RESEARCH ARTICLE

WEEDY RICE: AN INSIGHT INTO GENETIC DIVERSITY AND POPULATION STRUCTURE FOR EFFECTIVE WEED MANAGEMENT

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ABSTRACT

Weedy rice (*Oryza sativa* f. *spontanea*) is a conspecific weed that has invaded cultivated rice fields, effectively adapted to modern management practices, leading to substantial yield losses and reduced harvest quality. The understanding of weedy rice genetic diversity and population structure is critical in developing effective control measures. Twenty weedy rice populations were collected around the island and evaluated using 33 SSR markers. Our findings indicated that the genetic diversity (H_e) in a population of weedy rice was reasonably high (0.305 - 0.560). A high level of within-population (79%) and a low level of among-population (21%) genetic variation were found by analysis of molecular variance (AMOVA). There is no significant correlation across genetic and geographical distances (P>0.05). The unweighted pair group method with arithmetic mean (UPGMA) demonstrated that 20 populations were structured into two well-separated groups. Remarkably, many admixed individuals were evident according to the STRUCTURE. Weedy rice management strategies should focus on micromorphological monitoring to detect, group, and eliminate weedy rice in paddy fields, restricting the exchange of saved paddy seeds among geographical regions, increasing the production and distribution of certified seeds, the varietal recommendation for rice-growing regions to minimize mixing of varieties with different age classes, raising awareness are the recommendations to reduce the emergence of future weeds.

Keywords: Genetic variation, Invasive weeds, Rice ecosystem, SSR fingerprinting, Weed management

INTRODUCTION

Weedy rice, also known as *Oryza sativa* f. *spontanea*, is a member of the Poaceae family and is broadly distributed in rice ecosystems around the globe (Wang *et al*. 2022; Hsu *et al*. 2022). It is prevalent as a troublesome weed species in areas where direct-seeded rice (DSR) is practiced (Rao *et al*. 2007; Singh *et al*. 2013). Weedy rice is common in Asian rice fields, notably in India, Thailand, Vietnam, Malaysia, Sri Lanka, and the Philippines (Azmi *et al*. 2005; Abeysekera *et al*. 2010; Nadir *et al*. 2017). First reported in the Eastern province (Marambe and Amarasinghe 2000), currently distributed in all rice-producing regions of Sri Lanka (Ratnasekera *et al*. 2014). About 450,000 hectares of paddy lands in Sri Lanka are cultivated during the Yala season (minor growing season; March-September) supported by the first inter-monsoon (March-April) and the southwest

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monsoon (May-September), while 850,000 hectares are cultivated during the Maha season (main growing season; October-February) supported by the second inter-monsoon (October-November) and the northeast monsoon (December-February) (USDA 2020, Punyawardena 2008). Around 1.8 million farmer families are actively involved in paddy cultivation across the island (RRDI 2019). Currently, Sri Lanka produces 2.7 million metric tons of rice annually, which meets approximately 95% of local demand. It is expected that rice demand will increase by 1.1% annually. To achieve this requirement, rice production should increase by 2.9% per year (Weerakoon *et al*. 2011; Marambe *et al*. 2020; USDA 2020; De Silva *et al*. 2020). But, it is challenging because of substantial yield loss due to the heavy infestation of weedy rice in the Sri Lankan rice ecosystem.

Taxonomically, weedy rice and cultivated rice belong to the same species of the genus *Oryza* (*O. sativa*). Because of their close phylogenetic relationships and long-term sympatric distribution, weedy and cultivated rice show biological and developmental similarities (Hsu *et al*. 2022; Cao *et al*. 2006). Weedy rice may readily gain genetic diversity from crops via spontaneous hybridization and introgression, in which genes from a particular crop can be transmitted to a weedy species (Ellstrand *et al*. 1999). This mechanism increases the genetic diversity and phenotypic plasticity of conspecific weed species in agroecosystems. Moreover, in rice fields, gene flow from cultivated species to weedy rice is common, and farmers' selections against weedy types during rice weed management may result in diminished genetic heterogeneity in weedy rice across consecutive seasons. Even though, weedy rice has high genetic diversity and phenotypic plasticity, which contributes to its success as a competitor (He *et al*. 2014). It causes crop failures and is characterized by faster growth, high tillering, early shattering, and varied seed dormancy (Singh *et al*. 2013; Delouche *et al*. 2007). Weedy rice is morphologically diverse, with taller and more open panicles and weaker culms compared to cultivated rice (Delouche *et al*. 2007). Abeysekera *et al*. (2013) reported a distinct characteristic evident in the weedy rice populations of Sri Lanka, wherein the plant architecture exhibited considerable variability, with a recorded count of more than 4,849 morpho-types. Studies conducted in Matara (Southern Province) and Kurunegala (North Western Province) districts have shown that a limited number of prominent agro-morphological traits within the weedy rice ecotypes may be derived from a mixture of germplasm of cultivated or wild rice varieties (Ratnasekera *et al*. 2014). Subasinghe *et al*. (2007) used the Random Amplified Polymorphic DNA (RAPD) technique to determine the relationship between weedy rice, cultivated rice, and wild rice varieties available in Sri Lanka. The resulting UPGMA cluster diagram revealed four distinct clusters corresponding to different rice accessions, with weedy rice clustering together with cultivated rice varieties. The finding suggests a close relationship between these accessions.

Therefore, the processes that transmit and accumulate genetic diversity through pollination or seed dispersion in a particular region must also be revealed. An investigation on the genetic differentiation and population structure of weedy rice was thus carried out to confirm the existence of a rich within-population genetic diversity in the weedy rice populations sampled across the island. Further, the study evaluated the role of geographical genetic structure or population differentiation among weedy rice populations in Sri Lanka to identify the processes that transmit and accumulate genetic diversity. Based on the findings of the study, it is expected to suggest prevailing weedy rice management strategies to be adopted in Sri Lanka.

