New Rainfed-Drought Rice Variety developed through In Vitro Mutagenesis

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Abstract

In vitro mutagenesis (IVM), a technique combining tissue culture and gamma irradiation (30 Gy ^{60}Co), was performed with a modern upland rice variety NSIC 2001 Rc 9 (NSIC Rc9), locally known as Apo. The breeding strategy was implemented to improve the variety's grain quality, specifically its grain size and shape, and amylose content. The induced mutation strategy regenerated 55 IVM_1 families from which the IVM_2 population was generated comprising of 206 segregating plants. Phenotypic variability assessment conducted in 2009 wet season resulted in the selection of 150 plants with acceptable phenotypes. After a series of evaluations and selection for grain quality, drought stress tolerance and grain yield, seven elite lines were selected for multi-environment trial (MET) in four rainfed locations. The MET identified four lines ready for nomination to the National Cooperative Testing (NCT) for further evaluation and recommendation to the National Seed Industry Council (NSIC) as a new variety for release. In 2015, the line PR41395-NSICRc9-IVM2009DS 50-1-4 (IVM-032) was entered into NCT as one of the breeding lines evaluated under the rainfed-drought prone rice ecosystem. In 2019, the line was released as a new variety recommended for cultivation and consumption. The IVM-032 was officially registered as NSIC 2019 Rc572 and with the variety name of Sahod Ulan 28.

***Keywords**: gamma irradiation, induced mutation, mutation breeding, tissue culture, upland*

1. Introduction

The development of new rice genotypes resilient to environmental stresses in tandem with appropriate cultural management is one of the long-term solutions for adapting to the drastic effects of climate change on agriculture. Climate change has reduced yields of different crops from 3.5 to 13.4% (Ray *et al.*, 2019). For rice alone, yield reduction due to climate change-related phenomena such as drought and submergence was 0.3% average each year and 0.9% for wheat (Ray *et al.*, 2019). In contrast, drought-tolerant crops benefited from climate change. An increase in yields in sorghum by 0.7% in sub-Saharan Africa and 0.9% yearly in western, southern and southeastern Asia was observed (Food and Agriculture Organization, 2016).

For the past decades, various crop improvement strategies were employed in plant breeding to develop new rice lines with durable adaptation to changing climate. One of the most popular strategies resorted to was induced mutation (Suprasanna *et al.*, 2015). To date, about 3000 mutant crops were produced across the globe from which 833 were rice (Mutant Variety Database of the International Atomic Energy Agency [IAEA], 2020). These mutant varieties possess higher yields, disease resistance, improved quality and resilience to environmental stresses. Most of these mutant cultivars were released in marginal regions, thereby boosting their economic status (Mir *et al.*, 2020). From 1971 to 2013, the Philippines has released 11 rice mutants as varieties for commercial cultivation; two of which – the *Sahod Ulan 2* and *Sahod Ulan 11* – were developed by the Philippine Rice Research Institute (PhilRice). These mutants were characterized as improved lines in terms of agronomic features, abiotic stress tolerance, disease and pest resistance and grain quality (IAEA, 2020).

In vitro mutagenesis (IVM), which combines tissue culture and physical mutation employed in various crops, has been proven to generate novel genetic variations important to crop breeding (Larkin and Scowcraft, 1981; Brar and Jain, 1998). The technique is widely used in crop improvement specifically for abiotic stress tolerance (Flowers, 2004). The IVM was successful in inducing salt tolerance in various crops including rice (Saleem *et al.*, 2005), sugarcane (Nikam *et al.*, 2015), peanut (Wang *et al.*, 2015), rapeseed (Mosleh *et al.*, 2009) and wheat (El-Sayed *et al.*, 2007).

This paper presents the utilization of the IVM technique in the development of a new rice variety from the modern upland Philippine cultivar, *Apo*, officially registered as NSIC 2001 Rc 9 (NSIC Rc9) in 2001. NSIC Rc9 is known for its tolerance to drought and good performance under aerobic rice culture systems (Kato and Katsura, 2015). The NSIC Rc9-derived new variety was improved in terms of grain quality specifically its grain dimensions and amylose content through combined in vitro culture and gamma irradiation.

2. Methodology

Mature seeds of NSIC Rc9 were cultured in vitro in 2009 dry season (DS). The established seed culture protocol of the Plant Breeding and Biotechnology Division of PhilRice, Philippines was followed.

2.1 Callus Induction, Regeneration and Gamma Irradiation

Rough rice grains were dehulled using a rice tester (JLGJ2.5, Satake, Japan) and cleaned manually removing broken and mixed grains to maintain the purity of the genotype. Brown rice grains were sterilized with 50% (v/v) sodium hypochlorite with agitation at 200 rpm for 30 min using an orbital shaker (MaxQ2000, Thermo Scientific, United States) and rinsed three times with sterilized distilled water. The procedure was repeated and the surface-sterilized seeds were blot dried in sterile petri plates inside the laminar flow hood. The dried surface-sterilized seeds were cultured in the callus induction medium (CIM) containing Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal salts supplemented with 1-mgL^{-1} naphthalene acetic acid (NAA), 1-mgL^{-1} 2,4-dichlorophenoxyacetic acid and 1-mgL^{-1} 6-benzylaminopurine as plant growth regulators and solidified with 3-gL^{-1} agar (Pronadisa, Belman Laboratories, Quezon City) and 2-gL^{-1} gelrite (Sigma Aldrich, Singapore). The CIM were dispensed in Gerber bottles amounted to 30 mL and were autoclaved (TOMY SX-7000, Tomy Tech, United States) with 0.1 MPa for 15 min at 115 °C steam-sterilizing temperature. The cultures were incubated in the dark at 27±2 °C for two weeks until friable calli were formed.

The calli produced from the scutellar tissues of the cultured seeds were excised with sterile forceps and a scalpel. Callus tissues were separated from the endosperm and the coleoptile and transferred into a half-strength MS medium contained in a petri dish. The cultures were irradiated with 30 Gy of ^{60}Co gamma rays at the Philippine Nuclear Research Institute (PNRI), Quezon City. The irradiated calli were sub-cultured in a regeneration medium (RM) within 24 h after irradiation containing MS basal salts and supplemented with 0.5-mgL^{-1} NAA and 2-mgL^{-1} kinetin. The cultures were incubated under 16 h of continuous light from cool white fluorescent lamps followed by 8 h of continuous darkness per day at 25±2 °C for four to six weeks until regenerated shoots and roots have fully developed. Plantlets were hardened for three to five days under laboratory conditions and seven days under gradual sunlight exposure duration before transplanting them in the screen house for growing to maturity. The matured plants (IVM_1) produced the seeds, which were harvested and comprised the IVM_2 plant population, were subjected to evaluation and selection.

2.2 Variability Evaluation of IVM_2 Plant Population for Agro-morphological Traits

The IVM_2 seeds harvested from each IVM_1 plant were sown panicle-to-a-row under field conditions for agro-morphological trait variability evaluation. Each IVM_2 plant grown was characterized at vegetative and reproductive stages for variations induced in 12 morphological and six agronomic traits. Descriptive statistics to include frequency distribution, histograms, skewness, kurtosis, Shannon-Weaver diversity index (SWI) and cluster analysis were used to describe the variations. The SWI was computed based on the formula of Hutcheson (1970) (Equation 1).

$$H = \frac{-\sum_{i=1}^{n}\left(p_i * ln\, p_i\right)}{Log_2\,(n)} \quad (1)$$

where H is the Shannon diversity index; p_i is the fraction of the entire population made up of variation I (proportion of variant i relative to total population size); n is the population size.

