and Grain Yield (Table 1)

Panicle length of the PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10 (23.1 cm) was significantly higher by 5.52% (1.2 mm) compared to the wildtype, Samba Mahsuri-Sub1 with panicle length of 21.9 cm. In terms of no. of productive tillers, the mutant (25 tillers) was comparable to the wildtype (24 tillers). Though no significant difference was observed in grain yield of the mutant (9.290 tha^{-1}) and the wild type (8.360 tha^{-1}), the yield of the latter was increased by 11.1%. On the other hand, panicle length (24.4 cm), and no. of productive tillers (17 tillers) of the mutant line PR34358-5-Pokkali-AC37 (DrS93) from Pokkali was not significantly different compared to the wildtype (panicle length of 25.3 cm and productive tiller of 17). Grain yield of the Pokkali mutant line (9.750 tha^{-1}) was significantly higher than the wildtype (2.740 tha^{-1}) by 256%. The result of the evaluation for both genotypes showed the

efficiency of induced mutation in improving the agronomic traits and grain yield [7] of breeding line

Table 1. Agronomic traits and grain yield of the mutant lines in comparison with the wildtype, PhilRice, CES, 2018DS.

No.	Genotype	Grain Yield		Productive Tiller (no.)		Panicle Length	
		tha ⁻¹	RD/WT (%)	no.	RD/WT (%)	cm	RD/WT (%)
1	Samba Mahsuri-Sub1	8.360 ^a		25 ^a		21.9 ^c	
2	PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10	9.290 ^a	11.1	24 ^a	-1.36	23.1 ^{bc}	5.52
3	Pokkali	2.740 ^b		17 ^b		25.3 ^a	
4	PR34358-5-Pokkali-AC37 (DrS93)	9.750 ^{a*}	255.8	17 ^b	4.22	24.4 ^{ab}	-3.30
	Minimum	2.74		17		21.90	
	Maximum	9.75		25		25.30	
	Range	7.01		8		3.40	
	Mean	7.54		20.75		23.68	
	Standard Deviation	3.25		4.35		1.49	
	Coefficient of Variation	43.11		20.96		6.29	

Note: Means with the same letter are not significantly different at Alpha=0.05 by Tukey's comparison of means

*Significantly different to the wildtype by Dunnett comparison of means at alpha=0.05

RD – Relative difference/Wildtype (WT) = ((entry-check)/Check)*100

Evaluation of Yield Component Parameter

For yield component traits, results showed that *Samba Mahsuri-Sub1* (wildtype) had higher no. of spikelets/m² and no. of filled and unfilled grains/m² compared to the mutant line. However, a reduction in unfilled grains was obtained from the mutant line (319) compared to the wildtype (1,721), indicating a higher %fertility in the mutant. Due to increase in grain size and shape (*Figure 1a and 1b*), 1000 seed weight was also increased in the mutant line (25.6 g) compared to the wildtype, weighing 12.7 g (*Table 2*). These improvements in yield contributing traits would explain why PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10 had numerically higher yield than the wildtype, despite of the reduction in the no. of spikelets. This implies that the increase in grain yield of the mutant line was compensated by the reduction in unfilled grains, and the increase in weight of 1000 seeds and in the size and shape of the grains.

Whereas for *Pokkali*, the wildtype was significantly lower than its mutant line in terms of filled and unfilled grains, and in total no. of spikelets, but has heavier weight of 1000 seeds (*Table 2*). The heavier seed weight of the mutant can be attributed to its longer and wider grains (*Figure 1c and 1d*). The increase in the no. of spikelets of the mutant line PR34358-5-Pokkali-AC37 (DrS93) contributed to the increased in grain yield of the mutant line compared to the wildtype.

Table 2. Yield components of the mutant lines compared to the wildtypes, PhilRice, CES, 2018DS.