MATERIALS AND METHODS

Plant Material

The homogeneity of cultivated rice makes it easy to distinguish weedy rice by its characteristic traits, such as differences in seed and panicle features, typically exhibiting taller stature, profuse tillering, a more open or spreading growth habit, and weaker culms. Weedy rice plants were collected from 20 major rice-growing areas in Sri Lanka that had a recent history of heavy weed infestation, as confirmed by personal communication with

the Department of Agriculture, Sri Lanka (Fig. 1). One population was defined as approximately 25 weedy rice plants randomly sampled from each field, which covered an area of approximately 01 ha. Thus, a total of 500 individual weedy rice plants were collected across the entire geographical distribution in Sri Lanka. The sampled rice fields infested with weedy rice were at least 30 km apart from each other. To prevent repeated samples of the same genets (clones), the distance between sampled individuals was maintained at least 10 m apart. Green leaf samples were collected from rice fields in the Maha season from late February to March 2016. The green leaves were collected individually in the field, placed in zip-lock bags containing silica gel, and stored at 0-4 °C until DNA extraction. Information on population codes, the number of individuals sampled, locations, and GPS readings of the weedy rice populations are available from the first author upon request.

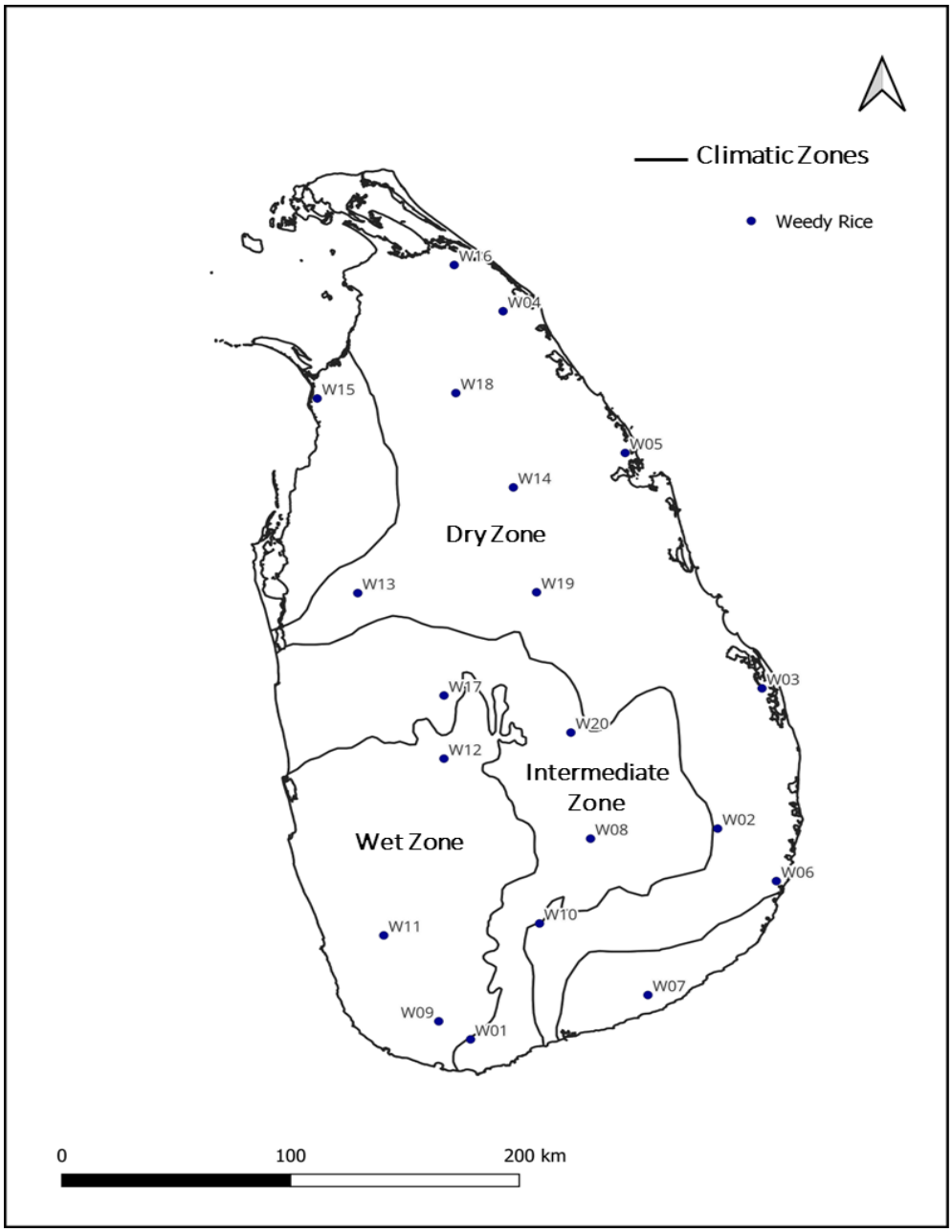

Figure 1: Geographical locations of 20 weedy rice populations. Each dot represents a different weedy rice population

Molecular Screening

Molecular studies were conducted at the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing, China, using the 20 mg silica gel-dried leaves collected from 500 weedy rice plants from Sri Lanka. Preliminary screening of 150 simple sequence repeat (SSR) primer pairs across representative individuals sampled from different populations revealed that 33 SSRs produced clear and reproducible products. These 33 microsatellite primer pairs created from cultivated rice, according to the RiceGenes Database (https://gramene.org), were used to assess the genetic variation of weedy rice in this study. The 33 loci were dispersed in all 12 chromosomes in the rice genome, with the polymorphism varying widely among loci. Detailed information on the primer pairs is available from the first author upon request. Primers were synthesized by ABI DNA Analyzer (Applied Biosystems, Foster City, CA, USA), with the forward primers labelled with blue (FAM), yellow (TAMAR), and green (HEX) fluorophores.

