

Diversity of agro-morphological traits of selected Philippine weedy rice accessions

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Abstract: Weedy rice (WR) is a major weed of cultivated rice (CR), particularly difficult to manage early on due to its morphological similarity to CR. Understanding its agronomic traits requires analyzing both quantitative and qualitative data, best addressed through multivariate analysis that capture the complexity and interrelationships among diverse traits beyond univariate methods. A study was conducted to characterize the agronomic traits of selected Philippine WR accessions from various provinces, identify key traits contributing to phenotypic variation, and classify WR accessions into distinct groups. The study evaluated 16 accessions, including 15 WR accessions and IR64 as the control. Plants were grown in a screenhouse, with each accession replicated 10 times in pots. Eighteen agro-morphological traits, including vegetative, structural, seed morphological, and phenological

traits, were assessed. Analysis of variance revealed significant differences between WR and CR, and among WR accessions. WR was generally taller with longer culms than CR, though some accessions, such as WR7 and WR62, matched IR64 in height. Most WR accessions matured earlier than CR. Factor analysis on mixed data (FAMD) showed that 100-seed weight, days to flowering, panicle initiation, maturity, plant height, culm length, leaf length, and pericarp color contributed most to the variation. WR accessions were clustered into six groups based on flowering time, maturity, awn length, tiller count, leaf width, culm length, height, leaf length, and pericarp color. This study provides insights into the phenotypic diversity of Philippine WR accessions, offering a better understanding of trait relationships that can inform targeted weed management strategies.

Keywords: Weedy rice morphology; Hierarchical clustering; Trait variation; Weed mimicry

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1. Introduction

Weedy rice (WR) is morphologically similar to the cultivated rice (*Oryza sativa* L.; CR) making it very challenging to manage as a weed. Its weedy characteristics include early maturity, shattering, greater seed dormancy, longevity, and red pigmented pericarp (Juliano et al., 2020). In addition, WR plants usually grow taller, faster, and profusely produce tillers. It competes with cultivated rice for limited resources such as light, water, space, and nutrients (Rao et al., 2007; Fogliatto et al., 2012; Anjali et al., 2018) resulting in yield losses of more than 50% (Ziska et al. 2015). As the trend in crop establishment shifts to direct-seeded rice (DSR) due to scarcity of labor and water in Asia, problems with WR will likely worsen (Durand-Morat et al., 2018).

The diversity observed in WR is dependent on ecotype and habitat (Shivrain et al. 2010). WR found in different Southeast Asian countries varies in terms of grain characteristics and competitiveness against cultivated rice (CR) (Chauhan, Johnson 2010). Philippine WR has higher grain yield, while those from Vietnam have higher growth potential, Thailand WR is the shortest, and Sri Lanka WR has diverse morphology in terms of tiller number, plant height, and panicle number (Chauhan, Johnson 2010). In Malaysia, four major clusters of WR have been found based on their morphology. These are (1) awned black and brown hull derived from wild *Oryza* population, (2) strawhull awnless derived from highly shattering CR, (3) brown hull WR, and (4) WR of mixed weedy morphotype suggesting multiple origin (Sudianto et al., 2016).

In the Philippines, the presence of WR has been reported in rice growing areas including Central Luzon, Nueva Ecija, Iloilo, North Cotabato, and Sultan Kudarat (Martin, 2014). Agronomic characterization studies have been conducted by Donayre et al. (2016) and Martin et al. (2020) for WR variants from Iloilo province and WR variants from Mindanao, respectively, but limited studies have been made to identify which traits contribute to the observed variations in the Philippine WR. Analyses such as factorial analysis on mixed data (FAMD) and hierarchical clustering on principal components (HCPC) become highly valuable and suitable to capture complexity and interrelationships among these diverse traits which traditional univariate analysis methods fail to capture. This study aimed to characterize the agronomic traits of selected WR accessions from different provinces of the Philippines. Specifically, this study sought to characterize the agronomic traits of Philippine WR

accessions compared to CR, IR64; identify key agronomic traits and its contribution to the total phenotypic variation among WR accessions; and classify the distinct groups of WR accessions through clustering to reveal natural grouping patterns within the phenotypic data.

2. Material and Methods

2.1 Seed collection and source

The WR seeds collected from different provinces of the Philippines were used in the study. Mature panicles of WR plants were individually collected from farmers' fields across various provinces in the Philippines. Each panicle, representing a single plant, was placed in a separate paper bag. Collection was conducted during the crop's maturity stage. WR plants were identified based on distinct phenotypic traits, including earlier maturity than CR, presence of awns, taller plant stature, ease of shattering, and structural differences compared to CR. The WR accessions used in this study were randomly selected from the broader collection to ensure representation from all collection locations. The number of accessions was limited due to the need for replication (10 times per accession) and available space in the screenhouse. After collection, seeds were dried to 13% moisture content and stored in a cold room until use. Details on genotype code, origin, collection date, and seed source for each WR accession are presented in Table 1.

2.2 Seed preparation and sowing

Before sowing the WR seeds, approximately 30 seeds of each selected WR populations were placed in a petri dish with distilled water and placed inside an oven at 35-37 °C

for 72 hours to break the dormancy of the seeds and induce germination. After 72 hours, 10 pre-germinated seeds of each WR population and a CR check (IR64) were directly sown in 20-cm diameter pots with soil. The morphological characterization experiment was conducted at a screenhouse located in the International Rice Research Institute, College, Los Baños, Laguna where the plants were grown until maturity. The plants were sown in November 2023 – February 2024 with average high temperature of 30.0 - 31.0 °C and an average low temperature of 21.0–24.0 °C. Each WR and CR rice accessions was replicated 10 times. Fertilizer was applied at the rate of 90–30–30 kg NPK ha⁻¹ at 10, 25, 45 days after sowing (DAS) and the plants were watered as needed to replicate field conditions in terms of water and nutrient management.

2.3 Agronomic and morphological characteristics

The agronomic traits of the WR plants and the IR64 (check) were measured and evaluated using the Standard Evaluation System (SES) for rice developed by International Rice Research Institute (2013). The parameters measured are listed in Table 2.

