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Comparison of Microsatellite DNA Fingerprints of Original Seed Files and Conserved Rice Germplasm Collections

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The understanding of the genetic identity and composition of germplasm collections conserved in a genebank is vital for the proper management of germplasm resources and utilization in various plant breeding programs. A study was conducted at the Genebank of the Philippine Rice Research Institute (PhilRice) to examine the genetic similarity of the original seed files (SF) and regenerated seeds conserved as an active collection (AC) using 18 microsatellite DNA markers and to explore their underlying intra-varietal heterogeneity. Here, 74 rice germplasm collections regenerated from 1992–2012 were randomly selected for the comparative DNA fingerprinting. The genetic similarity between the original SF and regenerated AC was examined through the Dice coefficient (DC) analysis, while the extent of intra-varietal genetic heterogeneity was determined *via* average-linkage cluster analysis based on binary genetic distance. Of the 74 collections studied, 68 showed paired clustering of SF and AC, indicating high genetic similarity. The DCs of these collections ranged from 0.79–1. On the other hand, SF and AC comparison of six germplasm collections yielded very low DCs ranging from 0.62–0.77, with their corresponding SF and AC pair dissociated in the dendrogram owing to their high intra-varietal genetic heterogeneity. The intra-varietal polymorphic alleles between SF and AC were observed in all markers analyzed – especially in RM562, RM547, and RM154 microsatellite markers –suggesting their potential use in assessing the levels of intra-varietal genetic heterogeneity in rice cultivars. This pilot study provides baseline information that may be used for potential integration of intra-varietal similarity or variability tests in the Genebank which are important aspects for effective germplasm conservation, management, and utilization. Also discussed in this paper are other insights regarding the intra-varietal genetic heterogeneity of the selected rice accessions.

Keywords: active collections, genetic similarity, intra-varietal heterogeneity, microsatellite DNA markers, rice germplasm

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INTRODUCTION

The PhilRice Genebank based in Nueva Ecija, Philippines is considered as the country's largest repository of Philippine traditional rice varieties. In the Genebank, rice germplasm collections are acquired and processed following standard operating procedures (SOPs) that included the preparation of SFs, which are representative original seeds secured during germplasm acquisition. The rest of the original seeds are then processed for regeneration (seed increase), characterization, then finally, *ex situ* conservation in a cold storage facility (10 °C) designated as ACs, where regenerated rice seeds are stored primarily for germplasm distribution and utilization. The SF serves as a resource for identity verification of conserved germplasm collections such as those in the AC. Thus, SFs play an important role in the Genebank to ensure correct germplasm identity and indicates efficiency in germplasm conservation.

With the advent of the Plant Variety Protection Act 2002 (Republic Act No. 9168) (PhilRice 2004), it has become apparent that identification of a particular cultivar through conventional phenotypic means will no longer suffice. In PhilRice, routine DNA analysis through microsatellites has been established as an effective DNA-based marker for DNA fingerprinting and identification of rice varieties (Dalusong *et al.* 2019). Microsatellite markers, also known as simple sequence repeats (SSRs), are stretches of DNA consisting of tandemly repeating di-, tri-, tetra-, or pentanucleotide units that are scattered throughout the genomes of most eukaryotic species (Powell *et al.* 1996). Because of their abundance and inherent potential for variation, these microsatellites have become a valuable source of genetic markers (Temnykh *et al.* 2001). In rice, microsatellites are abundant and well-distributed throughout the genome (Wu and Tanksley 1993; Akagi *et al.* 1996; McCouch *et al.* 1996, 1997, 2002). It has also been widely used in genetic diversity studies, as well as in genetic similarity analysis.

As a "cleistogamous" (self-pollinating) crop, rice is presumed to exhibit genetic homogeneity within a cultivar. However, wide intra-varietal genetic and phenotypic variations have been documented in rice, especially in traditional rice cultivars/landraces (Fukuoka *et al.* 2006a, b; Gautam *et al.* 2019). These variations are of important interest to genebank curators, plant breeders, agronomists, among others, to ensure the maintenance of genetic richness for utilization in crop improvement programs. In the PhilRice Genebank, intra-varietal phenotypic heterogeneity has been observed, especially in traditional rice varieties. However, the extent of genetic variations and/or genetic similarity within the germplasm accession from the time of collection has not been looked into.

This preliminary study was performed to examine the similarity and variations between the original and regenerated seeds of selected germplasm accessions in the PhilRice Genebank. For this, seeds obtained from AC and SF of 74 rice germplasm collections were retrieved to compare their DNA fingerprints using 18 microsatellite markers and to explore the possible intra-varietal heterogeneity exhibited by these collections. The information generated in this study provides baseline information that may be used to augment large-scale intra-varietal similarity or variability tests in the Genebank. Also, understanding the genetic composition of germplasm is a useful resource for germplasm conservation and utilization in plant breeding programs.