Plants with acceptable phenotypes were selected and evaluated for uniformity and stability at the IVM_3 generation.

2.3 Grain Yield Evaluation of the IVM Lines

The selected lines were evaluated for grain yield in several trials. In 2011 DS, the selections were initially evaluated for agronomic traits and yield under observational nursery (ON). In the 2011 wet season (WS) and 2012 DS, preliminary (PYT) and general yield trials (GYT) were also conducted, respectively, at PhilRice, Central Experiment Station (CES) in Nueva Ecija. The PYT was done under irrigated (IL) and managed drought stress (MDR) conditions. The trials were conducted at PhilRice, CES in Nueva Ecija. The data were analyzed using the Statistical Tools for Agricultural Research (STAR) version 2.0.1 (International Rice Research Institute [IRRI], 2013a) and Plant Breeding Tools version 1.3 (IRRI, 2013b). Experimental details of each trial are presented in Table 1.

Table 1. Experimental details of the field evaluation trials (PhilRice, CES)

Description	ON	PYT		GYT
		Non-stress (IL)	Stressed (MDR)	
Year/season	2011 DS	2012 DS		2012 WS
No. of entries	267	86		48
No. of check varieties	7	7		7
No. of replication	Unreplicated	3		3
Experimental design	Augmented	RCBD		RCBD
Date sown	Dec. 28, 2010	Dec. 20, 2011	Dec. 27, 2011	June 12, 2012
Date transplanted	Jan. 19, 2011	Jan. 12, 2012	Jan. 19, 2012	July 11, 2012
Plot size	4.2 m^2	6.72 m^2	5.04 m^2	10 m^2
No. of rows	5	8	6	10
No. of hills/row	21	21	21	25
Planting distance between rows and hills	20 x 20 cm	20 x 20 cm		20 x 20 cm

2.4 Performance of Multi-environment Trial (MET) of the IVM Lines

Lines identified with superior grain yield compared with the check variety were selected and forwarded to MET. Locations of the MET were fully characterized as a rainfed rice ecosystem based on weather parameters, specifically the frequent occurrence of precipitation. The trial was conducted in six locations across the country – CES, Nueva Ecija; Negros Occidental; Isabela; Cagayan; Batac, Ilocos Norte; and Iloilo – with three replications in each location. Lines in each and across locations were ranked according to grain yield.

2.5 Abiotic and Biotic Response, and Grain Quality Test of PR41395-NSICRc9-IVM2009DS 50-1-4 (IVM-032)

The selected line, PR41395-NSICRc9-IVM2009DS 50-1-4 (IVM-032), was evaluated for drought stress tolerance at seedling and reproductive stages in 2010 WS and 2011 DS, respectively. Blast resistance screening was conducted in 2011, 2014, 2016 and 2017 WS. Evaluation for grain quality traits – milling recovery, physical attributes and physicochemical properties – was carried out in 2014 WS. The established evaluation protocols for abiotic stress, pest and disease and grain quality were followed (National Cooperative Testing [NCT] Manual of PhilRice [1997]).

2.6 Performance of IVM-032 in the NCT

The IVM-032 was evaluated for field performance under the NCT in three rainfed locations – La Union, Negros Occidental and Cagayan from 2015 to 2017 WS.

2.7 Genetic Diversity Assessment

To further establish the variation of the IVM-032 from other IVM lines and the wildtype (NSIC Rc9), phenotypic and genetic diversity assessments were conducted (Figure 1). Phenotypic diversity assessment was done using morphological markers (phenotypic traits), which were analyzed by complete linkage cluster analysis of the STAR. On the other hand, genetic diversity assessment was carried out by simple sequence repeat (SSR) markers analyzed via simple matching coefficient using NTSYS version 2.0 (Exeter Software, India). The schematic diagram of the development and evaluation of IVM-032 is presented in Figure 1.

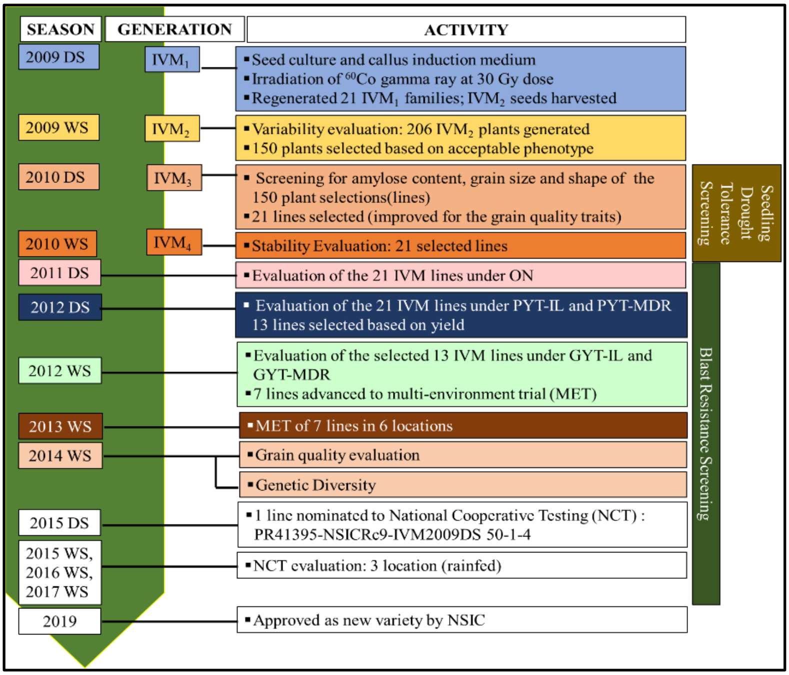


Figure 1. Schematic diagram summarizing the evaluation and selection of the IVM line derived from NSIC 2001 Rc 9

3. Results and Discussion

3.1 In Vitro Culture Response

Of the 300 dehulled rice grains cultured in CIM in 2009 DS, 187 (62.3%) seeds produced callus (Table 2) of which 120 callus pieces were irradiated with 30 Gy.

Table 2. In vitro response of NSIC Rc9 in callus induction and regeneration media (PhilRice, CES, 2009 DS)

Callus induction and irradiation	Frequency/percentage
No. of seeds plated (SP)	300
No. of seeds with callus formation (CF)	187
Percent CF/SP (%)	62.3
No. of callus irradiated (CI)	120
Plant Regeneration	
No. of regenerated callus	58
Percent regeneration/CI (%)	48.3
No. of plants regenerated	55
No. of plants regenerated/callus	1
No. of proliferated callus	15
Percent proliferated callus/CI (%)	12.5
No. of rooted callus	31
Percent rooted callus/CI (%)	25.8
No. of necrotic callus	16
Percent necrotic callus/CI (%)	13.3

Regeneration efficiency of 48.3% (58 calli) was obtained and each of the calli gave rise to an average of one plantlet. A total of 62 IVM_1 plants were recovered but only 21 (34%) plants survived to maturity under greenhouse conditions. From the 21 IVM_1 plants that produced seeds, 206 IVM_2 plants were generated. The responses of the culture seeds are presented in Figure 2.