2.4 Experimental design and analysis

The morphological characterization experiment in the screenhouse was a Completely Randomized Design (CRD) with 10 replications. The individual traits were subjected to analysis of variance (ANOVA) and the significance of the treatment means were compared using Tukey's HSD test at 5% level of significance. Factor analysis on mixed data (FAMD) was performed to estimate the contribution of various traits to the observed total phenotypic variation. FAMD is used to comprehensively understand how both quantitative

Table 1 - List of selected WR populations and their origin

No.	Genotype	Origin	Collection Date	Source of Seed
1	WR7	Sta. Barbara, Iloilo	October 2019	IRRI
2	WR13	Sta. Barbara, Iloilo	October 2019	IRRI
3	WR30	Leon, Iloilo	November 2019	IRRI
4	WR34	Leon, Iloilo	November 2019	IRRI
5	WR40	Nasugbu, Batangas	2019	IWEP-UPLB
6	WR41	Sariaya, Quezon	March 2019	IWEP-UPLB
7	WR42	San Juan, Batangas	2019	IWEP-UPLB
8	WR46	Calisitan, Muñoz, Nueva Ecija	October 2022	RCPC - Region 3
9	WR47	Calisitan, Muñoz, Nueva Ecija	October 2022	RCPC - Region 3
10	WR51	Tugal, Midsayap, North Cotabato	October 2018	PhilRice
11	WR52	Tugal, Midsayap, North Cotabato	October 2018	PhilRice
12	WR55	Sampao, Isulan, Sultan Kudarat	November 2018	PhilRice
13	WR57	General Santos, South Cotabato	Nov 2018	PhilRice
14	WR62	New Passi, Tacurong, Sultan Kudarat	Nov 2018	PhilRice
15	WR63	Centrala, Surallah, South Cotabato	Nov 2018	PhilRice
16	IR64	Cultivated Variety		IRRI

Table 2 - List of agronomic and morphological traits measured and its method of measurement

Agronomic Trait	Code	Method of trait measurement
Days to maturity	DTM	Number of days from seeding to grain ripening where 85% of the grains on the panicle are mature.
Days to panicle initiation	DTP	Number of days from seeding to panicle initiation.
Days to flowering	DTF	Number of days from seeding to flowering.
Plant height (cm)	PH	Measured from the soil surface to the tip of the tallest panicle using a meter stick.
Culm length (cm)	CL	Measured from the soil surface to the tip of the culm at booting stage using a meter stick.
Total number of tillers	TT	Number of tillers per plant counted at maturity.
Productive tillers	PT	Number of productive tillers per plant counted at maturity.
Leaf length	LF	The length of the leaf below the flag leaf measured at the heading stage using a meter stick.
Leaf width	LW	The width of the leaf below the flag leaf measured at the heading stage using a 0.01mm digital caliper.
Panicle length	PL	Length of panicle measured from the base to the tip using a meter stick.
Grain length (mm)	GL	Average length of 15 mature, fully-formed seeds measured after harvest using a 0.01mm digital caliper.
Grain width (mm)	GW	Average width of 15 mature, fully-formed seeds was measured after harvest using a 0.01mm digital caliper.
Pericarp color	PC	The color of the pericarp of 10 mature, fully-formed seeds of each WR variant and cultivated rice observed after harvest. The following colors were used to describe the pericarp: white, light brown, speckled brown, brown, red, or purple (Chang, Bardenas, 1965).
Awn length	AL	Average awn length of 15 mature, fully-formed seeds was measured after harvest using a 0.01mm digital caliper.
Presence or absence of awn	Awn	The presence of awns was observed for each WR accession.
100-grain weight	SW	Weight of 100 mature, fully-formed seeds dried to 13% moisture content.
Shattering grains	Sh	Seeds of each plant was observed for the presence of seed shattering ability at maturity.
Panicle threshability	Th	Estimated based on the percentage of shattered grains after firmly grasping and pulling the hand over the panicle. The rating scale from IRR's SES for rice was used: 1- difficult (less than 1%), - moderately difficult (1-5%), 5- intermediate (6-15%), 7- loose (26-50%), and 9- easy (51-100%).

and qualitative traits collectively contribute to phenotypic variation. While examining individual traits provides insight into specific characteristics such as seed morphology or plant height, FAMD allows for a multidimensional view by analyzing both categorical and continuous variables together. This approach highlights how traits interact and cluster, revealing patterns that might not be apparent in univariate analyses. FAMD also identifies which traits contribute most to the overall variance and clustering among WR accessions, helping to better understand the underlying biological groupings. Such insights are valuable for designing targeted management strategies, as they can indicate which combinations of traits contribute to competitiveness, adaptability, and persistence in rice farming systems. In addition, a clustering analysis using HCPC were also performed to determine which accessions are relatively similar based on the morphological traits. All these analyses were performed in R software using different packages including FactomineR (Lê et al., 2008), factoextra, and dplyr.

3. Results and Discussion

3.1 Vegetative and structural traits of weedy rice accessions

There were differences among WR accessions and IR64 for all traits. The vegetative and structural traits of the different WR accessions and the CR check IR64 are summarized in Table 3. Leaf length (LF) of WR accessions

such as WR7, WR34, WR46, WR47, and WR63 were longer than IR64. Leaf width (LW) varied among WR accessions but only WR40 had broader leaves than IR64. Several WR accessions have comparable LW with WR40, including WR13, WR42, WR46, WR47, WR51, WR55, and WR57 but were not wider compared to IR64. IR64, had the shortest culm length (CL) and plant height (PH) among all plants. WR7 and WR62 had closer PH to IR64. The longest CL among WR plants were WR51 and WR63 while the tallest WR accessions include WR13, WR51, WR52, WR55, WR57, and WR63. Height is often a distinguishing feature of WR, oftentimes it is used as an indicator to identify potential WR in the field. Panicle length (PL) was generally greater in WR compared to the cultivated check, IR64. WR57 had the longest PL while WR7, WR41, and WR42 had more comparable PL to IR64. Tillering capacity of WR and IR64 did not differ in terms of total number of tillers (TT) and productive tillers (PT). However, differences were observed among WR accessions with WR52, WR55, and WR41 producing more tillers than WR13, and WR30. Lastly, WR52 and WR 41 had more PT than WR13 and WR42.