DNA Extraction and PCR Amplification

The total genomic DNA was extracted from dried leaves of 500 samples using the Plant genomic DNA kit (Biomed DL114-01, China) following the CTAB protocol (Saghai-Maroof *et al*. 1984) with minor modifications. The DNA amplification was carried out using a 2,720 thermal cycler (Applied Biosystems, USA) in a 15 µl reaction mixture. Each reaction contained 5 µl buffer (ddH_2O), 7 µl 2 × Taq PCR MasterMix (0.1 U Taq Polymerase/ µl, 500 µM dNTP each, 20 mM Tris–HCl, 100 mM KCl, 3 mM $MgCl_2$) (TIANGEN KT201, TIANGEN Biotech (Beijing) Co., Ltd), 1 µl each primer (forward and reverse) (10 µM), and 1 µl template DNA. PCR cycling was performed at 94 °C, 3 min; 35 cycles of 94 °C, 30 s, 55 °C, 30 s, and 72 °C, 1 min; and 72 °C, 10 min for the final extension.

The PCR products (2 µl) were diluted with 8 µl of ultrapure water and scoured with 100% alcohol for 15 min. The diluted DNA was then dried and mixed with highly deionized formamide (Applied Biosystems, USA) and submitted to fragment analysis by capillary electrophoresis using an Applied Biosystems 3130 × l DNA Analyzer (Applied Biosystems, USA). The analysis of DNA fragment size and allele calling was performed using GeneScan and GeneMapper software (Applied Biosystems, USA), followed by manual allele binning. To analyze the FAM-, TAMRA-, and HEX-labelled DNA fragments with the ABI3130 series systems (Applied Biosystems, USA), the matrix standard kit was used to generate the "multi-component matrix". The Data Collection Software (Applied Biosystems, USA) was used to record the data of the multi-component matrix. The instrument automatically analyzed three different coloured fluorescent dye-labelled samples in a single capillary, and reference lanes were further verified by polyacrylamide gel electrophoresis. In the case of non-amplification, the PCR was repeated to exclude technical failure, and a null allele was recorded if both PCRs failed.

Statistical Data Analysis

All 33 SSR loci were evaluated for their adherence to the Hardy-Weinberg equilibrium and the presence of null alleles using the heterozygous deficiency method in Genepop v4.7 (Brookfield 1996). The presence or absence of linkage disequilibrium between loci was confirmed by testing for genotypic linkage disequilibrium. The genetic parameters for each population were assessed by calculating various parameters, including allelic richness (A), mean observed heterozygosity (H_0), percentage of polymorphic loci (P), and unbiased expected heterozygosity (H_e). All genetic diversity estimates were calculated using GenAlEx 6.5.01 software (Peakall and Smouse 2006). Additionally, the fixation index (F_{ST}) values were calculated using FSTAT version 2.9.3 and Arlequin 3.5 (Excoffier and Lischer 2010) to investigate the population differentiation. The AMOVA was performed in Arlequin 3.5 to determine the proportion of genetic variance explained by the differences within and among populations. Furthermore, a model-based program, STRUCTURE 2.3.4 (Pritchard *et al*. 2000), was used to infer the number of distinct genetic clusters and to assign individuals to a spe-

cific genetic cluster using default parameters. The program was executed with 20 independent runs for each value of K ranging from 1 to 10, each with 1,000,000 Markov chain Monte Carlo replications, following a 100,000 burn-in period. The admixture ancestry model and a correlated allele frequency model were used for all runs. The STRUCTURE HARVESTER online application was utilized to determine the estimated numbers of genetic components (K values) (Evanno *et al*. 2005; Verkuil *et al*. 2012). Clustering patterns and population structure inferences were determined throughout the K using the web tool CLUMPAK (Jakobsson and Rosenberg 2007; Kopelman *et al*. 2015). Both inter- and intra-specific genetic structures of the different populations were assessed using multivariate principal coordinates analyses (PCoA) through the GenAlEx 6.5.01 software (Peakall and Smouse 2006). The UPGMA clustering analysis of all populations was performed based on Nei's (1972) unbiased genetic distance using the PowerMarker software (Liu and Muse 2005), and the resulting tree was visualized with TREEVIEW ver. 1.52.

RESULTS AND DISCUSSION

Genetic Diversity

The 33 loci were dispersed across all 12 chromosomes of weedy rice, with the level of polymorphism varying widely among loci. These loci are co-dominant, highly polymorphic, and randomly distributed throughout the rice genome (Pusadee *et al*. 2013; Samal *et al*. 2018; Sandamal *et al*. 2018a), making them reliable for use in this study. The genotypic linkage disequilibrium among the loci did not show significant values (P>0.05), suggesting an absence of linkage disequilibrium between loci. All 33 loci displayed polymorphism (66.67% ~ 87.88%) among the 20 populations, with a total of 360 alleles identified. The most variable locus, RM426, had 39 alleles, while RM414 showed only two alleles across the populations, with an average of 18 alleles per population. Conversely, the number of effective alleles (N_e) was much lower than that of observed alleles (N_a), with an average of 3.802 and 2.49 alleles per locus, respectively. This indicates the presence of many rare alleles in weedy rice populations studied in Sri Lanka (Table 1). As measured by expected heterozygosity, the highest genetic diversity was found in the W3 population (H_e= 0.560), while the W2 population recorded the lowest (H_e= 0.305) (Table 1). The average genetic diversity of 20 weedy rice populations was relatively high (0.43) as measured by H_e, while varied considerably among weedy rice populations (Table 1). The majority of weedy rice populations had a high level of observed heterozygosity (H_o ranging from 0.465 to 0.573) and a wide range of inbreeding coefficients (-0.346 ~ 0.194) (Table 1), demonstrating both self-mating and cross-mating that have occurred in weedy rice populations. Moreover, as revealed by Dai *et al*. (2014), the genetic diversity of weedy rice can considerably increase its ability to compete with cultivated rice and the competitiveness of weedy plants.