No.	Genotype	Total no. of spikelets/m ²	No. of filled grains/m ²	Fertility (%)	No. of unfilled grains/m ²	1000 Seed Weight (g)
1	Samba Mahsuri-Sub1	9597 ^a	7876 ^a	82.07	1721 ^a	12.7 ^c
2	PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10	4751 ^{b*}	4433 ^{b*}	93.29	319 ^{b*}	25.6 ^{b*}
3	Pokkali	3225 ^c	3083 ^c	95.60	142 ^b	28.3 ^a
4	PR34358-5-Pokkali-AC37 (DrS93)	5241 ^{b*}	4765 ^b	90.91	476 ^{b*}	24.7 ^{b*}
	Minimum	3225	3083		142	12.70
	Maximum	9597	7876		1721	28.30
	Range	6372	4793		1579	15.60
	Mean	5703.50	5039.25		664.50	22.82
	Standard Deviation	2733.95	2026.23		717.43	6.92
	Coefficient of Variation	47.93	40.21		107.96	30.32

Note: Means with the same letter are not significantly different at Alpha=0.05 by Tukey's comparison of means

*Significantly different to the wildtype by Dunnett comparison of means at alpha=0.05



Figure 1. Grain size and shape of wildtype, Samba Mahsuri-Sub1 (a), compared to PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10 (b), and of wildtype, Pokkali (c) compared to PR34358-5-Pokkali-AC37 (DrS93) (d).

Evaluation for Grain Size and Shape

Grain Width (GW) of PR41905-Samba Mahsuri-Sub1-IVC2010DS 31-1-10 (10.2 mm) was significantly increased by 42.6% (3.1 mm) compared to the wildtype with grain width of 7.2 mm, shifting its classification from medium to long grains (PPV and FRA, 2007). Grain Length (GL) of the mutant line (2.5 mm) was significantly broader by 43.7% (0.8 mm) compared to the wildtype with 1.7 mm, shifting the grain width classification from narrow to broad grains (PPV and FRA, 2007). No significant changes in GS (grain shape) was observed from the mutant line.

On the other-hand, GW (8.3 mm) and GL (2.3 mm) of PR34358-5-Pokkali-AC37 (DrS93) was not significantly different from the wildtype with 8.9 mm GL and 3.1 mm GW, though numerically there was a reduction in GL and GW. The reduction in the grain dimension of the mutant line, shifted the classification of GS from intermediate grains (2.9 mm) to slender grains (3.6 mm).

Table 2. Yield components of the mutant lines compared to the wildtypes, PhilRice, CES, 2018DS.

No.	Genotype	Total no. of spikelets/m ²	No. of filled grains/m ²	Fertility (%)	No. of unfilled grains/m ²	1000 Seed Weight (g)
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Correlation Analysis: Grain Yield and Yield Component Parameter

Correlation analysis showed the relationship of the yield components and grain morphology to grain yield (Table 4). Moderate to strong positive correlation was observed between grain yield and productive tiller (0.47), no. of filled grains (0.46), total no. of spikelets (0.43) and grain shape (0.60). This indicates that when these parameters increases in value, the grain yield also increases. On the other hand, weak to moderate negative correlation was observed between grain yield and panicle length (-0.48), 1000 seed weight (-0.37), grain length (-0.12) and grain width (-0.55), which means a decrease in these parameters cause a decrease in grain yield.

Table 4. Pearson Correlation analysis of the traits in reference to grain yield, PhilRice, CES, 2018 DS.

Agronomic/Yield Component Trait	Grain Yield	Pr(> F)
Panicle length	-0.48	0.1177
Productive tiller	0.47	0.1239
Total number of spikelets/m ²	0.43	0.1611
Number of filled grains/m ²	0.46	0.1337
Number of unfilled grains/m ²	0.29	0.3548
1000 Seed Weight	-0.37	0.2385
Grain length	-0.12	0.6498
Grain width	-0.55	0.0654
Grain shape	0.60	0.0399

Pearson Correlation Value: 0-0.19 – very weak, 0.2-0.39- weak, 0.4-0.59-moderate, 0.6-0.79-strong, 0.8-1.0- very strong

The positive correlation of the yield component parameters viz., productive tiller, no. of spikelets, no. of filled and unfilled grains, contributed to the increase in grain yield of the mutant lines.