3.2 Seed morphological traits of weedy rice accessions

The seed morphological traits (Table 4) further reflected the diversity among the WR accessions. Although

average SW of WR accessions did not differ from IR64, values varied widely. Lighter seeds were observed in WR7, WR52, and WR55, while WR47, WR40, and WR42 had heavier seeds. WR accessions generally had shorter grain lengths than IR64 except for WR40 and WR42. The shortest grains were observed in WR7 and WR34. Similar grain width was observed between IR64 and WR accessions except for WR41, WR51, and WR57 which had slightly wider grains. The presence of awn (Awn) was observed in five accessions: WR40, WR41, WR42, WR57, and WR63. Among these, WR40 and WR63 had long awns, while WR41, WR42, and WR57 had shorter awns. These differences in awn presence and awn length (AL) may influence seed dispersal mechanisms. Pericarp color (PC) varied across accessions, from dark purple, red, brown, light brown, and white. Interestingly, WR40, WR42, and WR62 had white pericarp similar to IR64. Shattering (Sh), a usual weedy trait observed in WR (Wu et al., 2023), was exhibited in several accessions including WR30, WR46, and WR63. In addition, high threshability (Th) was observed in almost all accessions including IR64. Only WR40 and WR47 had intermediate Th ratings. Although Sh and Th traits are rarely connected, some Poaceae species naturally possess a weak disarticulation zone. This zone, where cells easily break apart, serves as a common mechanism influencing both Sh and Th in rice (Yu & Kellog, 2024).

3.3 Phenological development traits of weedy rice accessions

The differences in phenological development between WR accessions and the CR variety IR64 was presented in Table 5. Most WR accessions reached key developmental stages earlier than IR64. In general, IR64 exhibits a relatively longer duration for key growth stages compared to most WR accessions. A few WR accessions, notably, WR46 and WR47, had developmental timing similar to IR64. In contrast, WR30 showed earliest development, suggesting adaptation to evade control measures or compete early for resources. WR accessions generally exhibited faster phenological cycles which could enhance their persistence in agricultural ecosystems through early flowering and seed set.

3.4 Factor analysis on mixed data (FAMD)

In this analysis, 18 agronomic traits were studied, and five principal components (or “dimensions”) were retained based on their eigenvalues, all of which are greater than 1. These five components (Figure 1) account for 77.59% of the total variance indicating a significant portion of the variation within the dataset.

The first dimension (Figure 2a), which explains most of the variations (23.01%), is primarily associated with phenological agronomic traits and seed morphological traits such as DTF, DTP, DTM, PC, SW, GL, Th, and LW. The strong contribution of these traits suggests that they are very

Table 3 - Vegetative and Structural traits of selected weedy rice (WR) and IR64 used as check grown under screenhouse conditions in Los Baños, Laguna

Accessions	Leaf length (cm)	Leaf width (mm)	Culm length (cm)	Plant height (cm)	Panicle length (cm)	Total tillers	Productive tillers
WR7	61.94 ^{bc*}	11.54 ^{bcde*}	80.06 [*]	95.25 [*]	15.19 ^{bc*}	5.10 ^{abcde*}	2.60 ^{bc*}
WR13	60.74 ^{bcd}	12.00 ^{abcde}	118.10 ^{abc}	140.75 ^{abc}	22.65 ^{ab}	3.00 ^e	2.20 ^c
WR30	58.58 ^{bcd}	11.54 ^{bcde}	94.90 ^{ef}	114.80 ^{defg}	19.90 ^{ab}	3.20 ^{de}	3.10 ^{abc}
WR34	67.63 ^b	10.72 ^{cde}	92.90 ^{ef}	110.85 ^{efg}	17.95 ^{ab}	5.90 ^{abc}	3.90 ^{abc}
WR40	55.89 ^{bcd}	14.45 ^a	97.20 ^{def}	116.40 ^{defg}	19.20 ^{ab}	4.60 ^{abcde}	3.00 ^{abc}
WR41	60.26 ^{bcd}	11.78 ^{bcde}	100.30 ^{cde}	118.10 ^{def}	17.80 ^{abc}	6.10 ^{ab}	4.70 ^a
WR42	57.15 ^{bcd}	13.82 ^{ab}	115.20 ^{bcd}	130.80 ^{bcde}	17.60 ^{bc}	3.90 ^{bcde}	2.50 ^c
WR46	64.45 ^b	12.96 ^{abc}	100.30 ^{cde}	120.30 ^{cdef}	20.00 ^{ab}	4.80 ^{abcde}	2.80 ^{bc}
WR47	84.91 ^a	12.88 ^{abc}	100.80 ^{cde}	125.30 ^{cde}	24.50 ^a	5.70 ^{abcd}	3.00 ^{abc}
WR51	56.78 ^{bcd}	12.52 ^{abcd}	134.50 ^a	154.20 ^a	19.70 ^{ab}	3.80 ^{bcde}	2.70 ^{bc}
WR52	54.88 ^{bcd}	9.92 ^e	123.90 ^{ab}	142.60 ^{abc}	18.70 ^{ab}	6.70 ^a	4.40 ^{ab}
WR55	58.00 ^{bcd}	12.89 ^{abc}	118.15 ^{abc}	136.05 ^{abcd}	17.90 ^{ab}	6.00 ^{abc}	3.60 ^{bc}
WR57	63.08 ^b	12.72 ^{abc}	126.70 ^{ab}	151.00 ^{ab}	24.30 ^a	3.40 ^{cde}	2.60 ^{bc}
WR62	46.83 ^{cd}	11.47 ^{bcde}	83.76 ^{ef}	101.95 ^g	18.19 ^{ab}	4.40 ^{abcde}	2.90 ^{abc}
WR63	46.82 ^{cd}	10.13 ^{de}	129.70 ^{ab}	150.20 ^{ab}	20.50 ^{ab}	4.20 ^{abcde}	2.90 ^{abc}
IR64	45.93 ^d	11.66 ^{bcde}	54.30 ^g	64.50 ^h	10.20 ^c	5.30 ^{abcde}	3.10 ^{abc}

*Means within a column followed by the same letters are not significantly different at 5% level of significance based on Tukey's Honest Significant Difference (HSD) test.