Assessing genetic diversity and the probable mechanisms that contribute to the emergence and persistence of such diversity within and among weedy rice populations is critical for developing efficient weedy rice management techniques (Cao *et al*. 2006; Delouche *et al*. 2007). A comparison of weedy rice populations across various regions in China, using different molecular approaches, showed moderate to high genetic diversity (Han *et al*. 2020; Cao *et al*. 2006; Yu *et al*. 2005). Studies from the South Asian region reported low and high genetic diversity, suggesting that genetic diversity in weedy rice is variable and influenced by regional and local factors (Neik *et al*. 2019; Gealy *et al*. 2009; Song *et al*. 2014; Prathepha 2011). He *et al*. (2014) investigated the genetic diversity of 21 weedy rice populations from Sri Lanka and found an overall high genetic diversity (H_e = 0.62) and within-population genetic diversity (H_e = 0.37 -0.69) among the studied populations. The genetic diversity of Sri Lankan weedy rice, as revealed by previous studies (He *et al*. 2014), has declined over time, in contrast to the findings of the present study (H_e = 0. 0.43; ranging from 0.305 to 0.560).

Wongtamee *et al*. (2017) showed that the level of genetic diversity in weedy rice invading

Table 1: Genetic parameters characterizing 20 weedy rice populations in Sri Lanka

Pop	*N*	N_a	N_e	*P*	H_o	H_e	*F*	*I*
W1	25	3.606	2.523	81.82%	0.505	0.466	0.028	0.866
W2	25	2.667	1.655	75.76%	0.508	0.305	-0.346	0.503
W3	25	4.606	3.033	87.88%	0.501	0.560	0.194	1.079
W4	25	3.061	2.150	66.67%	0.480	0.355	-0.306	0.647
W5	25	3.273	2.380	75.76%	0.556	0.411	-0.230	0.743
W6	25	4.121	2.520	75.76%	0.490	0.422	-0.017	0.828
W7	25	4.303	3.020	78.79%	0.573	0.515	-0.069	1.002
W8	25	4.545	2.989	81.82%	0.501	0.522	0.106	1.034
W9	25	4.152	2.777	78.79%	0.465	0.478	0.130	0.933
W10	25	2.848	1.847	72.73%	0.479	0.327	-0.302	0.577
W11	25	4.303	2.973	75.76%	0.482	0.497	0.088	0.993
W12	25	3.576	2.486	72.73%	0.519	0.437	-0.095	0.825
W13	25	3.545	2.531	78.79%	0.524	0.441	-0.061	0.821
W14	25	3.545	2.203	72.73%	0.526	0.384	-0.182	0.713
W15	25	3.394	1.934	78.79%	0.547	0.367	-0.273	0.664
W16	25	4.182	2.671	78.79%	0.516	0.441	-0.065	0.878
W17	25	4.091	2.377	78.79%	0.508	0.424	-0.061	0.815
W18	25	4.303	2.677	81.82%	0.512	0.448	-0.003	0.892
W19	25	3.394	2.152	78.79%	0.541	0.385	-0.239	0.710
W20	25	4.515	2.921	75.76%	0.545	0.472	-0.079	0.947
Average	25	3.802	2.491	77.40%	0.514	0.433	-0.089	0.823

N, Number of samples; N_a, the average number of alleles; N_e, the average number of effective alleles ***P***, percentage of polymorphic loci; H_o, observed heterozygosity; H_e, expected heterozygosity; ***F***, inbreeding coefficient; ***I***, Shannon's information index.

rice fields decreases with each successive season, consistent with a gradual increase in homozygosity. The genetic heterogeneity in weedy rice has been reported to decrease due to gene flow from cultivated species to weedy types in rice fields, as well as farmers' selection against weedy types in subsequent seasons of rice farming. It is worth noting that although farmers mainly select phenotypes rather than genotypes, weedy rice maintains genetic diversity even when there is little to no morphological difference, as demonstrated by Wongtamee *et al*. (2017).

Population Structure

The weedy rice populations studied in Sri Lanka showed a high level of within-population genetic variation and a low level of among-population genetic variation, as estimated by AMOVA (Table 2). Of the total genetic variance observed, 21% (P<0.01) and 79% (P<0.01) were found to be structured among and within populations, respectively.

The analysis of pairwise F_{ST} measures showed a considerable degree of differentiation among weedy rice populations, with a wide distribution indicating low to high population differentiation (ranging from 0.011 to 0.553). The F_{ST} values were comparatively high when W2 or W4 populations were combined with other weedy rice populations. However, genetic differentiation between W12 and W20, W16 and W18, and W17 and W18 populations were not statistically significant (P>0.05). The matrix of pairwise F_{ST} values is available from the first author upon request.

The population divergence of Sri Lankan weedy rice populations did not follow the "isolation by distance" pattern. There was no significant correlation between genetic distance and geographic distance observed in this study [P (random Rxy) ≥ Rxy from the data, P = 0.015]. The Mantel test indicated a statistically non-significant low correlation (Rxy = 0.172). Interestingly, some populations collected from the same geographical regions with close spatial distance showed high population differentiation as measured by F_{ST}, which supports the Mantel test results (Fig. 2). However, populations from different geographical locations showed less population differentiation.

Table 2: AMOVA was performed on 500 weedy rice individuals collected from Sri Lanka using 33 SSR loci across 20 weedy rice populations

Group	df.	SSD	V	%
Weedy Rice				
Among populations	19	2190.19	2.18	21%***
Within populations	500	4230.50	8.46	79%***

df., degree of freedom; **SSD**, sum of squared deviations; **V**, variance component estimates; **%**, percentage of total variation, *** P<0.01.