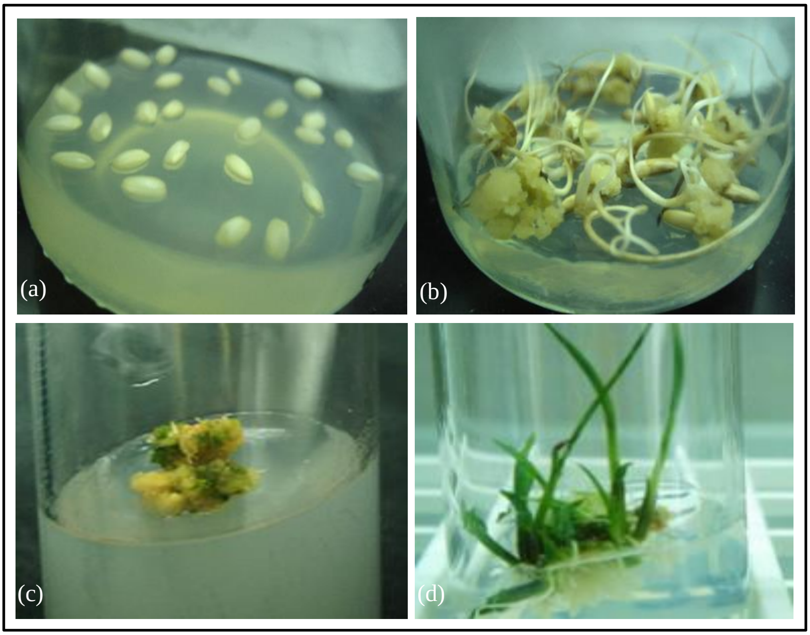


Figure 2. Seeds cultured in CIM (a); callused seeds, two weeks in CIM (b); callus with developed embryos, two weeks in RM (c); regenerated plants, four weeks in RM (d)

3.2 Variability Evaluation of Morphological Traits

The 206 IVM_2 plants generated were evaluated for variability in 12 morphological traits at vegetative and reproductive stages. In both stages, induced variation was observed in all of the traits evaluated, namely blade pubescence, blade color, basal leaf sheath color, blade angle, ligule color, collar color, auricle color, culm angle, flag leaf angle, panicle type, secondary branching and panicle exsertion (Figures 3 and 4). Similarly, variations were also observed among the six agronomic traits such as the number of days to heading (50%), the number of days to maturity (80%), plant height, culm length, panicle length and the number of productive tillers (Table 3) with the latter having the highest (CV = 36.9%) variability and days to maturity with the lowest (CV = 3.7%) variability.

Based on the morphological and agronomic traits, 150 (73%) plants with improved traits and good phenotypic acceptability were selected. The selections proved the efficiency of in vitro mutagenesis in increasing the mutation efficiency, thereby producing individuals containing at least one induced mutation introduced while retaining the existing good traits (Sharma and Singh, 2013).

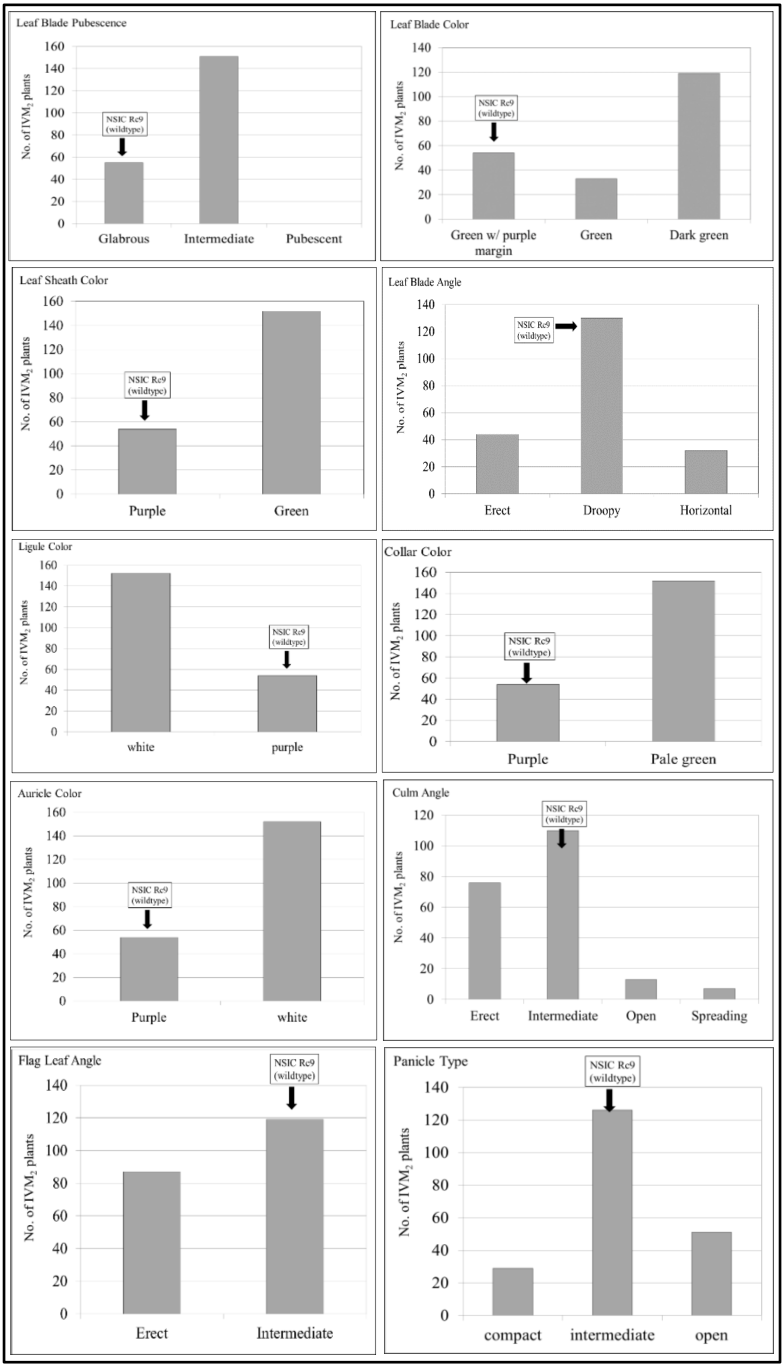


Figure 3. Frequency distribution and variability of the IVM_2 population for the 12 morphological traits evaluated (PhilRice, CES, 2009 WS)

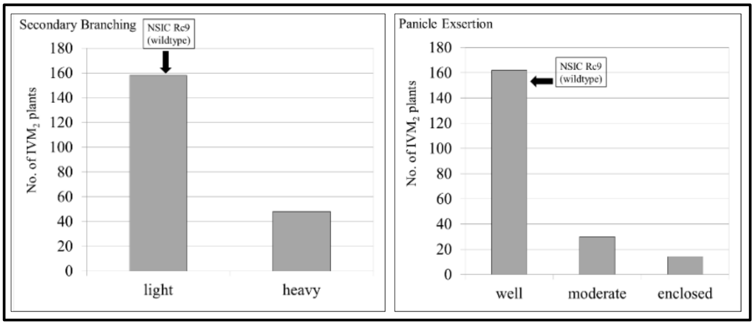


Figure 3 continued.