Grain yield and yield component traits are quantitative traits controlled by polygenes. The results of the evaluation showed that gamma radiation and tissue culture can induce mutation in quantitative characters. This generalization was proven by previous studies [8, 9, 10, 11, 12] that showed the effectiveness of mutation in inducing polygenic variability for quantitative traits important in crop breeding. Various research workers have reported that the possible mechanism of induced mutation for quantitative traits is micro-mutations [13, 14]. Micro-mutation or polygenic mutation is a mutation-al event that causes small modifications that are useful in improving quantitatively inherited traits of crops without affecting its major genotypic composition and phenotype [15].

CONCLUSION

Evaluation of the mutant lines and the wildtypes for agronomic traits and yield showed significant differences among traits. Grain yield was increased in mutant lines as a result of significant increase in yield contributing traits such as productive tillers, panicle length, spikelet no., grain weight and grain size and shape. Utilization of induced mutation viz., tissue culture and gamma irradiation, enhanced the genetic variability of the submergence tolerance Samba Mahsuri-Sub1 and the saline tolerant Pokkali resulting in the improvement of their yield component parameters and eventually their grain yield

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References

- [1] Worldometer – Real Time World Statistics, www.worldometer.com.
- [2] Philippine Statistics Authority, Rice and Corn Situation and Outlook Report, July 2018.
- [3] International Atomic Energy Agency (IAEA), Annual Report, 2016.
- [4] National Seed Industry Council, Bureau of Plant Industry, Released Variety, 2018.
- [5] Philippine Rice Research Institute, Field Operations Manual, 2007.
- [6] Protection of Plant Varieties (PPV) and Farmer's Right Authority (FRA), Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Rice, 2007.
- [7] A. Sharma and S.K. Singh, Inuced mutation- a tool for creation of genetic variability in rice (*Oryza sativai L.*), Journal of Crop and Weed 9 (1):132-138, 2013.
- [8] W.C. Gregory, Mutation Breeding, Plant Breeding (FREY, K.J., Ed.), Iowa State University Press, 1966.
- [9] Jegadeesan, Souframanien. (2016). Effect of Gamma Rays and Electron Beam on Various Quantitative Traits of Rice (*Oryza sativa L.*) in M1 Generation. Advances in Life Sciences. 5. 1876-1882.
- [10] Ramchander, S. *et al.* (2015). Quality characteristics of rice mutants generated through gamma radiation in White Ponni. International Journal of Agricultural Sciences, Volume 7, Issue 10, pp 714-718.
- [11] Nair, V.G. 1972. Induction of variability in quantitative traits of rice through mutagenic treatment. Agric. Res. J. Kerala, 10:119-124.
- [12] Rao, H.K.S. and A.R.G. Ayengar, 1963. Radiation induced high yielding mutant in rice. Indian J. Genet., 26:312-322.
- [13] Sharma B., Sharma S.K. (2004) Induced Mutations and Selection Techniques for Quantitative Traits. In: Jain H.K., Kharkwal M.C. (eds) Plant Breeding. Springer, Dordrecht.
- [14] Wani, Mohd & Kozgar, M & Tomlekova, Nasya & Khan, Samiullah & Gul, Alvina & Sheikh, Ahmad & Ahmad, Parvaiz & R Wani, M & Sheikh, Subzar & Tomlekova, N & Khan, Sami & G Kazi, A & Ur, Atta. (2014). Mutation Breeding: A Novel Technique for Genetic Improvement of Pulse Crops Particularly Chickpea (*Cicer arietinum L.*). 2. 10.1007/978-1-4614-8824-8_9.
- [15] Sharma, A.K., Sharma, Ramavtar, 2014. Crop Improvement and Mutation Breeding.