MATERIALS AND METHODS

Random Selection and Seed Preparation of Original SF and Conserved AC

The experiment was performed on 74 randomly selected collections from the 1992–2012 harvest (regeneration) to study the genetic similarity and variations between the original SF and conserved AC (Table 1). The regenerated rice seeds (designated as AC) of 74 collections were retrieved from PhilRice Genebank's 10 °C cold storage facility wherein seeds are primarily conserved for distribution/utilization. Meanwhile, the corresponding original SF of the collections was also retrieved, which were secured during germplasm acquisition. Due to a few numbers of seeds kept as SF, two representative seeds from SF and AC were sampled for DNA extraction and genetic comparison analysis.

Genomic DNA Extraction of Randomly Selected Germplasm Collections

The materials for DNA fingerprinting included a total of 148 samples, *i.e.* 74 from SF and 74 from their corresponding seed stocks in AC. Genomic DNA was extracted from the seeds using the ZR Plant/Seed DNA Miniprep kit (Zymo Research, USA). The two representative seeds (dehulled) were placed in a 1.5-mL microcentrifuge tube, then the DNA was extracted following the product's manual. The quality and concentration of genomic DNA extracted were subsequently checked using Nanodrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, USA), then the DNA was stored at –20 °C. The concentration of genomic DNA extracted from the seeds using the miniprep kit ranged from 15–38 ng μL^{-1}. The DNA concentration of all samples used was adjusted to 10 ng μL^{-1} before PCR (polymerase chain reaction) analysis.

Table 1. The 74 rice germplasm collections used in this study and their DCs between original SFs and regenerated seeds conserved in the AC.

No.	Accession no.[a]	Cultivar name	Biological status[b]	Source[c]	Regeneration year[d]	Collection year	DC
1	PRRI002616	Dinagahan	T/L	Samar (PHL)	1992 WS	1991	0.88
2	PRRI002622	Wag-wag	T/L	Northern Samar (PHL)	1992 WS	1991	0.79
3	PRRI002625	Bordagol	T/L	Iloilo (PHL)	1992 WS	1991	1
4	PRRI000806	Nagdami	T/L	Quezon (PHL)	1993 WS	–	0.96
5	PRRI002274	C-4 Dinorado	R	Quezon (PHL)	1994 WS	–	0.88
6	PRRI002536	Dagi	T/L	Laguna (PHL)	1997 WS	–	0.64
7	PRRI001078	Girona	T/L	Australia (AUS)	1999 WS	–	1
8	PRRI001712	TCF4-78	B	Nueva Ecija (PHL)	1999 WS	–	0.97
9	PRRI001713	BAS 4	B	Nueva Ecija (PHL)	1999 WS	–	1
10	PRRI001714	BAS 5	B	Nueva Ecija (PHL)	1999 WS	–	0.95
11	PRRI001786	Ginit-an	T/L	Batangas (PHL)	2000 DS	–	1
12	PRRI001794	Halay Bingi	T/L	South Cotabato (PHL)	2000 DS	–	1
13	PRRI001797	Milagrosa	T/L	Nueva Vizcaya (PHL)	2000 DS	–	0.97
14	PRRI004476	BPI Ri-20	R	Cagayan (PHL)	2000 DS	–	0.96
15	PRRI005682	IR42	R	Cagayan (PHL)	2000 DS	–	0.95
16	PRRI003604	C-4	R	Cagayan (PHL)	2000 DS	–	0.93
17	PRRI002344	Buric (Diket)	T/L	Cagayan (PHL)	2000 DS	–	1
18	PRRI001813	Campeña	T/L	Laguna (PHL)	2001 WS	–	0.96
19	PRRI000816	Kabuak	T/L	North Cotabato (PHL)	2001 WS	–	0.97
20	PRRI003048	Malkitran	T/L	Palawan (PHL)	2002 DS	–	0.94
21	PRRI003054	Malasay	T/L	Palawan (PHL)	2002 DS	–	1
22	PRRI003064	Penari	T/L	Palawan (PHL)	2002 DS	–	1
23	PRRI000631	Camoros	T/L	Quezon (PHL)	2005 DS	–	0.88
24	PRRI000645	Malagkit	T/L	North Cotabato (PHL)	2005 DS	–	1
25	PRRI005641	Lipunan	T/L	Maguindanao (PHL)	2008 WS	2006	0.88
26	PRRI005766	BPI Magcasar	R	South Cotabato (PHL)	2008 WS	2006	0.93
27	PRRI005770	RV 9	R	South Cotabato (PHL)	2008 WS	2006	0.81
28	PRRI005708	Catampal	T/L	South Cotabato (PHL)	2008 WS	2006	1
29	PRRI005771	Korean Tonner	R	South Cotabato (PHL)	2008 WS	2006	0.94
30	11595[a]	Malagkit	T/L	Batanes (PHL)	2010 WS	2010	0.93
31	PRRI005098	Americana	T/L	Indonesia (IDN)	2010 WS	2006	0.80
32	PRRI000827	C-21	R	Ilocos Sur (PHL)	2010 WS	–	0.97
33	PRRI005385	Mashuri	T/L	Malaysia (MYS)	2010 WS	2006	1
34	PRRI005470	Padi Ketumbar	T/L	Indonesia (IDN)	2010 WS	2006	0.96
35	PRRI007101	Red Tanggiling	T/L	Laguna (PHL)	2010 WS	2007	0.96
36	PRRI005520	Rampit	T/L	Indonesia (IDN)	2010 WS	2006	1
37	PRRI005294	Cina Mee	T/L	Indonesia (IDN)	2010 WS	2006	0.77
38	PRRI007125	Red Rice	T/L	Mountain Province (PHL)	2010 WS	2010	0.97