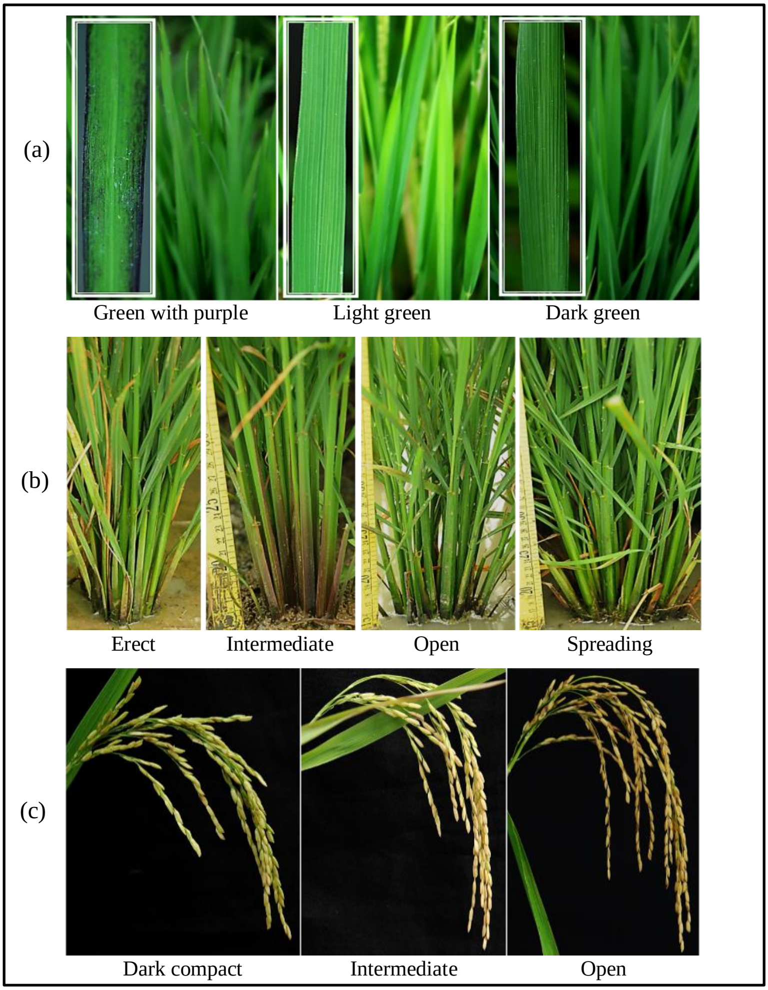


Figure 4. Variations in leaf blade color (a), culm angle (b) and panicle type (c) observed among the IVM$_2$ population (PhilRice, CES 2009 WS)

Table 3. Variability of the IVM_2 population for six agronomic traits (PhilRice, CES, 2009 WS)

Agronomic trait	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Days to heading (50%)	73	98	86	3.23	3.73
Days to maturity (80%)	103	128	116	3.23	2.77
Plant height (cm)	70	148.50	102	15.19	14.89
Culm length (cm)	53	128.50	81	15.13	18.71
Panicle length (cm)	16	34	21	3.0	14.10
No. of productive tiller/hill	4	32	14	5.24	36.87

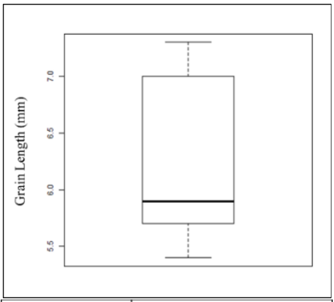
3.3 Screening for Grain Dimension and Amylose Content

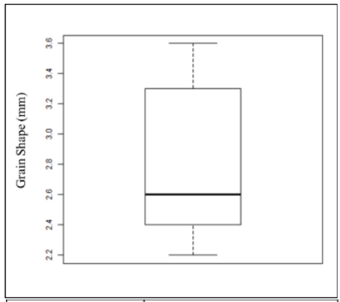
To identify the lines to be advanced further, grain size and shape and amylose content (AC) were evaluated in 2010 DS. The majority (55%) of the lines had medium grain length and intermediate (59%) grain shape. Intermediate amylose content was recorded for the majority (55%) of the lines (Figure 5).

Out of the 150 lines selected for phenotypic acceptability, 21 (14%) lines possessed a combination of improved grain quality traits and amylose content of 18-22% (Tables 4 and 5).

3.4 Grain Yield Evaluation of the Selected IVM Lines

The 21 selected IVM lines were successively evaluated to determine their grain yield performance under non-stress, irrigated lowland (IL) and managed-drought stress (MDR) conditions. Initially, the lines were evaluated in ON under irrigated conditions in 2011 DS (Figure 6). The lines flowered from 74 to 92 days after seeding (DAS) and yielded from 1.23 to 5.0 t ha^{-1} grains. The line IVM-032 was the highest yielding entry with 72% (2.1 t ha^{-1}) and 12% (0.5 t ha^{-1}) yield advantage over the wildtype, NSIC Rc9, which produced 2.9 t ha^{-1} and the highest yielding check, PSB Rc82, which made 4.5 t ha^{-1}.



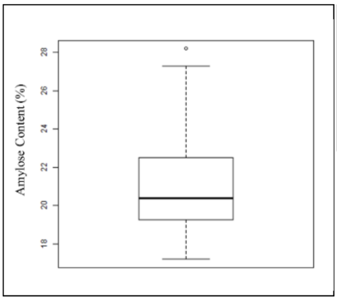


Grain length	Frequency	
	no.	%
Long	66	44.0
Medium	83	55.3
Short	1	0.7
Total	150	

Grain length (mm); extra long (EL) ≥ 7.5; long (L) 6.6 - 7.4; medium (M) 5.5-6.5; short (Sh) ≤ 5.4 and below

Grain shape	Frequency	
	no.	%
Intermediate	89	59.3
Slender	61	40.7
Total	150	

Grain shape (mm); slender (S) > 3.0; intermediate (I) 2.0-3.0; bold (B) < 2.0



Amylose content	Frequency	
	no.	%
High	4	2.7
Intermediate	82	54.7
Low	64	42.7
Total	150	

Amylose content
Waxy/glutinous (W) < 2.0%
Very low (VL) 2.1% - 10.0%
Low (L) 10.1% -17.0%
Intermediate (I) 17.1% - 22.0%
High (H) 22.1% and above

Figure 5. Frequency distribution of the selected IVM lines for grain size and shape, and amylose content (PhilRice, CES, 2010 DS)

In 2012 DS, the same selected lines were evaluated further in PYT under IL and MDR conditions. Under IL, lines IVM-032 and IVM-057 yielded 8.7 and 8.3 t ha^{-1}, respectively. Both were 50% (IVM-032) and 42% (IVM-057) significantly higher than the wildtype, NSIC Rc9 (5.8 t ha^{-1}). They had 8% (IVM-032) and 3% (IVM-057) more grain yield than the highest yielding check, PSB Rc14, which produced 8.0 t ha^{-1} (Table 6). Under MDR, lines IVM-032 (1.2 t ha^{-1}) and IVM-105 (1.2 t ha^{-1}) yielded significantly higher than the wildtype (0.6 t ha^{-1}) by 113 to 117%, respectively, and PSB Rc 14 (0.9 t ha-^{1}) by 250 to 575%, respectively. The stress tolerance index (STI) values of 19 (90%) IVM lines, ranging from 0.078 to 0.221, were higher than the wildtype (0.068), whereas the two (10%) lines (IVM-032 and IVM-105) had 0.221 and 0.275 STI value, respectively – higher than that of PSB Rc14 (0.140).