Table 4 - Seed morphological traits of WR and IR64 grown under screenhouse conditions

Accessions	100-seed weight (g)	Grain length (mm)	Grain width (mm)	Awn length (mm)	Pericarp color	Shattering	Threshability	Awn
WR7	1.41 ^{ef}	6.69 ^f	2.63 ^{bc*}	0 ^c	Dark Purple	-**	Easy	-**
WR13	1.57 ^{cde}	8.15 ^c	2.66 ^{bc}	0 ^c	Dark Purple	-	Easy	-
WR30	1.52 ^{def}	7.43 ^{de}	2.55 ^{bc}	0 ^c	Brown	+	Easy	-
WR34	1.46 ^{ef}	7.17 ^{ef}	2.91 ^{abc}	0 ^c	Dark Purple	-	Easy	-
WR40	1.93 ^{ab}	9.37 ^a	2.81 ^{bc}	21.66 ^a	White	-	Intermediate	+
WR41	1.69 ^{abcde}	7.95 ^c	3.67 ^a	6.98 ^{bc}	Brown	-	Easy	+
WR42	1.87 ^{abc}	9.18 ^{ab}	2.66 ^{bc}	7.20 ^{bc}	White	-	Easy	+
WR46	1.79 ^{abcd}	8.81 ^b	3.00 ^{abc}	0 ^c	Red	+	Loose	-
WR47	1.99 ^a	8.21 ^c	2.70 ^{bc}	0 ^c	Red	-	Intermediate	-
WR51	1.62 ^{bcde}	8.17 ^c	3.23 ^{ab}	0 ^c	Brown	-	Loose	-
WR52	1.22 ^f	8.31 ^c	2.75 ^{bc}	0 ^c	Brown	-	Loose	-
WR55	1.44 ^{ef}	7.83 ^{cd}	2.49 ^{bc}	0 ^c	Dark Purple	-	Easy	-
WR57	1.82 ^{abcd}	8.17 ^c	3.20 ^{ab}	11.74 ^b	Red	-	Easy	+
WR62	1.70 ^{abcde}	7.96 ^c	2.21 ^c	0 ^c	White	-	Easy	-
WR63	1.60 ^{cde}	7.88 ^{cd}	2.94 ^{abc}	28.72 ^a	Light Brown	+	Loose	+
IR64	1.73 ^{abcde}	9.64 ^a	2.38 ^c	0 ^c	White	-	Easy	-

*Means within a column followed by the same letters are not significantly different at 5% level of significance based on Tukey's Honest Significant Difference (HSD) test;

** "+" indicates presence of awns while "-" indicates absence of awns.

important in distinguishing the WR phenotypes from each other as these agronomic traits may potentially reflect the differences and distinctness of WR accessions in terms of phenological traits and seed characteristics. The variations observed may influence the management strategies of WR as these traits could influence the timing of WR maturity, complicating the post-emergent control measures in terms of synchronization with the CR. Johnson et al. (2004) reported that the critical periods to control weeds in rice fields are estimated between 29–32 DAS in the wet season (WS) and 4–83 DAS in the dry season (DS). The variation in timing of WR life cycle may allow the WR plants to escape control measures such as herbicide application and other mechanical weed control.

The second dimension (Figure 2b) which accounts for 19.5% of the variations is characterized by a strong correlation with six agronomic traits that are mostly composed of structural traits such as PH, CL, PL, PC, GW, AL, and Th. These agronomic traits show variations related to plant architecture and grain morphology. Several studies have shown that WR is generally taller compared to CR (Martin, 2014; Sánchez-Olquín et al., 2007). Similarly, Martin et al. (2020) observed significant differences in agronomic traits and grain characteristics of WR plants from Mindanao, Philippines and CR.

The third dimension (Figure 2c) accounts for 13.56% of observed variance, it emphasizes the importance of seed morphology and vegetative traits such as PC, LF, AL, presence of awn (Awn), and TT. Weedy characteristics such

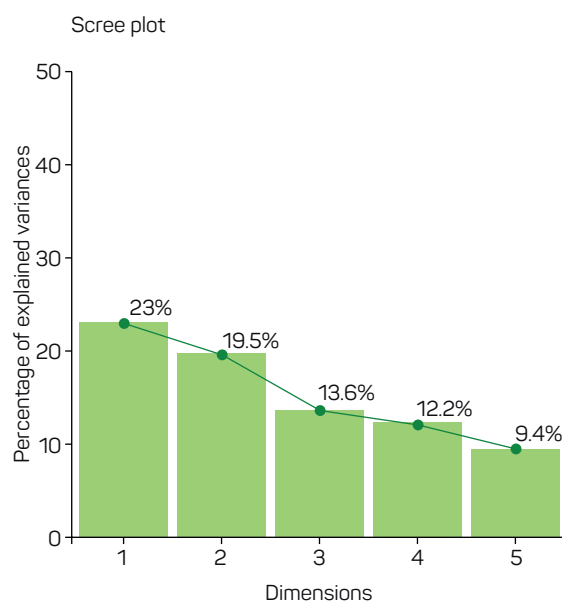


Figure 1 - Screeplot diagram of eigenvalues constructed on 18 morphological traits of 16 rice genotypes

as the presence of long awns is associated with seed dispersal (Ntakirutimana et al., 2019). Higher and more variability in growth parameters suggests stronger competitive ability and plasticity (Andres et al., 2015). Furthermore, the presence of awn, Sh, red PC, and black hull have been associated with seed dormancy (Gu et al., 2005).

