RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (3) 574-588, 2022
http://doi.org/10.54910/sabrao2022.54.3.10
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

GENETIC VARIABILITY IN WEEDY RICE BIOTYPES FOUND IN DIRECT-SEEDED RICE AREAS IN THE PHILIPPINES

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SUMMARY

Weedy rice can severely impact rice production through yield reduction because of its competitive ability to reduce growth resources for cultivated rice. The characteristic weedy traits have made weedy rice very challenging to manage as they have the same agro-morphological characteristics with cultivated rice. The study examined the relationships between selected cultivated rice (*Oryza sativa* L.) cultivars, weedy rice biotypes, and wild rice collected from different locations in the Philippines, using simple sequence repeat markers. Cluster analysis, using UPGMA, enabled the genetic differentiation and relationships examination of the test materials. Subgroups of 13 with at least two biotypes formed 100% similarity based on post-harvest data, with the cultivated rice cultivars forming one subgroup. With polymorphic SSR markers, five major clusters range from three (group I) to 62 biotypes (group III). There was 100% similarity observed for 15 subgroups ranging from two to 10 biotypes. The wild rice cultivars formed species-specific groupings. Subgroups with 100% similarity came from the same province; likewise, one with 100% similarity came from both Iloilo and Batangas; and still another from cultivated rice cultivar and weedy rice biotype from Pangasinan. The possible relationships of weedy rice biotypes with wild rice relatives (>65% similarity) include two biotypes related to *Oryza minuta*, one for *O. meyeriana*, and 22 biotypes for *O. rufipogon*. Concerning cultivated rice cultivars, high similarity (>80%) was observed in 22 biotypes closely related to PSB Rc 82, 13 biotypes to NSIC Rc 222, six to NSIC Rc 160, three biotypes each to NSIC Rc 215, NSIC Rc 152, NSIC Rc 64, NSIC Rc 18, and NSIC Rc 10, and one biotype each to IR64 and NSIC Rc 14.

Keywords: Rice (*Oryza sativa* L.) cultivars, weedy and wild rice, genetic diversity, genetic relationship, SSR markers

Key findings: Findings show a high genetic similarity of weedy rice biotypes to cultivated rice cultivars and wild rice populations. The study infers that where there are wild rice relatives, origin of weedy rice in these areas can be through hybridization between cultivated rice and wild rice, especially on the common *O. rufipogon*, the putative source of red pericarp in most weedy rice biotypes. But it can also take another route, such as, the de-domestication of cultivated rice cultivars giving rise to weedy traits.

To cite this manuscript: Juliano LM, Caguiat XGI, Evangelista EV, Martin EC, Ramirez AHM (2022). Genetic variability in weedy rice biotypes found in direct-seeded rice areas in the Philippines. *SABRAO J. Breed. Genet.* 54(3): 574-588. http://doi.org/10.54910/sabrao2022.54.3.10

Communicating Editor: Dr. Gwen Iris Descalsota-Empleo

Manuscript received: July 1, 2022; Accepted: August 14, 2022.

INTRODUCTION

Weedy rice (WR) is morphologically similar and shares a closely synchronized life cycle with cultivated rice. It is hard to differentiate at its early vegetative stage and shares traits with cultivated and wild rice. The shift from transplanted rice to direct-seeded rice (DSR) production makes WR a primary emerging constraint because of its competitive ability (Chauhan *et al.,* 2013). As direct seeding becomes the main method of crop establishment, the problem of WR escalates and persists, causing severe yield losses as high as 90% depending on WR density (Delouche *et al.,* 2007; Rathore *et al.,* 2017). In Asian countries, the adoption of direct seeding increases, thus an estimate of a simultaneous WR infestation in China, India, and Vietnam can result in a 1.9 million MT (0.5%) reduction in global long-grain rice output (Durand-Morat *et al.*, 2018). In the Philippines, DSR occurs in 35% of the 4.56 million ha harvested rice area, and the increasing adoption of DSR extends the areas infested with WR and the severity of infestation (Tanzo and Martin, 2015). As the shift to the cost-reducing direct-seeding practice continues, more challenges in weed management, especially WR, continue as selective herbicides have yet to be made available.

The weedy characteristics of WR, which make it very difficult to manage, include early emergence, vigorous growth, larger biomass, seed shattering, and variable seed dormancy. Weeds, like WR, are most troublesome because they tolerate stress better, and since they have growth resources similar to rice, they acquire these resources faster. An undesirable impact of WR infestation on rice production consists of yield reduction and poor quality of cultivated rice. With information on WR adaptive traits and how these traits are acquired, including its competitive ability, the agronomic implication requires the importance of controlling weedy rice at the seedling stage or at least before the flowering stage to reduce seeds in the soil. Hence, there is a need for a better understanding of WR evolution, ecology, and biology to identify weak points in their life cycles to develop effective WR management programs (Rathore *et al.,* 2017).

The primary gene pool of cultivated plants has three components—the cultivated plant, its wild ancestor, and in many cases, an associated congeneric weed (Harlan and de Wet, 1971). For Asian rice, the gene pool consists of cultivated rice *Oryza sativa L.,* its wild progenitor *O. rufipogon* Griff., and WR *O. sativa* f. *spontanea* Roshev. These three components are closely related because the common wild rice species, *O. rufipogon* is the putative ancestor of the Asian domesticated and cultivated rice *O. sativa* (Pusadee *et al*., 2013). Several distinct types of cultivated rice (including *japonica, indica,* and *aus* cultivars) have evolved through multiple domestication events, adaptation to the environment and rice-growing practices, and selection for agronomic and culinary traits (Londo *et al.,* 2006; Nirmaladevi *et al*., 2015; Bagati *et al*., 2016). It is assumed that the wild-weed-crop complex is found in rice agro-ecosystems throughout the native range of *O. rufipogon.* Previous studies reported the natural hybridization between *O. rufipogon*, weedy red rice, and *O. sativa* (Langevin *et al*., 1990; Song *et al*., 2003; Messeguer *et al*., 2004; Kuroda *et al*., 2005), and the wealth of data from these studies significantly enhanced our understanding of the origins of WR and its impact on rice production systems (Prathepha, 2009).