No.	Accession no.[a]	Cultivar name	Biological status[b]	Source[c]	Regeneration year[d]	Collection year	DC
39	PRRI005511	Padi Siak	T/L	Indonesia (IDN)	2010 WS	2006	1
40	PRRI005197	Padi Jarum Emas	T/L	Indonesia (IDN)	2010 WS	2006	0.87
41	PRRI005484	Umbang Kudung	T/L	Indonesia (IDN)	2010 WS	2006	0.94
42	PRRI006462	Gabura	T/L	Bangladesh (BGD)	2011 DS	–	0.77
43	11365[a]	Mindoro	T/L	Negros Occidental (PHL)	2011 DS	2009	0.96
44	7848[a]	IR86171-6-130-2	B	Laguna (PHL)	2011 DS	2008	0.83
45	PRRI000149	Cainte	T/L	Camiguin (PHL)	2011 DS	–	1
46	PRRI003549	Fancy	T/L	Ilocos Sur (PHL)	2011 DS	–	0.97
47	PRRI002345	IR 66	R	Cagayan (PHL)	2011 DS	–	0.83
48	8005[a]	IR84680-38-1-B	B	Laguna (PHL)	2011 DS	2008	0.93
49	PRRI006435	Digha	T/L	Bangladesh (BGD)	2011 DS	–	0.96
50	PRRI001199	O - 29	B	Nueva Ecija (PHL)	2011 DS	–	0.62
51	PRRI000763	M88-1-1	B	Laguna (PHL)	2011 DS	–	0.97
52	PRRI000656	Risco	T/L	Occidental Mindoro (PHL)	2011 DS	–	0.82
53	12059[a]	Maliket Variant	T/L	Ilocos Norte (PHL)	2012 DS	2011	0.96
54	12066[a]	Parina	T/L	Ilocos Norte (PHL)	2012 DS	2011	1
55	12071[a]	Zambales	T/L	Zamboanga Del Sur (PHL)	2012 DS	2011	0.98
56	PRRI005787	Binaka	T/L	Aurora (PHL)	2012 DS	2008	0.98
57	PRRI005703	Balatinaw	T/L	Zambales (PHL)	2012 DS	2008	1
58	PRRI005803	Galo	T/L	Aurora (PHL)	2012 DS	2008	1
59	PRRI005812	Inuway	T/L	Aurora (PHL)	2012 DS	2008	1
60	PRRI006208	Oyak	T/L	Kalinga (PHL)	2012 DS	2008	1
61	PRRI006127	Kamoros	T/L	Kalinga (PHL)	2012 DS	2008	1
62	PRRI006019	Goberno	T/L	Kalinga (PHL)	2012 DS	2008	1
63	PRRI005955	Bolinao	T/L	Kalinga (PHL)	2012 DS	2008	1
64	PRRI005809	Inantote	T/L	Palawan (PHL)	2012 DS	2008	0.98
65	PRRI005916	Pinarongpong	T/L	Palawan (PHL)	2012 DS	2008	1
66	PRRI005707	Blandi	T/L	Palawan (PHL)	2012 DS	2008	0.90
67	PRRI005792	Doryat	T/L	Palawan (PHL)	2012 DS	2008	1
68	PRRI006825	Dinorado	T/L	North Cotabato (PHL)	2012 DS	2006	0.93
69	PRRI006247	Sinadugan	T/L	Kalinga (PHL)	2012 DS	2008	1
70	PRRI000092	Binotete	T/L	Ilocos Norte (PHL)	2012 DS	–	0.63
71	PRRI000008	Aglipay	T/L	Nueva Ecija (PHL)	2012 DS	–	0.72
72	PRRI000162	Sinipit	T/L	Pangasinan (PHL)	2012 DS	–	0.93
73	PRRI006418	Biday	T/L	North Cotabato (PHL)	2012 DS	2006	0.98
74	6119[a]	Kadilag (Pilit)	T/L	South Cotabato (PHL)	2012 DS	–	0.92
Average DC							0.93