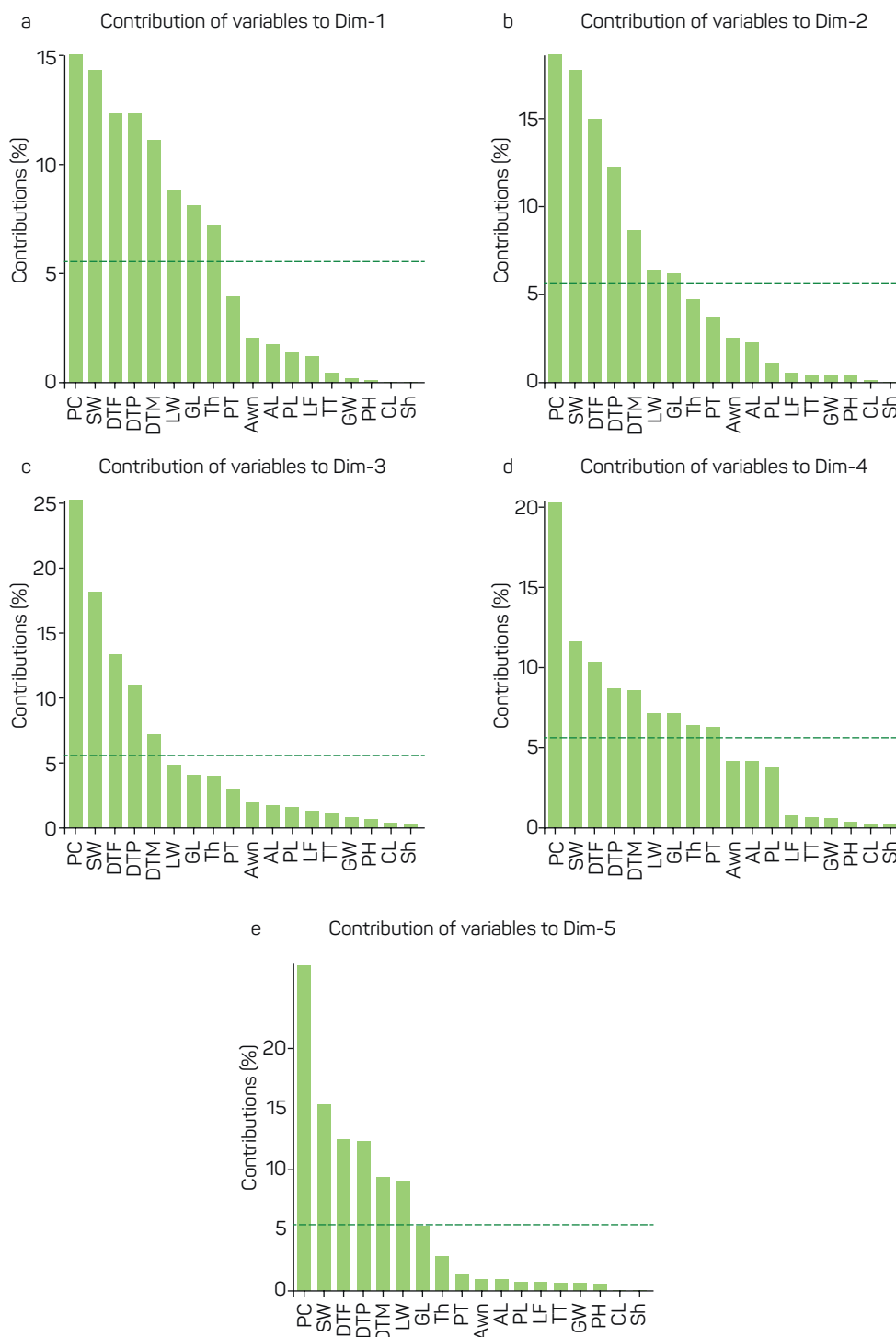


Figure 2 - Contribution of studied traits under different principal dimensions, each dimension is composed of trait that contribute to the variations in the dimension (a = Dimension 1 or phenological traits dimension, b= Dimension 2 or structural traits dimension, c= Dimension 3 or seed morphological and vegetative traits dimension, d= Dimension 4 or seed morphological traits dimension, and e= Dimension 5 or tillering ability and seed morphological trait dimension)

The fourth principal component (Figure 2d) is correlated to seed morphological traits, structural traits, and phenological traits such as Th, PC, LW, DTM, LF, TT, PL, DTF, and DTP accounts for 12.2% of the total variation

observed. The recurrence of phenological traits and seed morphological traits such as PC suggests its significance in the observed phenotypic diversity of WR accessions. Lastly, the fifth dimension (Figure 2e) focuses on total PT, TT, GW,

PC, Awn, and Sh, combining both seed morphology and reproductive traits, which are critical for understanding the adaptability and competitiveness of the WR phenotypes accounts for 9.4% of the total variation observed. Sh and presence of awn are mechanisms of WR for survival and seed dispersal, these traits are generally observed in WR plants (Osakina, Jia, 2023; Sudianto et al., 2016).

The factor maps for the first three dimensions obtained from FAMD, composed of individuals (WR accessions) and different categories (qualitative and quantitative variables), are presented in Figure 3. The factor maps show a visualization of the contribution of quantitative and qualitative traits for the dimensions. The quantitative traits that are found closer to an axis contributes more to that dimension and the arrow's color intensity indicates the strength of the contribution, with red arrows indicating strong contribution while blue/green indicates weaker contributions. The direction of the arrows reflects the value of the trait. High positive values indicate higher values such as taller plants or longer DTM while negative values reflect lower trait values such as low number of PT. In addition, traits that appear close to each other suggests that these traits are highly correlated while traits on opposite direction suggest inverse relationships. Meanwhile, qualitative traits are presented individually, the position and proximity of a qualitative trait and an accession reflects its association.