Genetic analysis studies showed that the weedy traits of WR biotypes have independent origins and have faster adaptation in the local environments where they grow. The introgression of dominant wild rice and/or crop-like alleles has been found in the adaptive traits of WR populations in areas with or without wild rice populations (Vigueira *et al.,* 2017; Li *et al.,* 2017). Genomic changes during rice de-domestication in cultivated rice and WR showed rapid local adaptation, selecting different subsets of adaptive genes in response to the different growing environments. With that, WR can use other adaptive genes than simply reversing domesticated genes to their wild types (Qiu *et al.* (2017). Studies on identifying genes underlying weedy traits and their origins can be a key in preventing the emergence of these traits as there are phenotypic traits strongly not selected during the rice domestication process (Adriansyah *et al*., 2021; Rani *et al*., 2022).

The seemingly recurrent genetic evolutions of WR have spurred several non-mutually exclusive hypotheses on the possible origins, according to Burgos *et al.* (2021): a) de-domestication of cultivated rice (Kanapeckas *et al.,* 2016; Li *et al.,* 2017; Qiu *et al.,* 2017), b) intervarietal hybridization among cultivated rice cultivars (Pusadee *et al*., 2013; Qiu *et al*., 2014; Ishikawa *et al*., 2005), c) crop-wild hybridization between domesticated and wild rice (Pusadee *et al.,* 2013; Song *et al*., 2014); and d) wild-derived origin from wild ancestors adapted in rice areas (Huang *et al*., 2017).

Hypothesis (a) has been the more likely WR evolution in different rice regions based on several morphological and genetic studies. For example, in regions where the japonica subspecies is predominantly cultivated, WR is phenotypically "japonica-like" (e.g., shorter grains and plant height); in indica-cultivated areas, WR is more "indica-like," e.g., longer grains, taller (Cho *et al.,* 1995; Bres-Patry *et al.,* 2001; Ishikawa *et al.,* 2005; Cao *et al.,* 2006). The endoferal hypothesis states that weedy crop relatives are derived directly from the crop, as a result of de-domestication with local weedy populations, possibly descending from either locally grown cultivars or distantly located cultivars through contamination (Gressel, 2005; De-Leon *et al.,* 2019).

However, studies in Thailand support hypotheses (b) and (c) wherein documented hybridization between cultivated rice and wild *O. rufipogon* as a likely source of WR origins existed (Prathepha, 2009). Population structure analysis identified a distinct genetic composition of WR in each separate region, which is an admixture of the local rice cultivar and *O. rufipogon,* thereby concluding that WR appears to emerge first from the hybridization of *O. rufipogon* and cultivated rice that coexisted in the agricultural landscape, particularly at the edges of cultivated fields (Pusadee *et al.,* 2013). Hypothesis (d) includes evidences that wild rice accessions have a role in the origin of WR with wild-like weeds having black hull, awn, and red pericarp like those in Nepal, India, and Sri Lanka (Huang *et al.,* 2017).

In the Philippines, WR was first reported in 1991 and recent surveys have shown that it has been increasingly infesting direct-seeded rice areas (reviewed by Juliano *et al.* 2020). In the survey of the Philippine Statistics Authority and PhilRice in 2011, WR was among the top 10 common weeds in rice fields (PSA, 2012). This sparked research interest as effective WR management is crucial to achieving rice sufficiency.

Except for surveys, limited phenotypic and genetic characterizations of WR biotypes exist. One published information showed high variability of WR phenotypes in Philippine rice fields using seed shape, which is known to have large genetic bases (Apuan *et al.,* 2011). The use of Geometric Morphometric (GM) tools, specifically Elliptic Fourier Analysis (EFA) and Multivariate Analysis in statistics, resulted in 64% of WR having phenotypic affinity to 13 wild landraces (AA genome) collected from 15 locations within West Africa, the Caribbean Islands, Latin America, India, Australia, South Asia, and Southeast Asia. Their study found that 10 populations have an affinity to *O. meyeriana* (GG genome) in the Philippines and Malaysia; both weedy populations from Misamis Oriental in Mindanao Island (WRMIS1) and Nueva Ecija in Luzon Island (WRNE2) have an affinity to PSB Rc 64 and Rc 82, respectively, while two populations from Iloilo in Visayas Island (WRILO1 and WRILO2) have an affinity to *O. latifolia* in Costa Rica.

This study aimed to determine the genetic relationships of WR biotypes collected in DSR areas with cultivated rice cultivars and wild rice accessions in the Philippines. It used microsatellite (SSR) markers, phylogenetic, population structuring, and genetic distance-based approaches to examine the possible genetic relationships of the rice materials. Based on these approaches, the study can gain insight into the possible evolutionary origins of Philippine weedy rice biotypes.

MATERIALS AND METHODS

Plant material

Researchers used 76 weedy rice biotypes collected across DSR areas in the country, 12 most planted cultivated rice (*Oryza sativa* L.) cultivars, and seven wild rice populations previously recanvassed by Caguiat *et al.* (2020) in the study (Table 1). The collection of most of the WR samples came from Iloilo province (Visayas) where 61%–100% of rice fields were planted with direct-seeded rice (Juliano *et al*. 2020). The coding of WR biotypes used the first letter of the province followed by a number, i.e., Pangasinan samples (P1 – P11), and the cultivated rice cultivars used the National Seed Industry Council (NSIC) naming as IR or Rc followed by the number, i.e., IR64, NSIC Rc 122. Previously, NSIC was called Philippine Seed

Table 1. Weedy rice biotypes, wild rice accessions, and selected cultivated rice cultivars used in the genetic study.