Table 4. Frequency of IVM lines with combined improvement in grain size and shape and amylose content (PhilRice, CES, 2010 DS)

Trait combination	Frequency	
	No.	%
Short-intermediate grains and low AC	1	0.7
Medium-intermediate grains and low AC	60	40.0
Medium-intermediate grains and intermediate AC	22	14.7
Long-slender grains and low AC	3	2.0
Long-slender grains and intermediate AC*	56	37.3
Long-slender grains and high AC	4	2.7
Long-intermediate and low AC	1	0.7
Long-intermediate and intermediate AC	3	2.0
Total	150	100

IVM-208 Short-intermediate

IVM-40 Medium-intermediate

IVM-70 Long-slender

*selected lines with AC = 18 to 22% only

Table 5. Selected IVM lines with improved grain length (GL) and grain shape (GS), and amylose content (AC) (PhilRice, CES, 2010 DS)

No.	IVM code	Line designation	GL		GS		AC	
			mm	Cl	mm	Cl	%	Cl
1	IVM-032	PR41395-NSIC Rc9-IVM2009DS 50-1-4	7.0	L	3.3	S	19.4	I
2	IVM-054	PR40059-NSIC Rc9-IVM2009DS 2-1-1-1	7.2	L	3.5	S	21.9	I
3	IVM-057	PR41395-NSIC Rc9-IVM2009DS 3-1-1	7.0	L	3.4	S	21.9	I
4	IVM-060	PR41395-NSIC Rc9-IVM2009DS 3-1-3	7.0	L	3.3	S	18.8	I
5	IVM-062	PR41395-NSIC Rc9-IVM2009DS 50-2-2	7.3	L	3.6	S	21.6	I
6	IVM-068	PR41395-NSIC Rc9-IVM2009DS 50-3-1	7.1	L	3.4	S	21.8	I
7	IVM-077	PR41395-NSIC Rc9-IVM2009DS 4-5-1	7.0	L	3.3	S	21.9	I
8	IVM-078	PR41395-NSIC Rc9-IVM2009DS 4-6-1	7.0	L	3.3	S	21.5	I
9	IVM-089	PR41395-NSIC Rc9-IVM2009DS 4-6-5	7.2	L	3.4	S	21.9	I
10	IVM-091	PR41395-NSIC Rc9-IVM2009DS 3-1-4	7.0	L	3.3	S	21.8	I
11	IVM-103	PR41395-NSIC Rc9-IVM2009DS 50-2-1	7.1	L	3.4	S	21.2	I
12	IVM-104	PR41395-NSIC Rc9-IVM2009DS 50-3-2	7.1	L	3.4	S	21.4	I
13	IVM-105	PR41395-NSIC Rc9-IVM2009DS 1-1-7	7.0	L	3.3	S	20.4	I
14	IVM-106	PR41395-NSIC Rc9-IVM2009DS 1-1-5	7.0	L	3.3	S	21.0	I
15	IVM-156	PR41395-NSIC Rc9-IVM2009DS 1-6-9	7.0	L	3.3	S	21.9	L
16	IVM-158	PR41395-NSIC Rc9-IVM2009DS 1-11-5	7.0	L	3.3	S	20.9	I
17	IVM-172	PR41395-NSIC Rc9-IVM2009DS 2-1-1	6.9	L	3.1	S	21.8	L
18	IVM-193	PR40059-NSIC Rc9-IVM2009DS 1-1-6	6.9	L	3.3	S	21.7	I
19	IVM-194	PR41395-NSIC Rc9-IVM2009DS 1-1-6	6.8	L	3.2	S	21.7	I
20	IVM-195	PR41395-NSIC Rc9-IVM2009DS 1-3-8	6.9	L	3.3	S	21.8	I
21	IVM-196	PR41395-NSIC Rc9-IVM2009DS 1-11-4	6.7	L	3.1	S	21.4	I

Cl - classification based PhilRice, NCT Manual (Philrice, 1997)

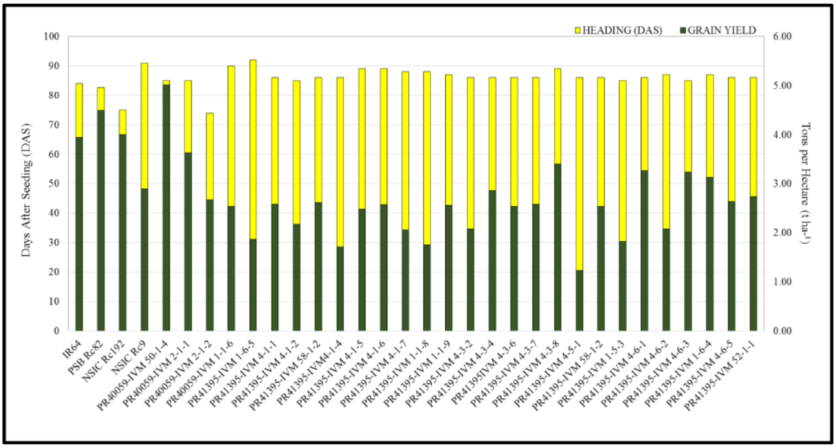


Figure 6. Initial evaluation of the IVM lines under ON (PhilRice, CES 2011 DS)

Table 6. Grain yield, yield reduction and STI of the selected IVM lines under PYT-IL and PYT-MDR (PhilRice, CES, 2012 DS)