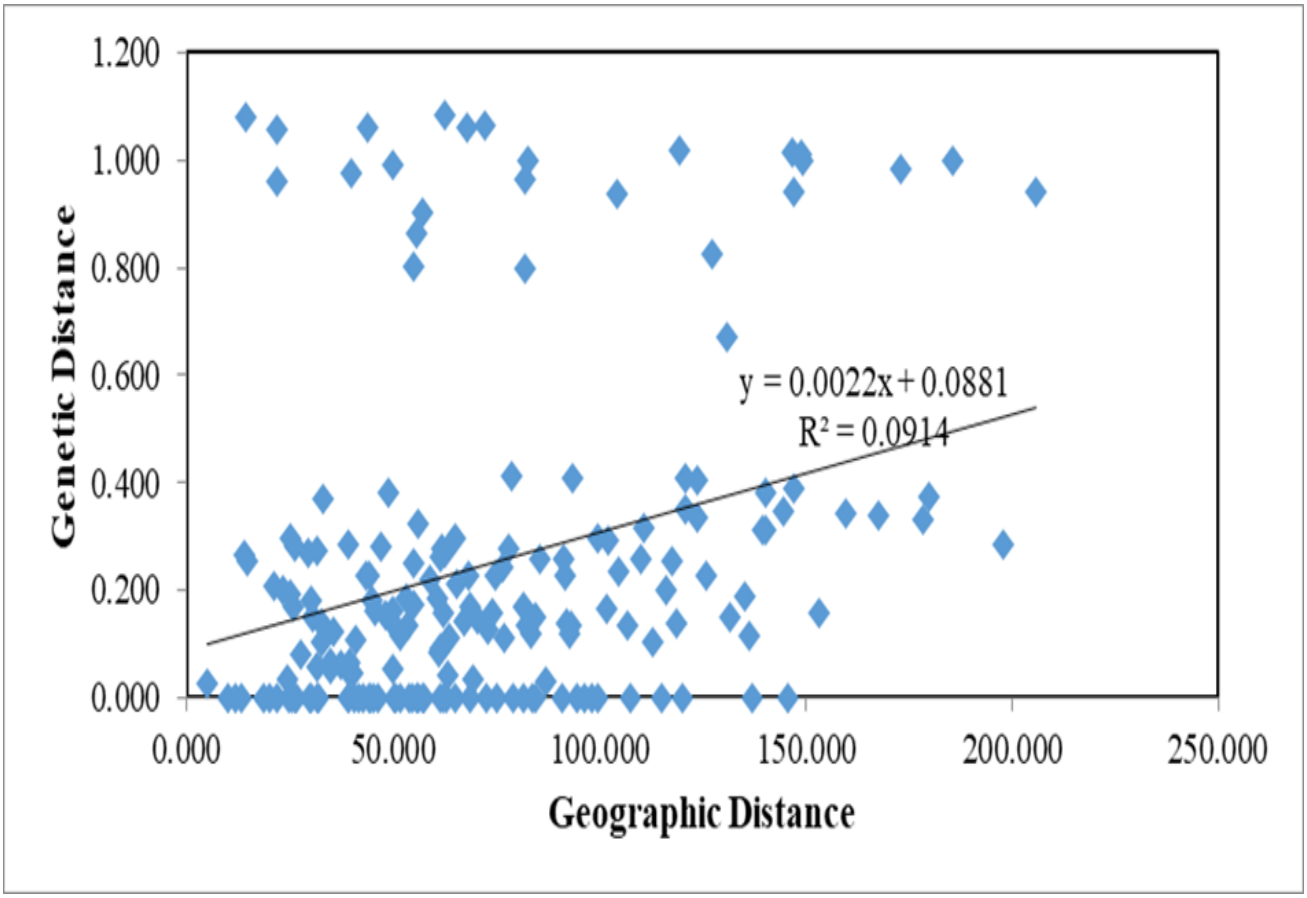

Figure 2: Mantel test for isolation-by-distance of geographic distance against genetic distance for 20 weedy rice populations in Sri Lanka estimated based on genotyping of the 33 SSR loci.

The UPGMA tree analysis revealed that the 20 weedy rice populations were clearly separated into two distinct major groups. Notably, the W4 population from Mullaithivu (Northern Province) and the W6 population from Panama (Eastern Province) in the coastal area formed a distinct cluster that was distantly separated from the others. Moreover, the W2 population from Siyambalanduwa (Uva Province), the W3 population from Batticaloa (Eastern Province), and the W1 population from Kamburupitiya (Southern Province) were also separated from the main cluster. The remaining weedy rice populations were divided into two separate clusters, as illustrated in Fig. 3.