No	Code	Barangay	Municipality	Province	Island	Hull Color	Pericarp Color	Awn
1	I1	Dapitan	Pototan	Iloilo	Visayas	Bronze	Red	Long
2	I2	Bantud	Dumangas	Iloilo	Visayas	Straw	Red	Long
3	I3	Pulao	Dumangas	Iloilo	Visayas	Brown/gray	Black	None
4	I4	Pulao	Dumangas	Iloilo	Visayas	Straw	Light green	Medium
5	I5	Pulao	Dumangas	Iloilo	Visayas	Straw	White	Medium
6	I6	Pulao	Dumangas	Iloilo	Visayas	Straw	White	Medium
7	I7	Pulao	Dumangas	Iloilo	Visayas	Bronze	Red	Long
8	I8	Pulao	Dumangas	Iloilo	Visayas	Straw	Red	Short
9	I9	Pulao	Dumangas	Iloilo	Visayas	Bronze	Red	Medium
10	I10	Pulao	Dumangas	Iloilo	Visayas	Bronze	Dark red	Medium
11	I11	Pulao	Dumangas	Iloilo	Visayas	Straw	White	Long
12	I12	Pulao	Dumangas	Iloilo	Visayas	Straw	Red	Short
13	I13	Pulao	Dumangas	Iloilo	Visayas	Bronze	Brown	None
14	I14	Pulao	Dumangas	Iloilo	Visayas	Straw	White	Medium
15	I15	Pulao	Dumangas	Iloilo	Visayas	Bronze	Red	None
16	I16	Pulao	Dumangas	Iloilo	Visayas	Straw	White	None
17	I17	Bangkal	Tigbauan	Iloilo	Visayas	Straw	Red	Medium
18	I18	Bangkal	Tigbauan	Iloilo	Visayas	Straw	Red	Short
19	I22	Bangkal	Tigbauan	Iloilo	Visayas	Straw	Red	Short
20	I24	Bangkal	Tigbauan	Iloilo	Visayas	Bronze	Red	None
21	I25	Bangkal	Tigbauan	Iloilo	Visayas	Straw	Red	Short
22	I26	Bangkal	Tigbauan	Iloilo	Visayas	Bronze	Black	None
23	I30	Ollo Baroc	Tigbauan	Iloilo	Visayas	Brown/gray	Red	Short
24	I31	Ollo Baroc	Tigbauan	Iloilo	Visayas	Straw	Red	Medium
25	I32	Ollo Baroc	Tigbauan	Iloilo	Visayas	Bronze	Red	Medium
26	I33	Anonang	Leon	Iloilo	Visayas	Bronze	Red	None
27	I34	Anonang	Leon	Iloilo	Visayas	Straw	Red	Medium
28	I35	Anonang	Leon	Iloilo	Visayas	Dark brown	Red	Medium
29	I36	Anonang	Leon	Iloilo	Visayas	Straw	Dark red	Long
30	I37	Anonang	Leon	Iloilo	Visayas	Straw	Red	Short
31	I38	Anonang	Leon	Iloilo	Visayas	Straw	Red	Short
32	I39	Anonang	Leon	Iloilo	Visayas	Straw	White	Short
33	I40	Anonang	Leon	Iloilo	Visayas	Straw	Red	Short
34	I41	Anonang	Leon	Iloilo	Visayas	Straw	Red	None
35	I42	Anonang	Leon	Iloilo	Visayas	Purple	Red	Medium
36	I43	Anonang	Leon	Iloilo	Visayas	Straw	Red	Medium
37	I44	Anonang	Leon	Iloilo	Visayas	Brown/gray	Red	Short
38	I45	Anonang	Leon	Iloilo	Visayas	Brown/gray	Black	Medium
39	I46	Anonang	Leon	Iloilo	Visayas	Purple/black	Dark red	Medium
40	I47	Anonang	Leon	Iloilo	Visayas	Brown/gray	Red	Short
41	I48	Anonang	Leon	Iloilo	Visayas	Straw	Red	Short
42	I49	Anonang	Leon	Iloilo	Visayas	Purple	Dark red	Short
43	I50	Anonang	Leon	Iloilo	Visayas	Straw	White	Medium
44	I51	Anonang	Leon	Iloilo	Visayas	Straw	Red	None
45	I52	Anonang	Leon	Iloilo	Visayas	Straw	Red	Short
46	B1	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Short
47	B2	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Short
48	B3	Bunducan	Nasugbu	Batangas	Luzon	Straw	Dark brown	Short
49	B4	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Medium
50	M2	Unknown	Unknown	Maguindanao	Mindanao	Dark brown	Red	Medium

Table 1 (cont'd).

No	Code	Barangay	Municipality	Province	Island	Hull Color	Pericarp Color	Awn
51	M1	Unknown	Unknown	Maguindanao	Mindanao	Straw	Dark Brown	None
53	B12	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	None
54	B11	Bunducan	Nasugbu	Batangas	Luzon	Bronze	Red	Short
55	B10	Bunducan	Nasugbu	Batangas	Luzon	Straw	Brown	None
56	B9	Bunducan	Nasugbu	Batangas	Luzon	Straw	Dark Brown	Medium
57	B8	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Short
58	B7	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Long
59	B6	Bunducan	Nasugbu	Batangas	Luzon	Straw	Red	Medium
60	B5	Bunducan	Nasugbu	Batangas	Luzon	Straw	Brown	Short
61	SK2	Didtares	Lambayong	Sultan Kudarat	Mindanao	Straw	Brown	None
62	SK1	Didtares	Lambayong	Sultan Kudarat	Mindanao	Straw	Light Green	None
63	C2	Sinawingan	Libungan	North Cotabato	Mindanao	Straw	Black	None
64	C1	Sinawingan	Libungan	North Cotabato	Mindanao	Straw	Red	None
68	P4	Calzada	Mabini	Pangasinan	Luzon	Straw	Red	Short
69	P5	San Roque	San Manuel	Pangasinan	Luzon	Straw	Red	None
70	P6	San Roque	San Manuel	Pangasinan	Luzon	Straw	Red	None
71	P7	San Roque	San Manuel	Pangasinan	Luzon	Straw	Red	None
72	P8	San Roque	San Manuel	Pangasinan	Luzon	Straw	Red	None
73	P9	San Roque	San Manuel	Pangasinan	Luzon	Bronze	Red	None
74	P10	San Roque	San Manuel	Pangasinan	Luzon	Bronze	Red	None
75	P11	San Roque	San Manuel	Pangasinan	Luzon	Bronze	Red	Short
76	T1	Balag	Concepcion	Tarlac	Luzon	Bronze	Red	Long
77	IR64	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
78	Rc222	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
79	Rc216	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
80	Rc152	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
81	Rc128	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
82	Rc160	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
83	Rc122	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
84	Rc64	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
85	Rc82	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
86	Rc18	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
87	Rc14	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
88	Rc10	IRRI*	Los Baños	Laguna	Luzon	Straw	White	None
89	O. minuta	Balian	Pangil	Laguna	Luzon	Straw	Red	Long
90	O. meyeriana	Magballo	Kabankalan	Negros Occ.	Visayas	Straw	White	None
91	O. meyeriana	Salong	Kabankalan	Negros Occ.	Visayas	Straw	White	None
92	O. meyeriana	Camansi	Kabankalan	Negros Occ.	Visayas	Straw	White	None
93	O. minuta	Salong	Kabankalan	Negros Occ.	Visayas	Straw	Red	Long
94	O. rufipogon	Lake Napalit	Pangantukan	Bukidnon	Mindanao	Straw	Red	Long
95	O. rufipogon	Lake Apo	Valencia	Bukidnon	Mindanao	Straw	Red	Long