The phenological traits dimension (23.0%; dimension 1) and the structural traits dimension (19.5%; dimension 2) that explain most of the observed variation in the data with 42.5% (Figure 3a). Phenological traits such as DTF, DTE, and DTP contribute strongly and the most to dimension 1 while structural traits such as PH and CL were found to contribute the strongest to dimension 2. Qualitative traits such as red PC, intermediate Th, and white PC contribute strongly to dimension 1 and these traits likely distinguish WR accessions from each other (Figure 3b). Meanwhile, light brown PC, loose Th, and Sh are strongly associated with dimension 2 (Figure 3b). The proximity of accessions to the qualitative traits reflects its association to it. WR42, WR46, and WR47 is closely associated with traits like red PC and intermediate Th, while WR30, WR34, and WR63 are closely associated with light brown PC and loose Th (Figure 3b).

The contribution of phenological traits dimension and vegetative and seed morphological traits (dimension 3) shows that DTP, DTE, DTM, and SW contribute most to dimension 1 while LF and TT have stronger influence in dimension 3 (Figure 3c). Dimension 3 contributes 13.6% to the total variation observed and it is mainly associated with vegetative traits and some seed morphological traits including LF which is moderately associated with DTP, DTE, and DTM suggesting that late maturing accessions may possess longer leaves. Meanwhile, SW and GL were found to be associated more to dimension 1 which suggests their contribution to phenological traits than vegetative traits. Figure 3c also shows PT and TT are exclusively found on dimension 3 which indicates their contribution to tillering and vegetative traits

rather than the phenological traits. In addition, the individual qualitative traits of dimension 1 and dimension 3 (Figure 3d) show that WR47 is associated with red PC, while WR34 and WR30 are linked to brown or dark purple PC.

The structural traits and vegetative and seed morphological traits explain 33.1% of the observed variation (Figure 3e). AL contributes strongly to the vegetative and seed morphological traits dimension (dimension 3) and it is inversely correlated with phenological traits such as DTP, DTE, and DTM which suggests that accessions with longer awns have shorter DTP, DTE, and DTM. In terms of qualitative traits (Figure 3f), IR64 has been observed to diverge from the weedy traits while aligning with non-weedy traits such as white PC and non-Sh.

These results are similar to the outcomes of phenotypic diversity studies conducted by Kanapeckas et al. (2018) on WR and Sharma et al. (2014) on CR. The WR study highlighted the significant contribution of presence of awns, seed Sh, and pigmented pericarp, hull color, maturity dates, and differences in plant architecture to the total variation observed in WR plants. Meanwhile, the CR study revealed the significant contribution of phenological and yield-related traits in CR including number of days to flowering, days to maturity, PH, number of PT, PL, and 100-seed weight to the observed variation in CR.

Overall, the FAMD analysis reveals that traits related to seed morphology, maturity, and plant architecture are key differentiators among WR accessions. Understanding the similarities and differences of WR accessions among each other and compared to CR will allow us to improve the effectiveness of the existing weed management practices. Identifying these key traits may help us tailor management practices that can significantly reduce the competitive advantage of WR in the field.

3.5 Grouping pattern of weedy rice based on morphological similarities

The hierarchical clustering analysis grouped the 16 rice accessions into six clusters (Figure 4, Table 6, and Table 7) with four accessions in cluster A, two accessions in cluster B, three accessions in cluster C, one accession in cluster D, four accessions in cluster E, and two accessions in cluster F. The results of this cluster analysis reveal distinct phenotype characteristics that may help in understanding the ecological and agronomic behavior of Philippine WR populations.

Cluster A which grouped WR34, WR55, WR30, and WR13, is characterized by similarities in phenological and morphological traits. WR plants in Cluster A mature earlier, with a mean of 73.97 days, compared to the overall mean of 83.74 days. Similarly, these plants initiate panicle development earlier, averaging 63.50 days, which is shorter than the overall mean of 71.29 days. They also flower earlier, with a mean of 65.25 days, compared to the overall mean of 73.12 days. Similar to the findings of Zhao et al. (2018), WR plants from three major cropping regions of China have been