[a]Collection numbers
[b]T/L – traditional cultivar/landrace; R – released variety; B – breeding line
[c]PHL – Philippines
[d]DS – dry season; WS – wet season

DNA Fingerprinting Using Microsatellite Markers

Eighteen (18) microsatellite markers across the rice chromosomes were used to generate DNA profiles and detect similarity/variation between the original SF and regenerated AC (Table 2). The selection of these markers was based on the validation studies by Dalusong *et al*. (2019). PCR analysis was done following the protocol of Perez *et al.* (2012) with modification. Each PCR reaction contains 2.0 µL of 10 ng µL^{-1} template DNA, 1.5 µL 5X PCR buffer, 0.25 µL 25 mM MgCl, 0.4 µL 5 mM dNTPs, 0.4 µL 10 µM forward primer, 0.4 µL 10 µM reverse primer, 0.7 µL of 500 U Taq polymerase, and 1.85 µL of sterilized distilled water. PCR was carried out in a programmable thermal cycler (PTC 100, MJ Research, Inc.) with the following profile: initial denaturation at 95 °C for 5 min, 29 cycles of denaturation at 94 °C for 1 min, annealing at 55–61 °C (depending on the requirement of each primer) for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 5 min. PCR amplicons were resolved through 8% non-denaturing polyacrylamide gel with 1X TBE buffer run on Dual Triple-Wide Mini-Vertical System (C.B.S. Scientific, USA) at 100 V for 1–2 h. The gel was stained with SYBR™ Safe stain (Invitrogen, USA) and documented through the GelDoc™ XR+ gel documentation system (Bio-Rad Laboratories, USA).

Gel Scoring and Analysis on the Genetic Similarity/ Variation of Original SF and Regenerated AC

Based on the polyacrylamide gel electrophoretogram obtained, shared alleles between SF and AC (placed side by side) for each microsatellite locus (18 markers) were converted into binary format before genetic analysis, *i.e.* the score of 1 for presence and 0 for the absence of the band. The binary data was used to compute for the genetic similarity value, the DC (Dice 1945) between SF and AC of each collection using the statistical software Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (version 2.10) (Rohlf 2000). To determine the extent of intra-varietal genetic variations, a dendrogram based on average-linkage cluster analysis of binary genetic distance tested with 1,000 bootstrap replicates was constructed using Pvclust R statistics package (Suzuki and Shimodaira 2006). Other figures/graphs were constructed using Prism 8 version 8.1.2 (GraphPad Software, Inc.).

RESULTS

The 74 rice germplasm collections randomly selected for this pilot study were composed mainly of traditional

Table 2. List and description of microsatellite DNA markers used in the study.

Microsatellite marker	Repeat motif	Chromosome locus	Position[a] (in bp)	Primer[b] sequence (5' → 3')		No. of alleles detected
				Forward	Reverse	
RM562	$(AAG)_{13}$	1	14,610,402	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC	6
RM3412	$(CT)_{17}$	1	11,566,961	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC	6
RM10890	$(TATC_{)5}$	1	14,743,438	GCTTCGGCTCTTCATTCACTGG	GCGATTATAGGAGCGCTATGTGG	3
RM10764	$(AT)_{28}$	1	12,078,205	AGATGTCGCCTGATCTTGCATCG	GATCGACCAGGTTGCATTAACAGC	4
RM521	$(TC)_{14}$	2	10,806,307	TTCCCTTATTCCTGCTCTCC	GGGATTTGCAGTGAGCTAGC	5
RM263	$(CT)_{34}$	2	25,889,828	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	5
RM154	$(GA)_{21}$	2	1,083,920	GACGGTGACGCACTTTATGAACC	CGATCTGCGAGAAACCCTCTCC	7
RM324	$(CAT)_{21}$	2	11,388,913	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	4
RM592	$(ATT)_{20}$	5	2,736,620	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA	8
RM164	$(GT)_{16}TT(GT)_4$	5	19,114,842	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	7
RM586	$(CT)_{23}$	6	4,709,090	ACCTCGCGTTATTAGGTACCC	GAGATACGCCAACGAGATACC	6
RM445	$(AG)_{12}$	7	17,409,772	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC	4
RM547	$(ATT)_{19}$	8	5,586,081	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG	8
RM331	$[(CTT)_4GTT]_2(CTT)_{11}$	8	12,288,130	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG	5
RM566	$(AG)_{15}$	9	14,651,176	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	6
RM171	$(GATG)_5$	10	19,048,795	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	2
RM536	$(CT)_{16}$	11	8,891,825	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	3
RM202	$(CT)_{30}$	11	8,908,399	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA	6
Average no. of alleles						5.3