Board (PSB), hence the cultivars, such as, PSB Rc 18.

The phenotypic characterizations of the WR biotypes included seed morphology, such as, hull color, tip color, awn size and color, and seed pericarp. Selection of the most commonly used, released, and cultivated inbred rice cultivars and wild rice accessions for comparison came from collections in representative locations in Luzon, Visayas, and Mindanao. Seeds of all the test plants were pre-germinated in Petri dishes lined with moist filter paper. Once the radicle protruded from the seed coat, seeds were sown in plant boxes in the screenhouse and allowed to grow until the right time for leaf collection.

Table 2. Simple-sequence repeats (SSRs) used in the study.

SSR Markers	Chr. No.	SSR motifs	Forward Sequence	Reverse Sequence
RM495	1	(CTG)7	Aatccaaggtgcagagatgg	Caacgatgacgaacacaacc
RM11904	1	(TGC)7	Agcttctgagccattgagacagg	Catcaacatttgcagcaacagc
RM113	1	(CA)8	Caccattgcccatcagcacaac	tcgccctctgctgcttgatggc
RM514	3	(AC)12	Agattgatctcccattcccc	cacgagcatattactagtgg
RM16945	4	(AAAT)9	Agcctgagcctgaatttgaacg	aaagatgtgtgctgccaagagg
RM334	5	(CTT)20	Gttcagtgttcagtgccacc	gactttgatctttggtggacg
RM162	6	(AC)20	Gccagcaaaaccagggatccgg	caaggtcttgtgcggcttgcgg
RM19574	6	(AAAT)6	Tcatcacaagctcgtaatcagg	ccagagaataagaggacatgacg
RM447	8	(CTT)8	Cccttgtgctgtctcctctc	acgggcttcttctccttctc
RM152	8	(GGC)10	Gaaaccaccacacctcaccg	ccgtagaccttcttgaagtag
RM447	8	(GA)16	Acgggcaatccgaacaacc	tcgggaaaacctaccctacc
RM328	9	(CAT)5	Catagtggagtatgcagctgc	ccttctcccagtcgtatctg
RM6051	9	(CCG)10	Aggctgatccaagatccatg	cccggaggctgattcttg
RM24843	9	(CTCC)5	Gccctacgtcagcgaagagtgg	agacgcagataaggcaggcaagc
RM23679	10	(AGAA)10	Tttgagccaaatccaaacccaacc	accaacatcccacacagtgaacacc
RM25934	10	(CAT)7	Tttgagccaaatccaaacccaacc	accaacatcccacacagtgaacacc
RM171	10	(GATG)5	Aacgcgaggacacgtacttac	acgagatacgtacgcctttg
RM25934	10	(CAT)7	Tttgagccaaatccaaacccaacc	accaacatcccacacagtgaacacc
RM27233	11	(CTG)8	Cccatgtacctgtgaggactgc	gttagggttctgatgctttgttgc
RM144	11	(ATT)11	Tgccctggcgcaaatttgatcc	gctagaggagatcagatggtagtgcatg

Genomic DNA extraction

The modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) extracted total genomic DNA from the leaves of 21-day-old seedlings. The purity and concentration of DNA were determined spectrophotometrically (Nanodrop deNovix DS-11 Spectrophotometer, USA) at 260nm and 280nm, while its quality was determined using agarose gel electrophoresis (Cleaver Scientific, UK). The DNA samples with Tris-EDTA (TE) buffer were diluted with sterile distilled water for the amplification of SSR primers, which were previously optimized by Caguiat *et al.* (2021) as shown in Table 2 and used in the analysis of genetic diversity.

Polymerase chain reaction (PCR) analysis

PCR test was conducted in a reaction of 5.6µl volume containing 5× PCR buffer, 5µm deoxynucleotide triphosphate (dNTPs), 25mM of MgCl2, 10mM of forward and reverse primer, 5 units of *Taq* DNA Polymerase, and the template DNA. PCR amplification was performed using a thermal cycler (PCRmax Alpha Cycler 1, USA), according to the cycle profile: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 2 min, and final extension at 72°C for 7 min. PCR-amplified products were subjected to electrophoresis in 8% polyacrylamide gel in 1× Tris/Borate/EDTA (TBE) buffer at 100 volts with a running time of 75 min. The gels were stained with gel red for 10 min. DNA bands were visualized under UV light using the AlphaImager gel documentation system (Biorad Gel Doc XR+ Imaging System, USA).