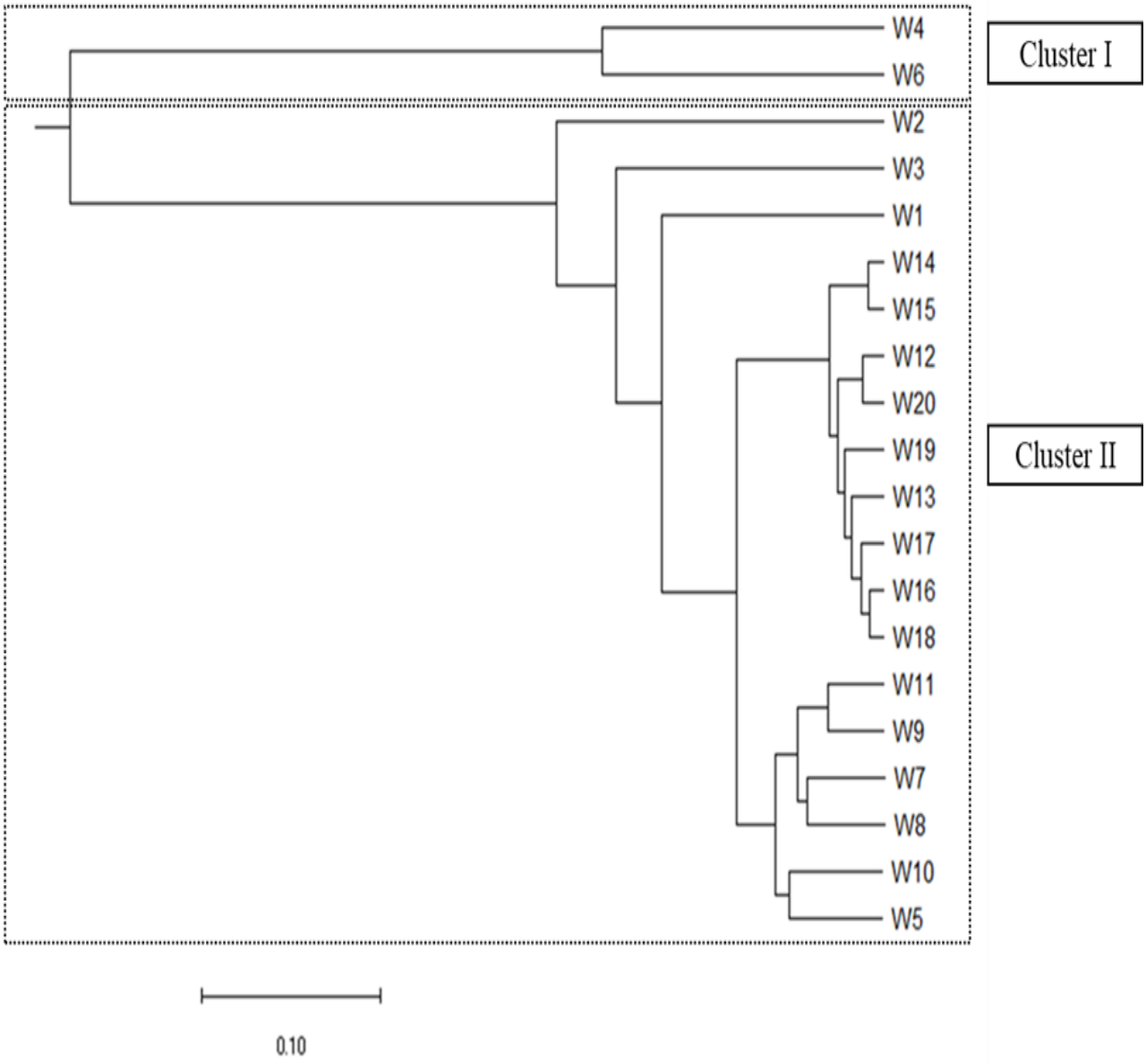

Figure 3: Dendrogram (UPGMA) constructed based on polymorphisms of 33 SSR loci in 20 weedy rice (*Oryza sativa* f. *spontanea*) populations, using Nei's unbiased genetic distance (Nei 1972). The bar represents genetic distance.

To investigate the genetic structure of weedy rice individuals and populations, the STRUCTURE analysis was conducted. The analysis revealed a clear peak in ΔK, as supported by Evanno *et al*. (2005), indicating the presence of two genetically similar subgroups (ΔK = 3001.92; Fig. 4) within weedy rice populations.

Similar to the UPGMA analysis, the W4 (Mullaithivu; Northern Province) and W6 (Panama; Eastern Province) populations were found to be distinct from other populations (Fig. 4) based on the STRUCTURE analysis. At K=2, the orange colour population component was predominant in W4 and W6 populations, and W1 (Kamburupitiya; Southern Province) represented a comparatively low proportion, while the blue component was predominant in most of the weedy rice populations. Notably, the STRUCTURE results revealed the presence of admixed individuals in the weedy rice populations. At K=10, the W2 population (Siyambalanduwa, Uva province) was differentiated from the other populations (Fig. 4), which is consistent with the UPGMA results (Fig. 3 and Fig. 4). At K=3, all weedy rice populations were structured into three main groups, with the dark purple component largely representing the W1 and W2 populations, W4 and W6 represented orange component while other represented blue component.

A principal coordinate analysis (PCoA) was conducted to assess the genetic relationships, diversity, and gene flow patterns among Sri Lankan weedy rice populations at the individual level. The scatter plot of the first (37.8%) and second (7.02%) principal components re-