[a]The physical position of markers is based on reference genome of Nipponbare or IR36 that can be found on Gramene database [[b]Source: https://archive.gramene.org/markers/; McCouch *et al.* (2002)]

cultivars/landraces (n = 58), and the rest were released varieties (n = 9) and breeding lines (n = 7) (Table 1). The rice seeds used from the AC were obtained from the 1992–2012 regeneration activities of PhilRice Genebank. The alleles detected at each microsatellite locus ranged from two (RM171) to eight (RM547 and RM592) with a total of 95 alleles detected and an average of 5.3 alleles per locus (Table 2). The DC computed by comparing the DNA fingerprints of SF and AC of 74 collections ranged from 0.62–1, with 1 considered as completely genetically similar between SF and AC (Table 1; Figure 2). An average DC of 0.93 was obtained, indicating that majority of the germplasm collections tested have a high DC genetic similarity value between SF and AC (Table 1). Of the 74 collections studied, 68 showed high genetic similarity between SF and AC through paired clustering tested with 1,000 bootstrap reiterations (Figures 1 and 3). The DCs of these collections ranged from 0.79–1 (Table 1; Figure 2). Of these 68 collections, 25 have DC genetic similarity value of 1 between SF and AC (Figure 3), while the remaining 43 collections with similar SF and AC have DCs of 0.79–0.98 (Table 1). Also, six germplasm collections exhibited high intra-varietal genetic heterogeneity that comparison of their SF and AC yielded low DCs ranging from 0.62–0.77 (Table 1; Figures 2 and 3). In addition, their corresponding SF and AC pairs were non-clustered and dispersed in the dendrogram (Figure 1). These collections include five traditional varieties: PRRI000092 (Binotete) (DC = 0.63), PRRI002536 (Dagi) (DC = 0.64), PRRI000008 (Aglipay) (DC = 0.72), PRRI006462 (Gabura) (DC = 0.77), PRRI005294 (Cina Mee) (DC = 0.77), and one breeding line: PRRI001199 (O-29) (DC = 0.62) (Table 1; Figure 2).

DISCUSSION

The molecular and morphological identity of germplasm collections in a genebank is vital to the effective management, conservation, and utilization of germplasm resources of any research institution. In this preliminary study, microsatellite markers were utilized to examine the genetic similarity and variations between the original SF and regenerated AC. Microsatellite DNA markers or SSRs are valuable as genetic markers because they are codominant, capable of detecting high levels of allelic diversity, and assayed inexpensively by PCR (McCouch *et al.* 1997). In this study, the extent of intra-varietal genetic heterogeneity between the original SF and regenerated AC was determined *via* analysis of the genetic distance, which is used to determine the effective genetic deviations between samples or populations (Nei 1978).

Comparative DNA fingerprinting analysis between SF and AC showed that 68 collections (out of 74) were clustered in pairs in the dendrogram based on the average-linkage method (Figure 1), thus showing the genetic similarity between SF and AC. More than half of these collections (43 collections) displayed high genetic similarity between SF and AC (as shown by paired clustering) but with degrees of intra-varietal variations (DC of 0.79–0.98) (Table 1; Figure 1). The rest of these rice collections (25 collections) showed a complete genetic match (with DC of 1) between SF and AC (Figure 3; Table 1), suggesting genetic homogeneity in these collections but not guaranteed. Additional/other markers may be employed for further validation. Meanwhile, six collections (five traditional rice collections and one breeding line) exhibited high intra-varietal heterogeneity between SF and AC, as shown by low DC values ranging from 0.62–0.77 (Table 1; Figures 2 and 3), and showing dissociation of their corresponding SF and AC pair in the dendrogram (Figure 1). The intra-varietal heterogeneity or heterogeneity within the population/cultivar has been well-documented in rice, especially in traditional varieties/landraces (Fukuoka *et al.* 2006a; Gautam *et al.* 2019). Although generally low-yielding, traditional, or heirloom varieties persisted due to their adaptability to various ecological and local niches leading to high yield stability (Dwivedi *et al.* 2016). Because of their innate heterogeneity, traditional varieties are known to possess desirable traits such as good eating quality, disease resistance, tolerance to abiotic stresses, *etc.* (Lapitan *et al.* 2007; Dwivedi *et al.* 2016). In the Philippines' "Red rice" cultivars – the molecular bases of the intra-varietal heterogeneity, particularly the grain coloration – have been investigated through AFLP analysis and complemented with studies on the farmer's dynamic practices (Bertuso *et al.* 2005). Moreover, Olufowote *et al.* (1997) tested rice genebank accessions and observed that even modern varieties and pure lines with homogenous plant phenotype may still exhibit high intra-varietal genetic heterogeneity.