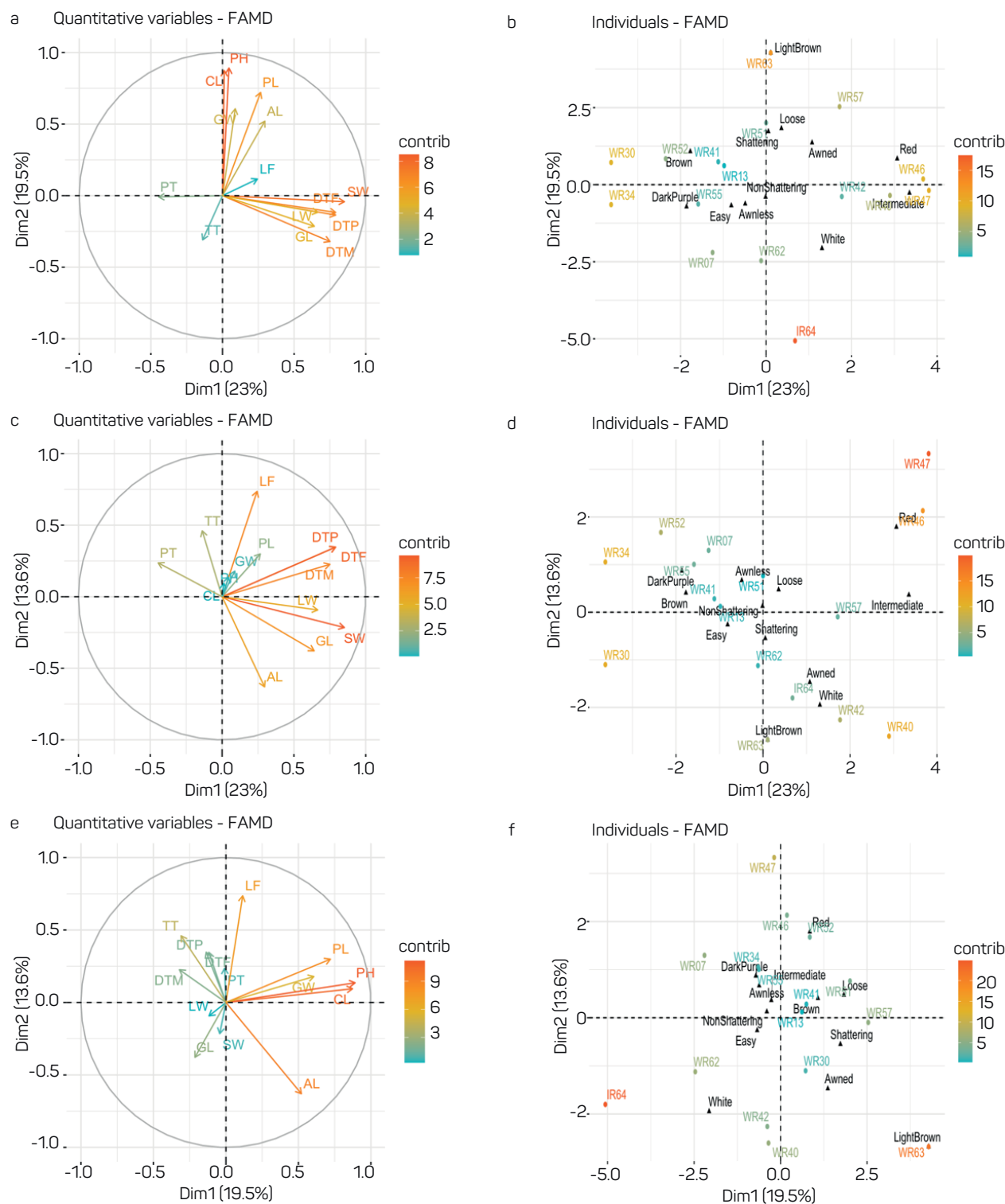


Figure 3 - Factor maps for the first three dimensions obtained from the FAMD analysis. (a,c,e): Quantitative variables (b,d,f): Individual WR accessions and qualitative variable categories colored according to their contribution to dimensions

found to mature and flower earlier than the CR. While Costa Rican WR studied by Sánchez-Olquín et al. (2007) has been found to reach anthesis and maturity earlier than the CR varieties. Early maturity is an important trait contributing to

the persistence of WR in the field as it can escape harvesting and increase its number in the seed bank (Zhao et al., 2018).

In contrast to cluster A, WR plants in Cluster F (WR46 and WR47) show later development stages. They initiate

panicle growth at an average of 83.2 days, which is about 12 days later than the overall mean. Flowering in this cluster occurs at an average of 85 days, later than the overall mean of 73.12 days, and they reach maturity at 95 days, longer than the overall mean of 83.74 days. Several studies show a wide range of flowering time and maturity of weedy accessions (Shivrain et al., 2010; Ahmed et al., 2012). Furthermore, WR plants in Cluster F have longer leaves, with an average length of 74.68 cm, compared to the overall mean of 58.99 cm.

WR has been known to have diverse PC including red, purple, black, brown, white, and green (Han et al., 2022). Clusters A and F have been found to be strongly associated with their respective PC: dark purple for Cluster A and red for Cluster F. Red pericarp is conferred by the functional alleles of the *Rc* gene which is also associated with seed dormancy (Cui et al., 2016), this suggests that WR accessions in these clusters may possess greater seed dormancy.

Cluster B composed of WR52 and WR41 is distinguished by vegetative traits, particularly a high number of PT and TT. WR plants in this cluster exhibited more PT, with an average of 4.55, compared to the overall mean of 3.13. Similarly, the TT was higher, averaging 6.40, in comparison to the overall mean of 4.78. High tillering capacity of WR plants provide them a competitive advantage against CR as

high weed density generally decreases yield potential of CR due to competition (Olajumoke et al., 2016).

Cluster C (IR64, WR07, WR62) is characterized by shorter PH, PL, CL, and smaller GW. The CR, IR64, share certain phenotypic traits with WR accessions found in cluster C such as shorter PH (87.23 cm), CL (72.71 cm), PL (14.53 cm), and smaller GW (2.41 mm). These characteristics suggest that WR plants found in the same cluster may have adapted to mimic CR varieties such as IR64, making it more difficult to identify and manually remove in the field.

Cluster D, the only cluster which has only one WR accession– WR63, is distinguished by a significantly longer AL of 28.72 cm compared to the overall mean of 4.77 cm. Awns can assist in seed dispersal mechanisms of WR. This result was similar to the findings of Hoyos et al. (2019) on Colombian WR which identified presence of awn, apiculus color, and AL as important predictors in morphological groupings of Colombian WR. Awned seeds can attach to animals, machinery, or humans, promoting the spread of these accessions to new areas. Additionally, awns can contribute to the persistence of WR in the seed bank due to their ability to remain dormant or delay germination, complicating eradication efforts (Ntakirutimana et al., 2019). The management of WR with long awns may require a focus on controlling