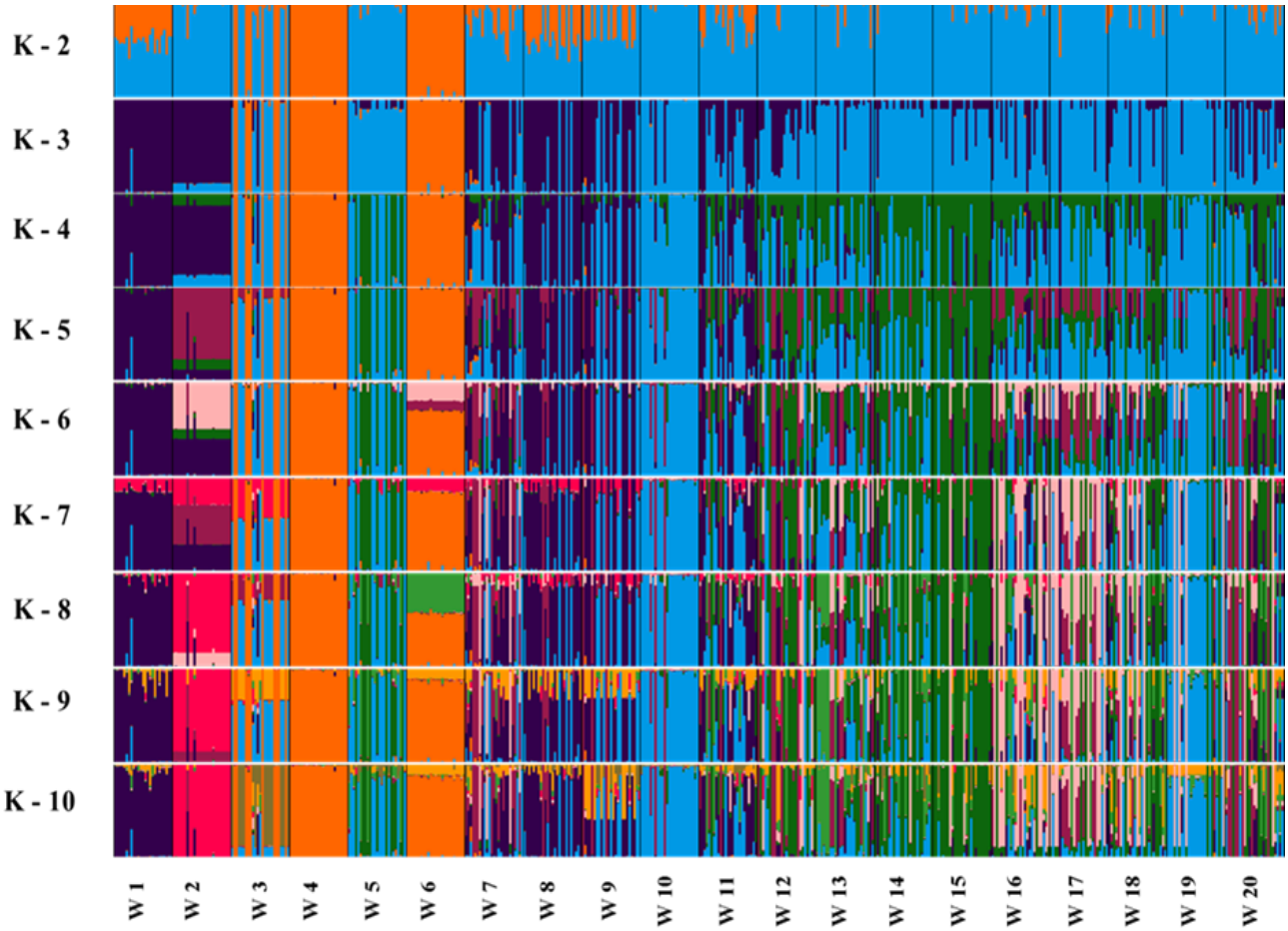

Figure 4: STRUCTURE graph showing genotype clustering of 20 weedy rice populations by model-based population assignment at K from 2 to 10. Each vertical bar represents an individual, with its assignment probability to genetic clusters represented by different colours.

vealed a clear genetic differentiation and variability within the populations (Fig. 5). The PCoA also corroborated the presence of two major groups, with W3 (Batticaloa; Eastern Province), W4 (Mullaithivu; Northern Province), and W6 (Panama; Eastern Province) being located in a distinct region of the plot due to their unique genetic characteristics (Fig. 5). Notably, the W3 (Batticaloa; Eastern Province) population exhibited a wide range of genetic diversity, with individuals scattered across a large area of the plot. Moreover, individuals from different populations tended to cluster together, indicating genetic similarity among populations. However, some overlapping of weedy individuals from different geographic regions was also observed.

Weedy rice had infested rice fields in Sri Lanka over a long period. The present results revealed that weedy rice has evolved to resemble cultivated rice, possibly due to the crop mimicry induced by human selection through rice farming (Wongtamee *et al*. 2017). In contrast to previous studies (Cao *et al*. 2006; Jiang *et al*. 2012; Reed and Frankham 2003), our study found a lower level of genetic differentiation among weedy rice populations (Table 2). Such a distinct pattern suggests that gene flow between populations is limited. However, weedy rice, a self-pollinating plant, had substantial within-population variation, suggesting gene flow, which is frequent in outcrossing species (He *et al*. 2014). These findings confirm that the seed-mediated gene flow has enhanced genetic diversity within weedy rice populations, as previously reported in other studies (Pusadee *et al*. 2009; Parzies *et al*. 2004), and low genetic differentiation among weedy rice populations, as in cultivated rice (Pusadee *et al*. 2009), leading to their low temporal genetic structure. Seed-mediated gene flow is a significant factor in the rapid dissemination and infestation of weedy rice in Sri Lanka (He *et al*. 2014).

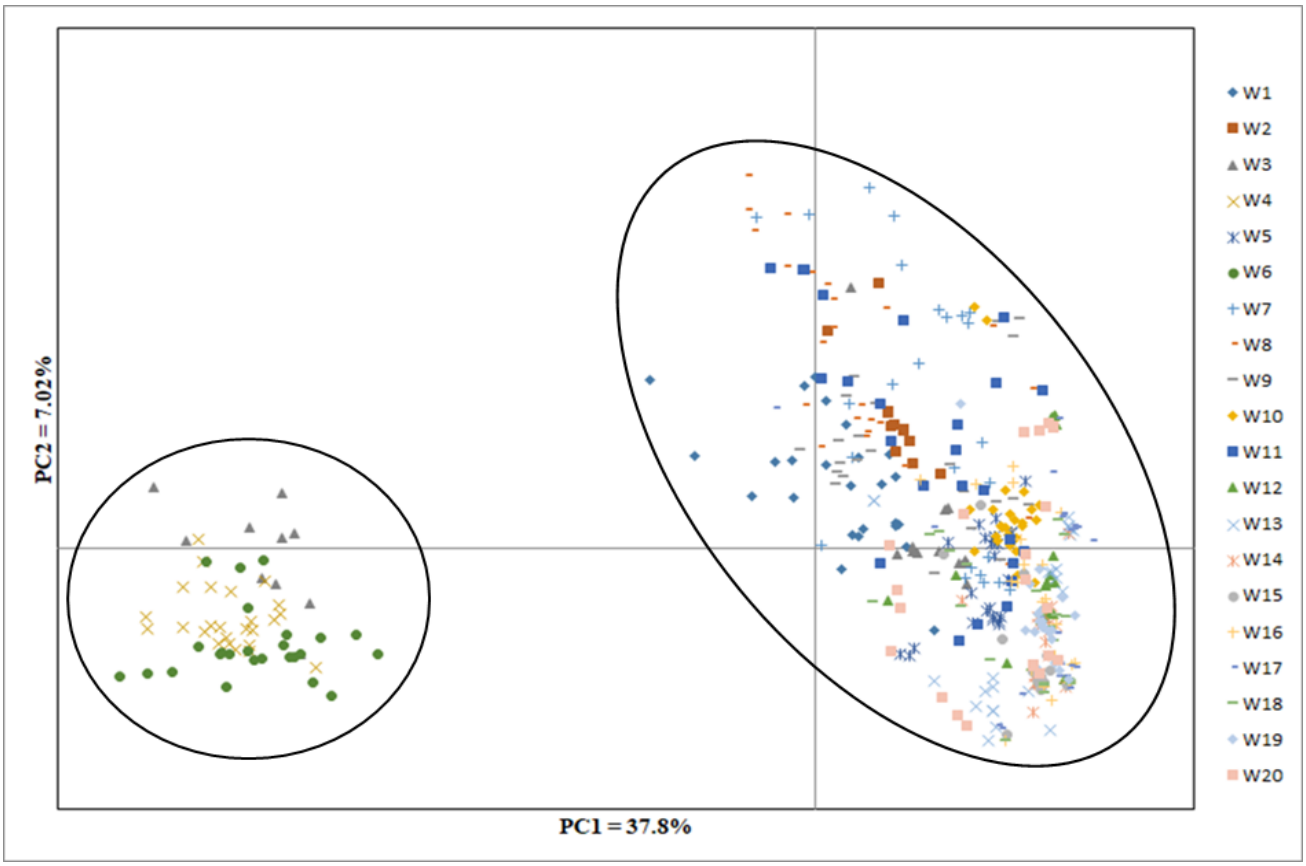

Figure 5: Scatter plot showing the first and second main components (PCoA) for 500 individuals of weedy rice collected from Sri Lanka based on the variation of 33 SSR loci.