Data analysis

Amplified products from microsatellite analysis were scored qualitatively for the presence (scored "1") and absence (scored "0") of each marker allele genotype combination. The genetic index for each SSR marker—including major allele frequency, genotype number, allele number, gene diversity, heterozygosity, and the polymorphism information content (PIC)—was determined using the PowerMarker software version 3.25. Performing the analysis and dendrogram construction used the Unweighted Pair Group Method with Arithmetic Average (UPGMA) through the NTSYSpc software version 2.02. Genomic and phenotypic data analysis. Population genetics parameters estimate genetic diversity and differentiation among cultivated rice cultivars and weedy rice accessions. The genetic diversity was estimated in seven provinces and cultivated rice groups based on 88 SSR loci.

The calculated genetic parameters included: (i) number of observed alleles per locus (Na); (ii) number of effective alleles per locus (Ne); (iii) observed heterozygosity (Ho); (iv) Nei's unbiased expected heterozygosity (He); and (v) fixation index (F, i.e., inbreeding coefficient). These analyses were conducted using the software GenAlEx ver. 6.5.31 (Peakall and Smouse, 2012).

RESULTS

Weedy rice (WR) biotypes collected in direct-seeded rice in the Philippines were highest in the Visayas at 59%; Luzon with 33%; and Mindanao at only 8% (Figure 1). The collection was conducted in seven provinces wherein Iloilo had the most (59%), followed by Batangas (17%), and Pangasinan (14%) (Figure 2).

Figure 1. Collection sites of weedy rice across Luzon, Visayas and Mindanao.

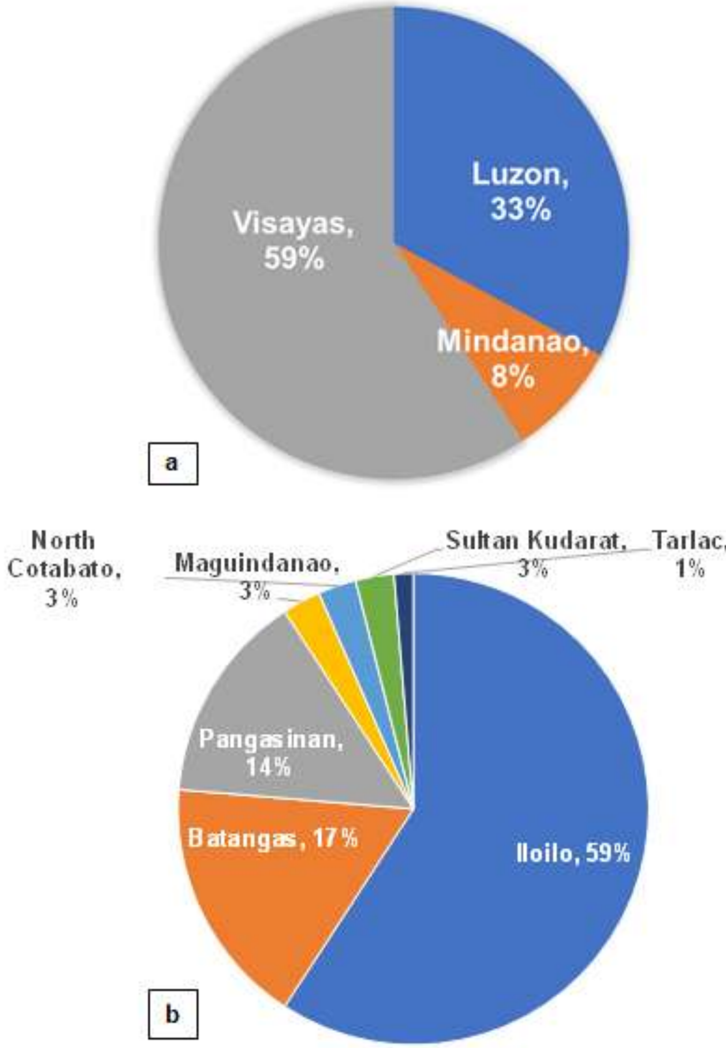

Figure 2. Distribution of weedy rice in the Philippines showing a) island-level, b) per province level.

To determine the relationships between the WR biotypes and cultivated rice cultivars, the study conducted cluster analysis using simple matching UPGMA with an overall high diversity of 68%. Thirteen subgroups of at least two populations formed 100% similarity based on post-harvest data (Figure 3). Four subgroups consisted of WR biotypes from Iloilo, two from Pangasinan, and one from Batangas. Two WR subgroups formed had Iloilo and Batangas, and one WR subgroup each for Iloilo and Cotabato, Iloilo and Pangasinan, and Batangas and Pangasinan. All cultivated rice cultivars (CRV) formed one subgroup.

On the other hand, a separate cluster analysis used polymorphic SSR markers for WR biotypes, cultivated rice, and wild rice accessions (Figure 4). Five major clusters ranged from three (I) to 62 WR biotypes (III). A 100% similarity was observed for 15 subgroups ranging from at least two to 10 WR biotypes. The wild rice relatives formed species-specific grouping for *O. minuta*, *O. rufipogon,* and *O. meyeriana* with one outlier. Four pairs of WR biotypes from Iloilo had 100% similarity (I45 and I46, I34 and I50, I3 and I4, I16 and I40), and ten biotypes formed one group. Batangas formed three WR biotypes (B8, B7, B6), while Pangasinan formed three WR biotypes (P1, P2, P3) and a pair (P6 and P9). Mixed-province subgroup with 100% similarity was with Iloilo and Batangas (I39, I41, and B2). Notably, NSIC Rc 216 and Rc 152, PSB Rc 18 and Rc 10, and PSB Rc 14 and WR P5 formed separate subgroups at 100% similarity.