No.	IVM code	Grain yield (t ha^{-1})				STI
		IL	MDR	Reduction[#]		
				t/ha	(%)	
1	IR64	6.9^{abc}	0.2^{e}	6.7	97.1	0.028
2	PSB Rc14	8.0^{ab}	0.9^{b}	7.2	89.3	0.140
3	NSIC Rc9	5.8^{cd}	0.6^{cd}	5.2	90.1	0.068
4	NSIC Rc222	6.4^{bc}	0.6^{cd}	5.8	90.5	0.080
5	IVM-032	8.7^{a*}	1.2^{a*}	7.5	85.7	0.221
6	IVM-054	7.0^{abc}	0.6^{cd}	6.4	91.2	0.087
7	IVM-057	8.3^{ab*}	0.7^{bcd}	7.6	92.0	0.110
8	IVM-060	7.1^{abc}	0.59^{cd}	6.5	91.6	0.086
9	IVM-062	7.7^{abc}	0.6^{cd}	7.1	92.3	0.093
10	IVM-068	6.3^{bcd}	0.5^{d}	5.7	91.4	0.069
11	IVM-077	8.0^{ab}	0.7^{bcd}	7.3	91.9	0.105
12	IVM-078	7.29^{abc}	0.7^{bcd}	6.6	90.3	0.105
13	IVM-089	6.3^{bcd}	0.6^{cd}	5.7	90.2	0.079
14	IVM-091	7.4^{abc}	0.7^{cd}	6.7	91.1	0.098
15	IVM-103	5.9^{cd}	0.7^{bcd}	5.2	87.8	0.086
16	IVM-104	8.1^{ab}	0.6^{bcd}	7.5	92.2	0.103
17	IVM-105	7.0^{abc}	1.2^{a*}	5.8	82.6	0.175
18	IVM-106	7.5^{abc}	0.7^{bcd}	6.8	90.6	0.107
19	IVM-156	7.6^{abc}	0.6^{cd}	7.0	91.9	0.096
20	IVM-158	6.7^{abc}	0.8^{bcd}	6.0	88.8	0.103
21	IVM-172	7.7^{abc}	0.7^{bcd}	7.0	91.1	0.107
22	IVM-193	6.7^{d}	0.8^{bcd}	6.0	88.4	0.107
23	IVM-194	4.31^{d}	0.7^{bcd}	3.6	84.1	0.060
24	IVM3-195	5.7^{cd}	0.7^{bcd}	5.1	88.4	0.078
25	IVM3-196	6.9^{abc}	0.7^{bcd}	6.2	90.5	0.091

Means with the same letter are not significantly different by Tukey's comparison of means at α = 0.05; [*]significantly superior than the highest yielding check; [#]significant by comparison of condition using LSD, split plot analysis.

Figure 7 presents the water table, soil moisture content and occurrences of rainfall during the trial, indicating the occurrence of drought episodes. Drought stress had significantly affected the grain yield of the genotypes by causing a yield loss of 83-97%. The highest yield loss and lowest yield reduction were obtained from IR64 and IVM-032, respectively. Significant delay in heading days and reduction in plant height was also observed among the genotypes.

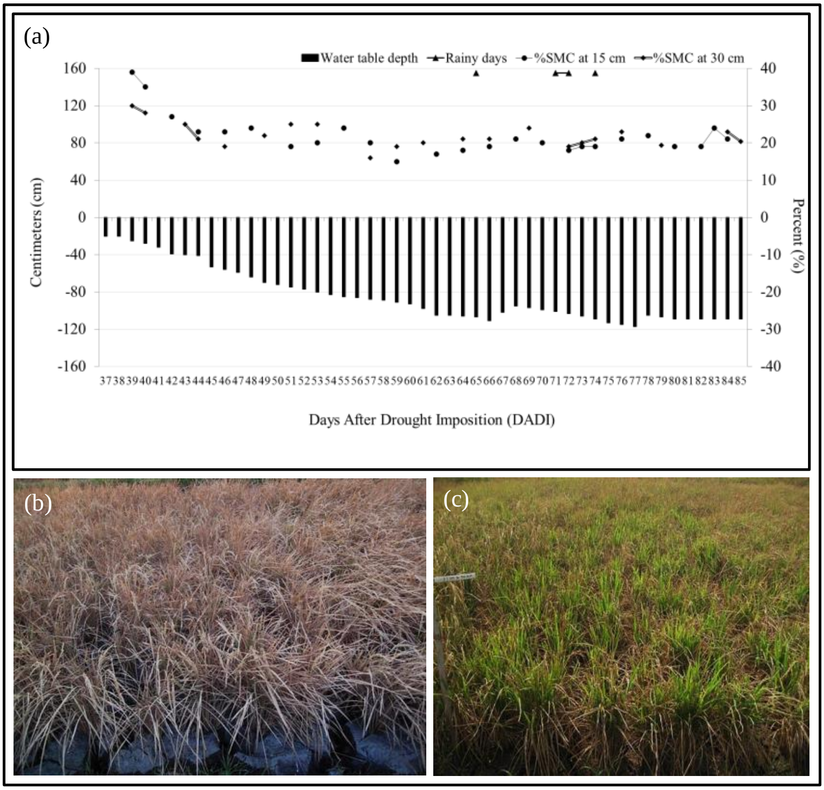
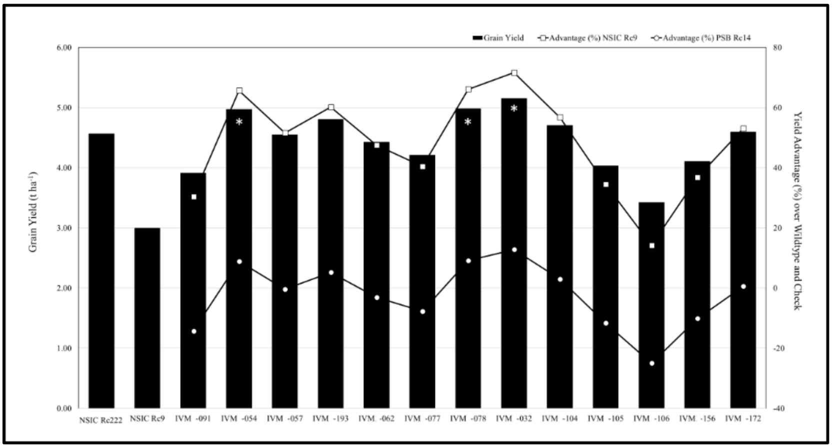


Figure 7. Water table, soil moisture content and rainfall data of PYT (PhilRice, CES, 2012 DS) (a): 79 days after drought imposition (b) and 21 days after rewatering (c)

Limitation in water supply affects plant growth and development, specifically cell division and enlargement due to impaired enzymatic activities, thereby affecting crop stand (Taiz and Zeiger, 2010). Drought delays flowering – the longer the delay, the higher is the penalty to grain yield (Fukai and Cooper, 1999).

3.5 Selected Lines for GYT

Based on grain yield under IL and MDR conditions and STI, 13 (62%) IVM lines were selected. These lines were evaluated under replicated GYT with bigger experimental plots in 2012 WS. The GYT was conducted in a 10 m^2 plot size to further validate the yield response of the selections. The result of the evaluation identified three (24%) lines (IVM-078, IVM-054 and IVM-032), which afforded significantly higher yield than the highest yielding check (NSIC Rc222) with 4.57 t ha^{-1} (Figure 8) by 9-13%.



Means with the same letters are not significantly different by Tukey's comparison of means at α = 0.05; *significantly higher than the highest yielding check.

Figure 8. Grain yield of the IVM lines, GYT (PhilRice, CES, 2012 WS)

IVM-032 was the highest yielding entry (5.16 t ha^{-1}). Based on grain yield, a total of seven (54%) lines were selected and advanced to MET.