The high genetic distance among genetically separated populations (W4 and W6) suggests that they originated from *O. nivara,* which co-occurs near commercial rice fields and adapts to take advantage of cultivated habitats due to continuous habitat disturbances and ongoing selection (De Wet and Harlan 1975; Vaughan *et al*. 2001). Besides, high-admixed nature among weedy rice populations has been observed, likely due to the long-term subsequence introgression and interaction with the different *Oryza* components of the prevailing rice ecosystem. Progenitors of cultivated rice, either *O. rufipogon* or *O. nivara*, surround commercial rice cultivation areas in Sri Lanka, coexisting in the edges and may contribute to gene flow to weedy rice through the overlap of their flowering periods and wind and insect-mediated pollen dissemination (Sandamal *et al*. 2018b,c, 2021, 2022; Samal *et al*. 2018; Wijerathna *et al*. 2021; Ratnasekera *et al*. 2022). The pattern of individuals of weedy rice populations tended to cluster together, showing their genetic similarity among populations due to long-distance seed-mediated gene flow. However, considerable overlaps among weedy individuals were observed in weedy populations representing different geographical locations. Therefore, population differentiation due to isolation by distance was not evident in the finding of this study.

Additionally, the evolutionary process of Sri Lankan weedy rice is believed to have two origins. Rice is grown in Sri Lanka under various physical environments with different altitudes, soils, and hydrological regimes. The rice-growing altitude ranges from 0 to 900 m above sea level, and the temperature ranges from 30 °C at sea level to about 15 °C at the highest elevations (Dhanapala 2007). These regional and local factors influence the diversity and infestation of weedy rice. The cultivated rice varieties are mainly *indica* type, improved, traditional cultivars, and exotic germplasm imported from countries like Indonesia used to develop high-yielding inbred varieties, resulting in high levels of genetic diversity due to the accumulation of different genetic components (Dhanapala 2020). Traditional cultivars are cultivated to a smaller extent compared to the high-yielding varieties. The genetic background of traditional cultivars is unclear (Tennakoon *et al*. 2020). Thus, weedy rice in the southern and eastern coastal areas showed close similarity to wild rice species, while weedy rice in the inland area showed similarities to traditional rice cultivars (Abeysekera *et al*. 2013). Marambe (2005) further demonstrated that rice grown in abandoned paddy fields in Sri Lanka led to the development of feral forms, which could potentially evolve into weedy types through the introgression of genes via the de-domestication of rice cultivars. Volunteer weeds in rice fields naturally mutated and evolved into weedy rice over generations by enriching soil seed banks (Hasangi *et al*. 2019), which could have interacted and contaminated with coexisting *O. sativa* and nearby wild species over time.

Farmers in Sri Lanka typically grow the same rice variety for 10-15 years, using their own saved rice seeds from one season to another due to the non-availability of certified paddy seeds to meet the current demand (He *et al*. 2014). Additionally, saved seeds are exchanged among farmers and between geographical regions. As reported by Ratnasekera *et al*. (2014), the seed-mediated gene flow between different rice-planting regions has promoted a serious infestation of weedy rice throughout the country. Because of this complex system, most weedy rice populations may originate primarily or secondary from indica-type cultivars, as reported from other regions (Londo and Schaal 2007; Goulart *et al*. 2012; Zhang *et al*. 2012). Therefore, new focuses on the rice-growing system are necessary to provide new clues to elucidate possible mechanisms of the origin of the weedy rice population in different localities across the country.

Strategies to Prevent the Invasion of Weedy Rice in Sri Lanka

The capacity of weedy rice to adapt and become troublesome is facilitated by phenotypic plasticity, which promotes population growth in disturbed settings. Phenotypic plasticity has been shown to represent the genetic correlation between certain phenotypic characteristics (Sandamal *et al*. 2018d; Wijerathna *et al*.

2022). Due to their similar morphology to co-existing cultivars, micromorphological monitoring is crucial to detecting, grouping, and eliminating weedy rice in paddy lands (Fakhr *et al*. 2022). Therefore, identifying the trends in genetic diversity and population structure in weedy rice populations can help scientists to estimate possible gene flow in a particular region and design appropriate weedy rice management techniques in different geographical regions (He *et al*. 2014). Controlling the spread of seed paddy contaminated with weedy rice seeds can help to avoid introducing the weed into clean farmlands or less-infested areas, accomplished via other efficient weed management strategies. It is challenging to eradicate weedy rice after it has been established in a rice field as it is rapidly established within a few seasons of introduction through seed enrichment in soil seed banks (Delouche *et al*. 2007). Based on the outcome of the present study, the recommendations for effective management of weedy rice are; (a) restricting the exchange of saved paddy seeds from infested areas to other geographical regions, (b) increasing the production and distribution of certified seeds, (c) developing new strategies for area-selection to seed farming with less contamination of wild taxa, (d) careful monitoring and evaluation of seed farms to avoid contamination of weedy rice seeds, (e) varietal recommendation for rice-growing regions to minimize mixing with other cultivated rice varieties, (f) raising awareness on weedy rice and its negative effects, and (g) further investigation of both the origin and the impact of weedy rice.

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AUTHOR CONTRIBUTION

S. G., D. R., B. M., and A. T. conceived the ideas. A. T. and S. S. collected samples and the data. A. T. analyzed and interpreted the data, and A. T., S. G., D. R., B. M., and S. S. wrote the paper.

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