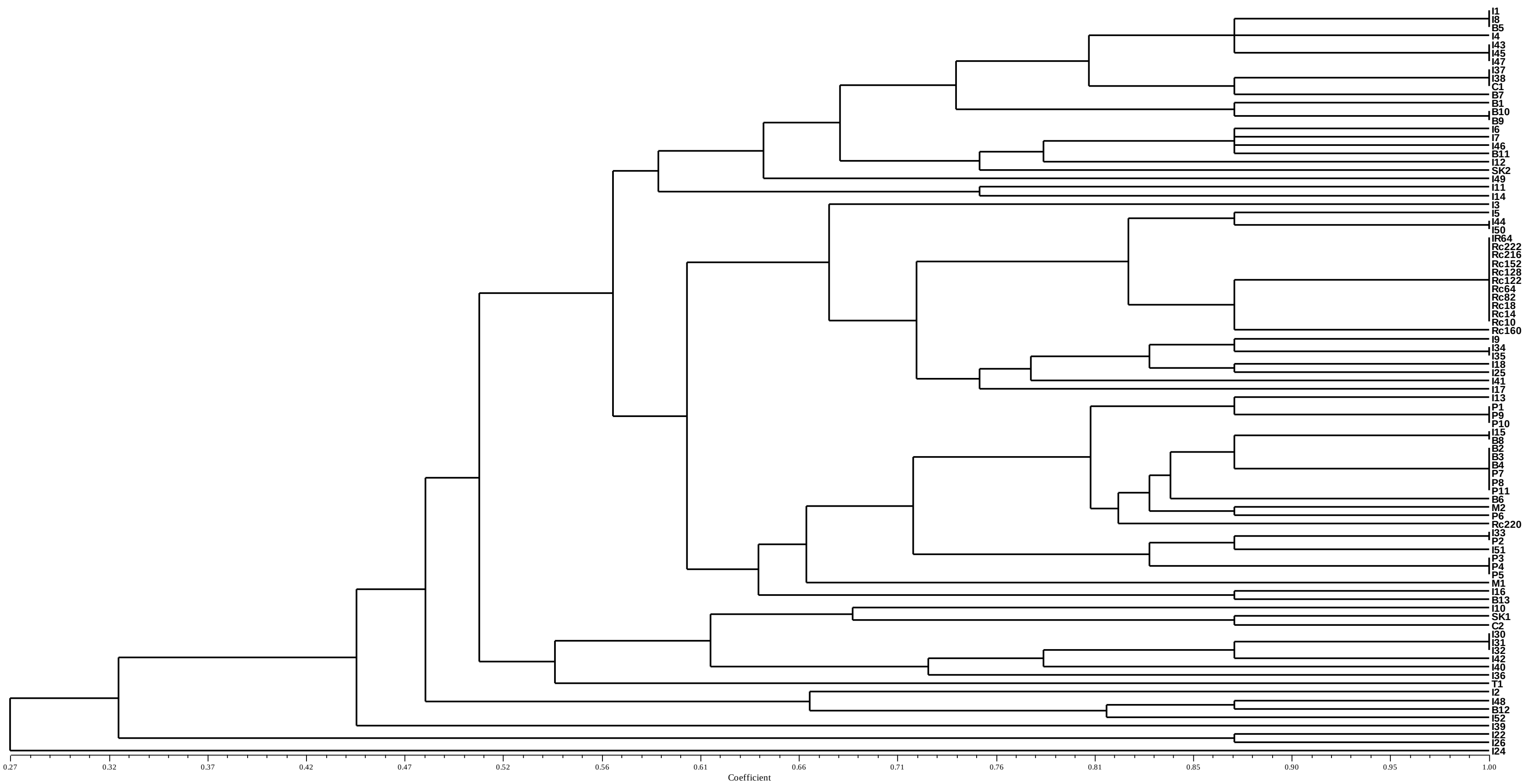

Figure 3. Cluster analysis of the weedy rice in the Philippines using UPGMA based on post-harvest data.