A lot of factors may be attributed to the intra-varietal genetic heterogeneity in rice such as outcrossing or occurrence of open florets and, in rare cases, spontaneous mutation, all of which indirectly allowed varying degrees of intra-varietal as well as inter-varietal heterogeneity over several years or decades (Olufowote *et al.* 1997; Gautam *et al*. 2019). Apart from these, the possibility of inclusion of seed admixtures could contribute to high heterogeneity and low genetic similarity. In the present paper, although the small sampling of seeds in a random fashion (two seeds each from SF and AC) may have partially represented the variations within the cultivar, some insights could still be drawn with regards to their intra-varietal heterogeneity. The intra-varietal polymorphic alleles between SF and AC were observed in all 18 markers – especially in RM562,

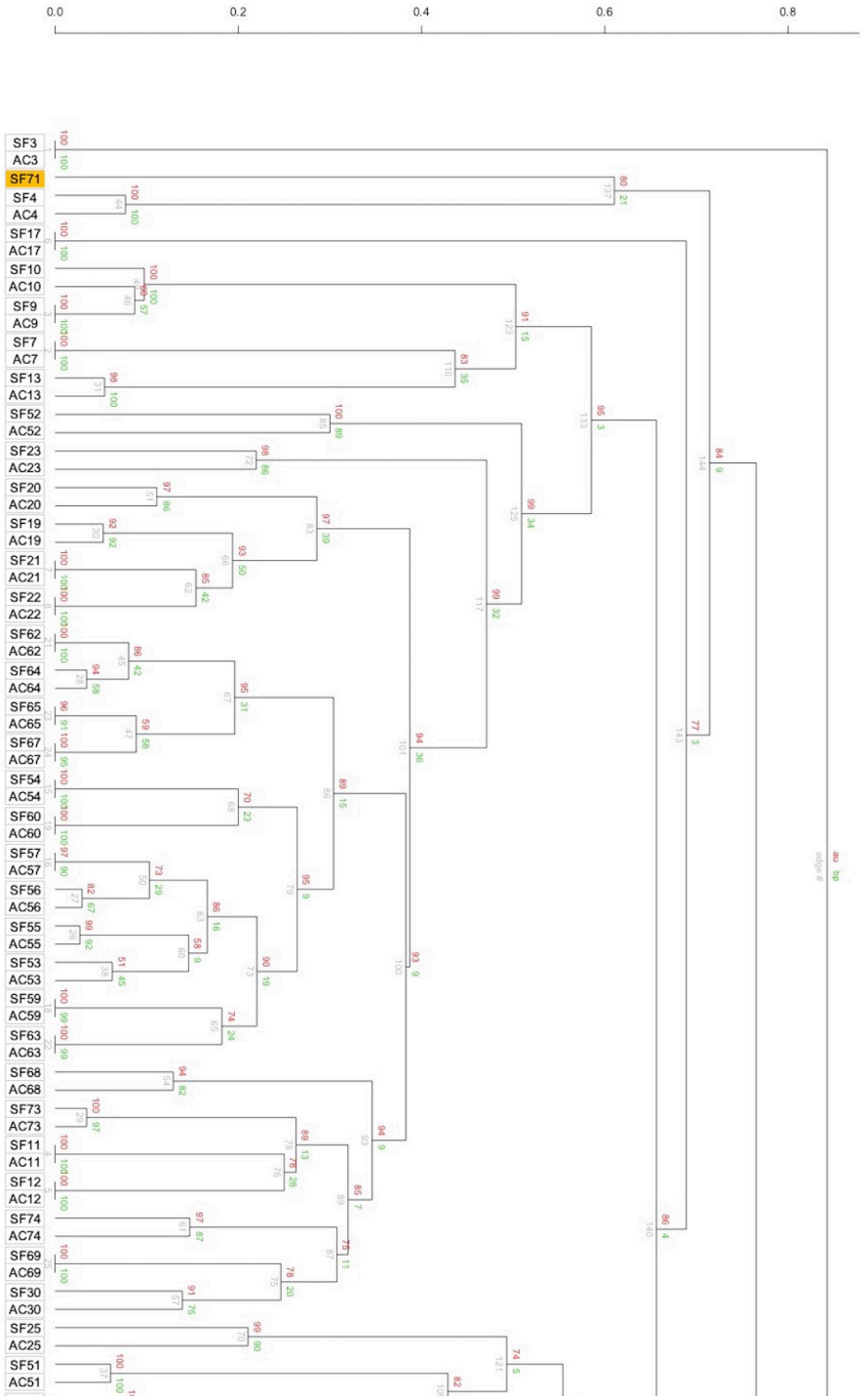

Figure 1. Paired clustering of 74 rice germplasm collections based on average-linkage of binary genetic distance tested using 1,000 replicates of bootstrapping. Collections with high genetic similarity between SF and AC are clustered in pair, while collections with high intra-varietal heterogeneity are spread across the dendrogram. The SF and AC pair for these collections were colored accordingly. Node numbers in red are approximately unbiased (AU) *p*-values while those in green are bootstrap probability (BP) values. The germplasm numbering is presented in Table 1. SF – seed files; AC – active collection