3.5.1 MET of the Selected IVM Lines from GYT

In 2013 WS, the selections were evaluated in MET across six locations, namely Nueva Ecija, Ilocos Norte, Isabela, Cagayan, Negros Occidental and Iloilo. In all locations, IVM-032 ranked as the highest yielder among all the IVM lines evaluated. The yield recorded for IVM-032 2.74, 3.26, 3.99, 1.71, 3.58 and 4.52 t ha^{-1} in Nueva Ecija, Negros Occidental, Isabela, Batac, Ilocos Norte, Cagayan and Iloilo, respectively (Figure 9). Mean yield of IVM-032 across locations was 2.95 t ha^{-1}.

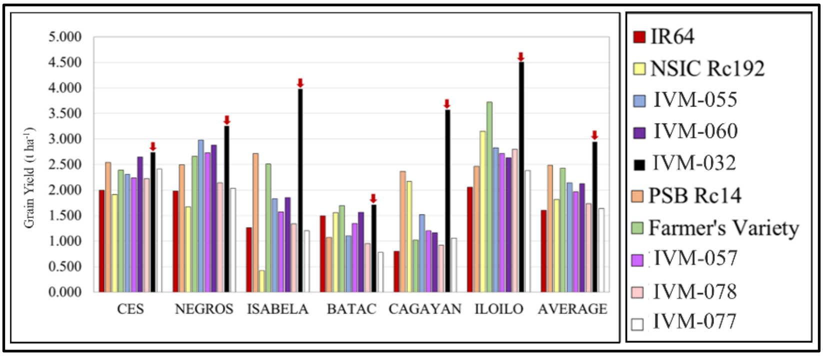


Figure 9. Performance of GYT selected IVM lines in MET with six locations (2013 WS)

Considering the performance of IVM-032 across the MET locations, it was nominated to the National Cooperative Testing (NCT) (PhilRice, 1997) for further evaluation and eventual recommendation to the National Seed Industry Council (NSIC) for approval as a new variety.

3.5.2 Evaluation of IVM-032: Biotic, Abiotic Stress Tolerance and Grain Quality

From the evaluation of the IVM lines for blast resistance (SES for Rice [IRRI, 2013c]) in 2011 to 2017 WS, the selected line scored resistant (R) at PhilRice, Isabela and Bicol, intermediate (I) at PhilRice, Midsayap and the University of the Philippines Los Baños (UPLB) and either resistant, intermediate or susceptible (S) at PhilRice, CES. Across locations and seasons, the mean rating of IVM-032 was 4.4 (intermediate resistant) (Table 7).

Screening of the line for drought tolerance at the seedling stage (Figure 10) was conducted in 2010 WS and 2011 DS under screen house conditions. In both seasons, IVM-032 scored tolerant with a recovery rate of 60-66%, significantly higher than the wildtype (18%) and the tolerant check, PSB Rc14 with 37% (Table 8).

Mutation broadens genetic bases of both qualitative and quantitative inherited traits including drought tolerance by creating beneficial and highly inherited variations (Mwadzingeni *et al.*, 2017). It has also been proven that its application on cultivated germplasm creates superior, environment resilient and well-adapted variants (Shu *et al.*, 2012).

Table 7. Response of IVM-032 to rice blast

Year/season	Location	Rate	Score
2011 WS	PhilRice, Isabela	2.00	R
2014 WS	PhilRice, CES	5.00	I
2016 WS*	PhilRice, CES	9.00	S
	PhilRice, Midsayap	4.00	I
	PhilRice, Isabela	3.50	R
	UPLB	7.00	S
	CBES, Bicol	3.00	R
2017 WS*	PhilRice, CES	7.00	S
	PhilRice, Midsayap	5.00	I
	PhilRice, Isabela	1.00	R
	UPLB	5.00	I
	CBES, Bicol	1.00	R
Season/location mean		4.40	I

IVM-032 (R)

Resistant check: SHZ-2 (I)

Susceptible check: IR42 (I)

Figure taken from 2011 WS screening, PhilRice, CES; *NCT data; R – resistant; I – intermediate resistant; s – susceptible

Table 8. Drought tolerance response of IVM-032 at seedling stage (PhilRice, CES)

Entry	Drought recovery rate#								
	2010 WS			2011 DS			Mean		
	% Rec	Scale	Score	% Rec	Scale	Score	% Rec	Scale	Score
IR64 (Susceptible)	37.3	7	S	21.7	7	S	29.5	7	S
PSB Rc14 (Tolerant)	47.3	5	MT	54.5	5	MT	60.9	3	T
NSIC Rc9 (Wildtype)	18.0	7	S	25.4	7	S	21.7	7	S
IVM-032	60.0*	5	MT	66.6*	3	T	63.3	3	T

#SES for Rice (IRRI, 2013c); *significantly higher than PSB Rc14 by Dunnett's test of means at $\alpha = 0.05$; s – susceptible; T – tolerant; MT – most tolerant

Characterizing the lines for grain quality (GQ) parameters in 2014 WS showed that the lines had improved in terms of head rice recovery, chalkiness, grain size and shape, and amylose content (Table 9). Compared with the grade 2 class of the wildtype, the mutant was a premium grade. Chalkiness was also shifted from very chalky (aa) to grade 2. Grain dimensions were also improved from medium-intermediate to long-slender. Crude protein and amylose content were also reduced, thereby improving its cooked rice texture (Juliano *et al.*, 2009). Based on the evaluated grain quality traits, the general acceptability of the mutant line was improved from very good to excellent.

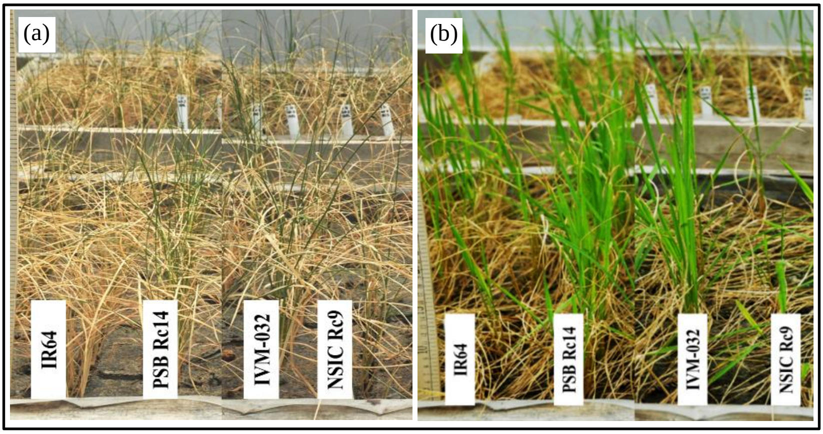


Figure 10. Entries before re-watering (a) and 10 days after re-watering (b), screen for drought tolerance at seedling stage (PhilRice, CES, 2011 DS)

Table 9. Grain quality traits of IVM-032 (PhilRice, CES, 2014 WS)

Grain quality parameters	NSIC Rc9		IVM-032	
	%	Class*	%	Class*
Brown rice	77.2	F	79.3	F
Milled rice	71.4	Pr	72.0	Pr
Head rice	43.1	G2	61.3	Pr
Chalky grains	35.3	aa	6.9	G2
Grain shape (L/W)	6.0	M	6.7	L
Grain length (mm)	2.4	I	3.2	S
Crude protein	10.4		8.9	
Amylose content	24.9	H	18.5	I
Gel. temp.	3.0	HI	3.0	HI

*Based on NCT Manual (PhilRice, 1997); F – fair, Pr – premium, G2 – grade 2, M – medium, L – long, I – intermediate, S – slender, H – high, HI – high-intermediate, aa – above average

Grain quality is one of the most important target traits in breeding cereal crops. As the genetic variability of modern rice cultivars becomes narrow and sources of genes for grain quality are becoming limited, creating variability through induced mutation has been one of the most important tools to improve rice (Viana *et al*., 2019).