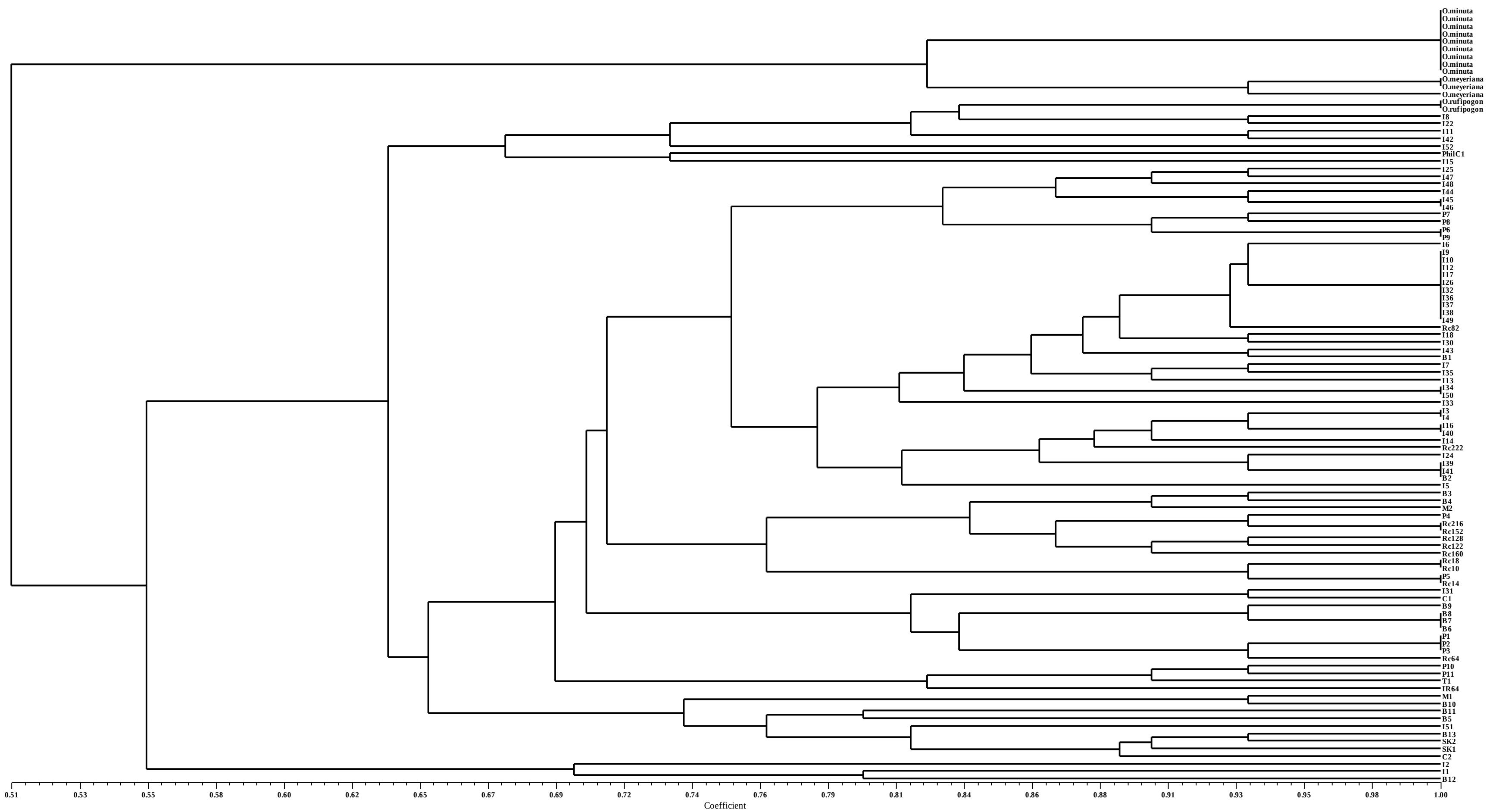

Figure 4. Cluster analysis of the weedy rice and wild rice in the Philippines based on SSR markers

Table 3 shows the possible relationships of the biotypes with wild rice relatives (>66%) and CRV. For wild rice populations, mixed-province WR biotypes (from Iloilo and Pangasinan) are closely related to *O. minuta;* only WR biotype P5 to *O. meyeriana;* and more diverse WR biotypes from Iloilo, Batangas, and Sultan Kudarat were related to *O. rufipogon.* For the CRV, high similarity (>80%) was observed for WR biotype B3 and six CRVs; WR biotypes P4, P5, and four CRVs; WR biotypes I43, I22, M2 and three CRVs; and 13 WR biotypes with two CRVs.

There were WR biotypes with high similarity to both wild rice populations and CRVs. Based on similarities, the WR biotype P5 has high similarity to *O. meyeriana, O. minuta,* and cultivated rice cultivars PSB Rc 10, Rc 14, Rc 18, and NSIC Rc 160. Of the 28 WR biotypes related to *O. rufipogon*, 12 were also related to cultivated rice cultivars (NSIC Rc 222, Rc 122, PSB Rc 82, Rc 18, and Rc 10). However, 17 distinct WR biotypes were highly similar only to wild rice accessions and 25 WR biotypes only to CRVs.

In terms of genetic diversity and inbreeding level among 76 WR biotypes from the seven provinces, an average of 3.6 alleles per locus was detected across 88 SSR loci, but they exhibited a moderate level of genetic diversity (He = 0.202) compared with the other populations (He = 0.142 to 0.4). Among the WR populations, there was a 2.56 average number of effective alleles (Ne) with 0.94 Shannon's information index (I) and 0.55 expected heterozygosity (He). In general, the average levels of these parameters, Ne, I, and He, were higher in the CRVs than with WR populations, although a considerable variation in these parameters was observed among the populations. Of the WR populations, Batangas WR biotypes had the highest levels of genetic diversity for the three parameters, while Tarlac WR had the lowest Ne (1.88), and Maguindanao WR biotypes had the least He at 0.42 (Table 4).

In terms of pairwise comparison at the provincial level, Cotabato WR had the most genetic distance (0.46) with the CRVs, while Iloilo had the least genetic distance (0.15). Among the WR population, the highest genetic distance was recorded between Maguindanao and Tarlac (0.51), while the least was between Pangasinan and Iloilo (0.11) (Table 5).

Table 3. Similarity of weedy rice biotypes with wild rice accessions and selected cultivated cultivars in the Philippines.

Released cultivars / wild rice relatives	Weedy rice biotypes
O. meyeriana	P5
O. minuta	I2, P5
O. rufipogon	I3, I4, I5, I6, I8, I11, I13, I14, I16, I22, I24, I35, I39, I40, I41, I42, I43, I46, I48, I51, I52, B1, B2, B13, B11, B10, B5, SK1
IR64	P11
NSIC Rc 222	I3, I4, I16, I22, I39, I40, I41, I48, B2, B3, B4, P6, P9
NSIC Rc 216	B3, M2, P4
NSIC Rc 152	B3, M2, P4
NSIC Rc 128	I34, I50, B3
NSIC Rc 160	I30, B3, B4, M2, P5, P11
NSIC Rc 122	I39, I41, B2, B3, B4, P4
PSB Rc 64	P1, P2, P3
PSB Rc 82	I6, I9, I10, I12, I16, I17, I18, I26, I32, I35, I36, I37, I38, I40, I43, I49, P1, P2, P3, P4, P6, P9
PSB Rc 18	I22, I43, P5
PSB Rc 14	P5
PSB Rc 10	I22, I43, P5

Table 4. Parameters of genetic diversity in weedy rice and cultivated rice groups based on 88 SSR loci.

Province	Ne	I	He
Cultivated cultivars	2.90	1.00	0.62
Batangas	3.48	1.00	0.68
North Cotabato	2.33	0.87	0.56
Iloilo	3.41	0.98	0.65
Maguindanao	2.00	0.65	0.42
Pangasinan	2.68	0.95	0.60
Sultan Kudarat	2.12	0.76	0.52
Tarlac	1.88	0.61	0.44
Mean (WR only)	2.56	0.94	0.55

Ne, number of effective alleles, I = Shannon's information index (Shannon, 1948), He, Nei's unbiased expected heterozygosity

Table 5. Pairwise population matrix of Nei genetic distance on weedy rice and cultivated rice groups based on 88 SSR loci.