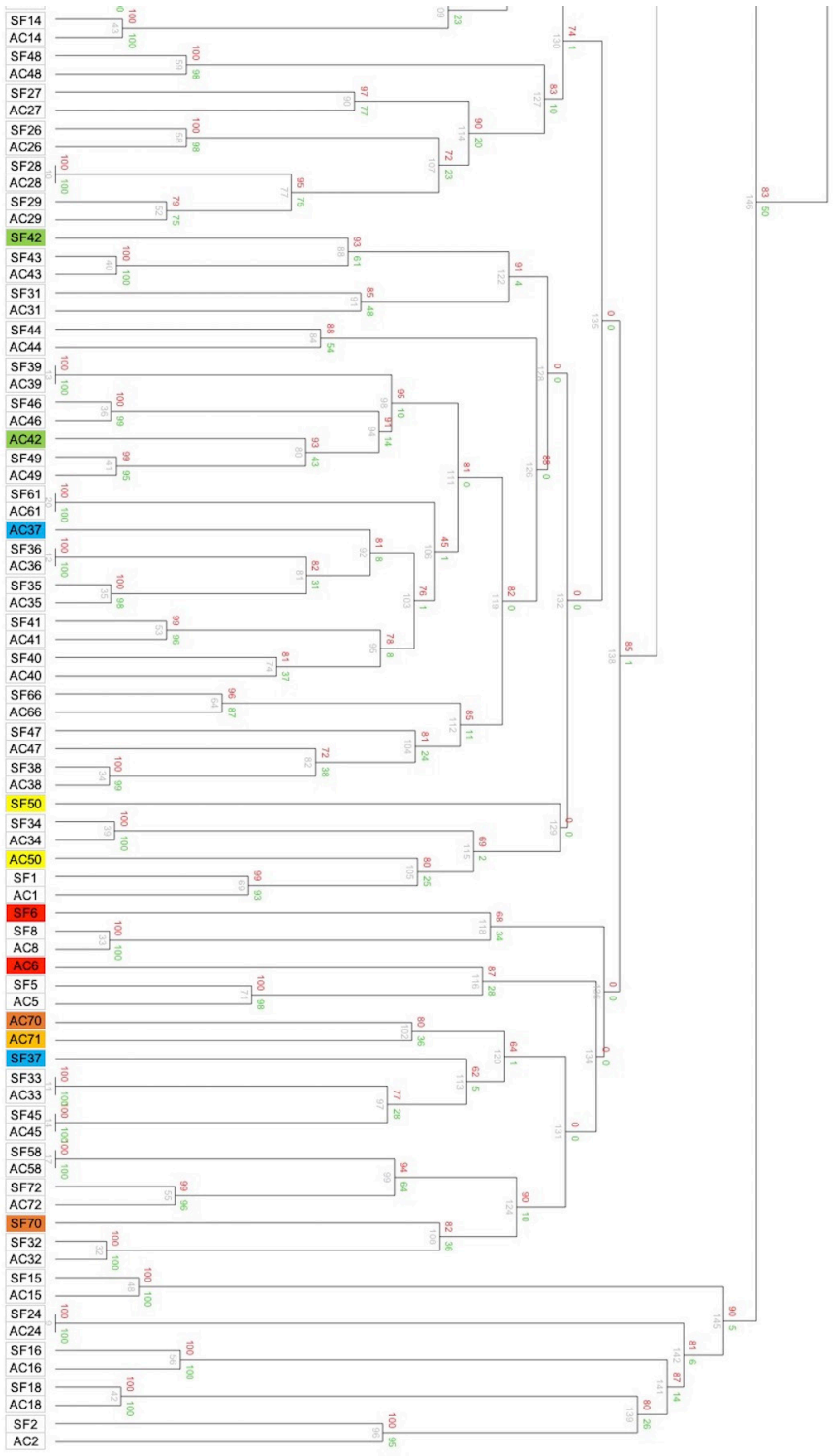

Figure 1. continuation

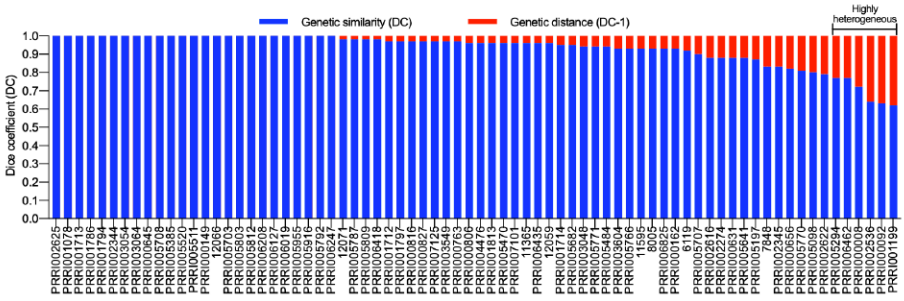

Figure 2. Graphical representation of the genetic similarity (blue bar) and distance (red bar) between SF and AC of 74 rice germplasm accessions based on DC.