3.5.3 Phenotypic and Genotypic Diversity Assessment

To establish further the variability IVM-032 and four other promising IVM lines from the wildtype, NSIC Rc9, phenotypic diversity assessment was carried out using the morphological and agronomic traits of the wildtype and the mutant lines (Table 10).

Cluster analysis generated a dendrogram that separated the elite mutant lines from the wildtype (Figure 11a). On the other hand, genotypic diversity assessment using SSR markers generated the same dendrogram pattern (Figure 11b), where the IVM lines were clearly separated from NSIC Rc9. These results indicate that the mutant lines, particularly IVM-032, were entirely different from the wildtype phenotypically and genetically.

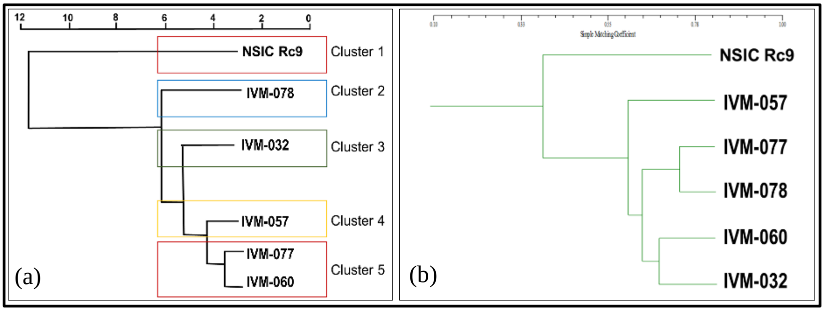


Figure 11. Phenotypic (a) and genotypic (b) diversities of IVM-032 and other promising lines from NSIC Rc9 in comparison with the wildtype (PhilRice, CES)

3.6 NCT Evaluation

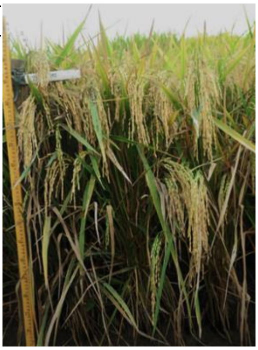
IVM-032 was nominated to NCT in 2015. The trial was conducted for three wet seasons in 2015, 2016 and 2017 in three locations, namely Mariano Memorial State University (MMSU), La Union; PhilRice, Negros; and Southern Cagayan Research Center, Cagayan. Across locations, the line yielded 1.40, 3.67 and 2.56 t ha^{-1}, in 2015, 2016 and 2017, respectively. Across seasons and locations, the mean yield of the line was 2.77 t ha^{-1}, which was 43% higher than the check variety, NSIC Rc192 (1.94 t ha^{-1}).

IVM-032 was consistently resistant to blast across seasons and locations of evaluation (data presented in the previous section). The line also passed the grain quality evaluation of the NCT for milling recovery, physical attributes and physicochemical properties which led to an overall acceptability rate of 93.3 and 60.0% of its cooked and raw rice, respectively.

Based on the NCT results, the line was recommended by the Rice Technical Working Group (RTWG) during its meeting in 2019 at the Philippine Carabao Center (PCC), Science City of Muñoz, Nueva Ecija and approved by the NSIC as a new variety for rainfed-drought prone rice ecosystem. It was officially registered as NSIC 2019 Rc 572 with the variety name *Sahod Ulan 28*.

Table 10. Agro-morphological traits of NSIC Rc9 and IVM-032 (PhilRice, CES)

Trait	NSIC Rc9	IVM-032
Blade pubescence	Glabrous	Glabrous
Blade color	Purple margins	Dark green
Leaf sheath color	Purple	Green
Leaf blade angle	Erect	Erect
Ligule color	purple	Whitish
Ligule shape	2-clept	2-cleft
Collar color	Green	Pale green
Auricle color	Purple	Pale green
Culm angle	Erect	Erect
Internode color	Green	Green
Culm strength	Intermediate	Moderate
Flag leaf angle	Erect	Erect
Panicle type	Intermediate	Compact
Secondary branching	Clustering	Absent
Panicle exsertion	Well exserted	Well exserted
Panicle axis	Droopy	Droopy
Awning	Absent	Absent
Apiculus color	Red	Straw
Stigma color	purple	White
Sterile lemma color	Straw	Straw
Panicle shattering	Moderate	Low
Spikelet fertility	Highly fertile	Highly fertile
Leaf senescence	Late	Late
Panicle threshability	Easy	Easy
Lemma and palea color	Straw	Straw
Lemma and palea pubescence	Short hairs	Short hairs
Seed coat color	Light brown	Light brown
Endosperm type	Non-glutinous	Non-glutinous
Days to 50% heading	89 DAS	85 DAS
Days to 80% maturity	120 DAS	111 DAS
Plant height	121 cm	98 cm
Panicle length	23 cm	25 cm
Culm length	98 cm	73 cm
Productive tiller (no.)	13	16


IVM-032


NSIC Rc9

4. Conclusion and Recommendation

In vitro mutagenesis induced genetic variability in the Philippine upland rice variety NSIC Rc9 resulting in the generation and identification of promising lines with improved agro-morphological traits, grain yield and grain quality traits, while retaining some of the variety's desirable traits such as drought tolerance and disease resistance. The evaluation and development process of the mutant lines was conducted from 2009 to 2015 WS until stable, elite IVM lines were identified. Phenotypic and genotypic assessment using morphological and SSR markers established the variation of the mutant lines from the wildtype, NSIC Rc9. This indicates that the IVM lines had a unique genetic pool different from the wildtype inferring that new gene forms conferring important traits for rice breeding may have been induced.

From the identified elite lines, one line – the PR41935-NSIC Rc9-IVM2009DS 50-1-4 (IVM-032) – was nominated to NCT in 2015 WS. Passing the NCT standards from a series of evaluations for agronomic traits and yield, insect pest and disease resistance, drought tolerance and grain quality resulted in the approval and registration of the elite breeding line as a new variety for cultivation in the rainfed-drought prone rice ecosystem in 2019. The line was registered as NSIC 2019 Rc 572 with the variety name *Sahod Ulan 28*.

This research had shown the benefits of utilizing mutation breeding in the development of stable and superior rice genotypes in a short period of time (six years). The technology also provides an opportunity in generating rice genotypes with superior traits and that are resilient to the changing environment.

In light of the new advanced technologies, it is recommended that *Sahod Ulan 28* together with the other elite IVM lines developed should be further characterized at the molecular level. The characterization would primarily aim to identify new single nucleotide polymorphism/s (SNP/s) that may lead to the determination of novel genes. This may also elucidate further the effect of induced mutation combined with tissue culture on the rice genome.

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