Rice biotypes	Batangas	North Cotabato	Iloilo	Maguindanao	Pangasinan	Cultivated cultivar	Sultan Kudarat	Tarlac
Batangas	-							
North Cotabato	0.20	-						
Iloilo	0.12	0.33	-					
Maguindanao	0.26	0.45	0.36	-				
Pangasinan	0.13	0.24	0.11	0.26	-			
Cultivated cultivar	0.25	0.46	0.15	0.32	0.18	-		
Sultan Kudarat	0.17	0.14	0.27	0.37	0.28	0.39	-	
Tarlac	0.38	0.46	0.35	0.51	0.26	0.39	0.42	-

DISCUSSION

The information on the relationships of the *Oryza* spp. complex allows us to get information on the genetic identity of WR biotypes in the Philippines. Farmers often refer to WR as contamination or off-type, which is attributed to have come from the soil. But most are not aware of the origins of the different morphological characteristics, i.e., generally taller, purple color in the stem, grains with awn, different hull color, and pericarp (Tanzo and Martin, 2014).

Relative to cultivated rice, Philippine WR are generally (1) taller, with longer, droopy leaves and panicles than cultivated rice; (2) with seeds that shatter at or even before maturity; (3) with grains that are either awned or awnless (cultivated rice have no awns) and with red or white pericarp; and (4) with seed characteristics including weight, length, width, and presence and length of awn differing among weedy rice variants (Guzman, 1996; Baltazar and Janiya, 2000; Chauhan and Johnson, 2010; Martin *et al.,* 2014; Donayre *et al*., 2016). In this study, the characteristics of the majority of WR biotypes showed the presence of awns (generally attributed to wild rice), colored hulls, and pigmented pericarps. Grouping the WR biotypes according to grain hull color, pericarp color, and presence of awns showed 65% having straw-colored hull, 22% bronze hull, and 13% with dark colored hull (purple, black, deep brown, or gray). Of the 76 WR samples, 64 had awns, with three partially-awned or absent and only 12 without awn. Further distinguishing the WR biotypes through pericarp color, only seven have white pericarp and the rest with colored pericarp: light green, pink to red, and 10 biotypes with very dark brown to black pericarp, regardless of hull color and presence or absence of awn. Grains can be long and slender, medium, to bold, but a good diversity of the characteristics of WR is shown to be a result of the segregation after the natural crossing or natural hybridization among weedy types and the continuous succession of rice cultivars of different statures, canopy structures, maturities, grain types, and others (Delouche *et al.,* 2007; Ziska *et al.,* 2015). Hence, one cannot remove de-domestication as an important contributor to the emergence of weedy rice biotypes in DSR areas. On the other hand, WR weedy characteristics include seed shattering and seed dormancy, which are traits of wild rice species (Gressel, 2005).

Overall, the genetic analysis using SSRs in this study provides an outlook on the diverse possible ancestry of WR biotypes in DSR areas with contributions of both cultivated and wild rice and they are not at all related to each other. Of the wild rice relatives, more WR biotypes are highly similar to *O. rufipogon.* The common wild rice (*O. rufipogon*) has been found to contribute to the evolution of sympatric weedy rice in South and Southeast Asia, however, studies do not show direct evolution of WR from *O. rufipogon,* indicating crop-wild hybridization (reviewed by Burgos *et al.,* 2021). In Malaysia and Thailand, studies on WR using SSRs and SNPs revealed natural gene flow and hybridization between known wild rice and cultivated rice as the major causes of occurrence and spread of WR biotypes (Pusadee *et al.,* 2013; Song *et al.,* 2014; Neik *et al.,* 2019; Vigueira *et al.,* 2019; Wedger *et al.,* 2019). Characterization of WR phenotypes in South Asia, and their associated candidate genes, contributes to the emerging understanding of mechanisms by which WR evolves worldwide. It suggests that standing ancestral variation is often the source of weedy traits in independently evolved groups highlighting the reservoir of genetic variation that is present in CRVs and wild rice and its potential for phenotypic evolution (Huang *et al.,* 2018).

However, where no wild rice is present, such as, in South Korea and the US, the more likely evidence of origin is from de-domesticated crop cultivars (i.e., *japonica*-derived South Korean WR as reported previously by Cho *et al.,* 1995; He *et al.,* 2017). Similarly, Li *et al.* (2017) found that two major WR (straw hull and black hull awned) in the USA and some Chinese accessions descended primarily from domesticated ancestors (*indica* and *aus* rice cultivars though not grown in the USA). Environmental adaptive mechanisms can also affect the presence or absence of WR traits in several populations.

Understanding that WR evolution affects efficient control is needed as information on its origin, including the underlying weedy traits, such as, seed shattering and dormancy, and enhanced photosynthesis, among others, may control the continuing evolution of WR groups and the emergence of these traits (Ziska *et al.,* 2015). The competitiveness of WR highly favors its successful establishment and achieves considerable advantages over CRV. Hence, distinguishing the traits that allow WR to establish successfully is a crucial factor to consider in any WR management strategy.

CONCLUSIONS

In closing, the study presents evidence of the different origins of weedy rice biotypes in direct-seeded rice areas with ancestries from cultivated rice and wild rice populations. Further, from the high genetic similarity of weedy rice biotypes to wild rice populations, that possible hybridization occurred between cultivated rice and wild rice, especially on the common *O. rufipogon*, the putative source of the red pericarp in most weedy rice biotypes. However, in weedy rice biotypes with high similarity to cultivated rice varieties, the other route of origin can be the de-domestication of improved rice cultivars giving rise to weedy traits. Hence, the need to further study the adaptive mechanisms of weedy rice traits, including their interactions with cultivated rice varieties as inputs to the stability of selecting traits during the breeding process for rice improvement.

ACKNOWLEDGMENTS

The authors would like to acknowledge the invaluable assistance of Ms. Mary Ann M. Rañeses of the Genetic Resources Division of the Philippine Rice Research Institute (PhilRice), and Dr. Virender Kumar and Mr. Bjorn Manuel of the International Rice Research Institute (IRRI). Likewise, also grateful to Mr. Constante T. Briones of PhilRice for the English language editing.

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