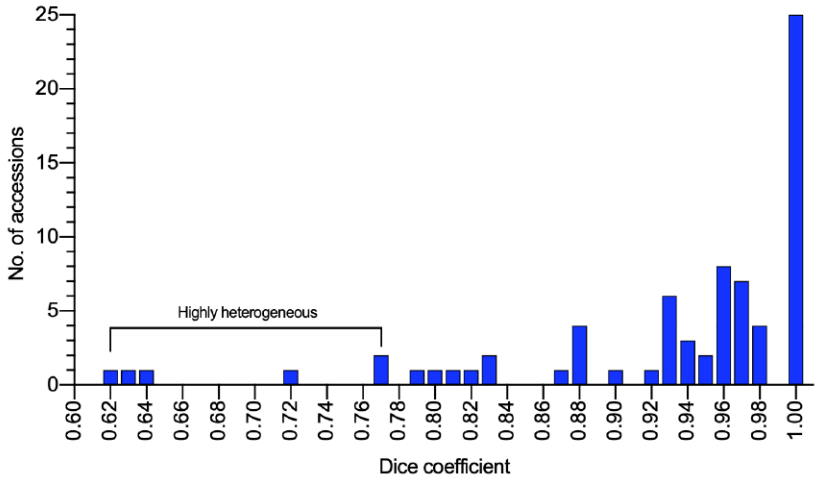

Figure 3. The frequency and distribution of 74 rice germplasm accessions according to their DCs. Six accessions have DC of 0.62–0.77, indicating high intra-varietal genetic heterogeneity as shown by average linkage cluster analysis (see Figure 1).

RM547, and RM154 microsatellite markers (Figure 4). This finding suggests that these three markers may be included in assessing the levels of intra-varietal genetic heterogeneity in rice cultivars. Among the rice accessions studied, O-29 (PRRI001199), Binotete (PRRI000092), and Dagi (PRRI002536) showed the lowest DC values (0.62, 0.63, and 0.64, respectively) (Table 1; Figure 2) and, therefore, exhibit the most intra-varietal allele polymorphisms.

In summary, using microsatellite DNA markers, we have successfully obtained an overview of the genetic composition of some of the conserved collections in the PhilRice Genebank through comparative DNA fingerprinting of original SF and regenerated AC. We have also provided insights regarding the innate intra-varietal heterogeneity of some collections, which may be used in other studies dealing with variations within the cultivar. From the 74 randomly selected collections with regeneration activities during the years 1992–2012, 68 showed high genetic similarity between the SF and AC. This indicates the robustness of the procedures being followed to conserve and maintain the genetic integrity of the rice accessions in PhilRice Genebank. On the other hand, six collections exhibited cases of very high intra-varietal genetic heterogeneity. This result may indicate the presence of inherent genetic variability within these accessions that could be possible sources of novel alleles or traits for rice genetic improvement. The results of this pilot study provide baseline information on the potential integration of intra-varietal similarity/variability tests at the molecular level with the current SOPs for germplasm conservation in the Genebank. The use of DNA marker technology is now an emerging and important aspect for effective germplasm conservation, management, and utilization.

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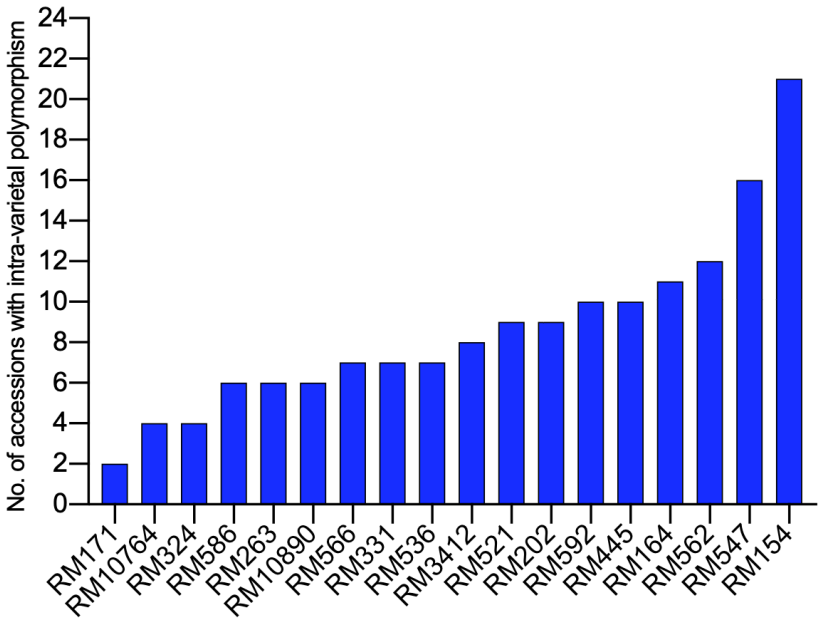

Figure 4. The frequency of markers with intra-varietal allele polymorphism between SF and AC.

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