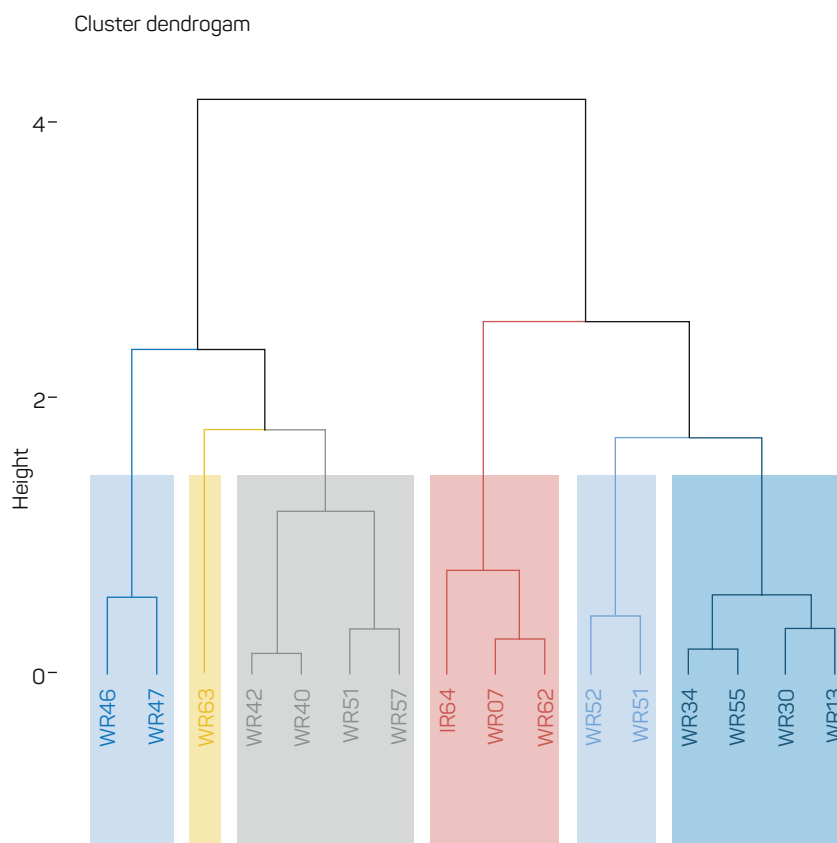


Figure 4 - Hierarchical clustering on principal components, created using 5 dimensions (77.59% of explained variance) from the factor analysis of mixed data (FAMD) model showing the clustering of WR accessions

Table 6 - Weedy rice clusters and their respective quantitative variables from the hierarchical analysis

Cluster	Variable	Mean in category	Overall mean	V-test	P-value*
A	Days to maturity	75.25 ± 5.42	83.744 ± 7.17	-2.65	0.0080
A	Days to panicle initiation	63.50 ± 5.33	71.288 ± 6.55	-2.66	0.0079
A	Days to flowering	65.25 ± 5.42	73.119 ± 6.57	-2.68	0.0074
B	Total productive tillers	4.550 ± 0.15	3.125 ± 0.67	3.12	0.0018
B	Total tillers	6.400 ± 0.30	4.756 ± 1.10	2.20	0.0280
C	Grain width	2.407 ± 0.17	2.799 ± 0.35	-2.10	0.0358
C	Panicle length	14.527 ± 3.30	19.018 ± 3.30	-2.53	0.0114
C	Culm length	72.707 ± 13.10	104.423 ± 20.57	-2.87	0.0041
C	Plant height	87.233 ± 16.31	123.316 ± 22.85	-2.94	0.0033
D	Awn length	28.720 ± 0.00	4.769 ± 8.55	2.80	0.0051
E	Leaf width	13.378 ± 0.79	12.063 ± 1.20	2.46	0.0139
F	Days to panicle initiation	83.200 ± 2.800	71.286 ± 6.55	2.66	0.0078
F	Days to flowering	85.000 ± 2.700	73.119 ± 6.574	2.65	0.0082
F	Leaf length	74.680 ± 10.230	58.992 ± 9.045	2.54	0.0111
F	Days to maturity	95.000 ± 2.700	83.744 ± 7.171	2.30	0.0216

*Variables with p-values < 0.05 represent significant deviations from the overall mean, indicating their distinct contribution to the definition of each cluster as determined by v-test analysis

Table 7 - Weedy rice clusters and their respective qualitative variables from the hierarchical analysis

Cluster	Pericarp Color	Cla/Mod	Mod/Cla	Global	v-Test	p-Value
A	Dark Purple	75	75	25	2.205	0.0275
F	Red	66.67	100	18.75	2.241	0.025

seed production and dispersal, along with post-harvest practices to reduce seed bank replenishment.

Cluster E is distinguished by having significantly larger leaf width. These accessions are WR40, WR42, WR51, and WR57 which have a mean leaf width of 13.38 mm compared to the overall mean of 12.06 mm. This trait may imply higher photosynthetic capacity and better light capturing efficiency resulting in higher biomass of WR plants. Plant architecture and nutrient utilization affect rice yields. Leaf width has been proven to also affect the regulation of grain and panicle traits (Fu, 2016). In addition, leaf width, leaf length, and its thickness are positively correlated to the number of spikelets, filled grain per panicle, panicle weight, and overall grain yield (Jun et al., 2006). A study by Zhu et al. (2020) revealed association of the leaf width gene to plant architecture and grain size through nitrogen transfer.

While the collection sites or the origin of these WR accessions may be an important factor in their groupings, no clear clustering patterns were observed based on origin and ecotype, except for Cluster F which included two WR accessions from irrigated rice fields of Muñoz, Nueva Ecija. This may be due to the limited sample size, and further studies with more WR accessions are needed to validate this observation.

The results of the cluster analysis supported the results of the FAMD, as phenological traits (DTM, DTF, DTP), tillering ability (TT and PT), plant size (PL, CL, PH, LF), and seed morphology (GW, AL, and PC), were the factors associated with the grouping into six clusters. The traits that highly contribute to the variations observed in Philippine WR accessions are generally weedy traits that are distinct to WR. The clustering of WR plants with IR64, a cultivated variety, provides us insights on which agronomic traits may be usually mimicked by WR. Crop mimicry is a common observation of weeds to evade weed management strategies such as in the case of *Echinochloa colona* which resembles rice seedlings even farmers have difficulty identifying it and manually removing it in their fields (Pannell, Farmer 2016). In addition, it is suggested that weed mimicry is a weed adaptation to avoid herbicide applications. As new weed control technologies emerge to differentiate weeds, the importance of understanding mimicry in weed science is crucial for its success (McElroy 2014).

4. Conclusions

WR characterization studies in the Philippines have primarily focused on surveying, understanding the competitive ability of WR, and characterizing its agronomic

traits. Philippine WR accessions exhibit diversity with its varying agronomic traits. However, there are limited studies to explore and understand the contributions of significant agronomic traits to the variations observed in Philippine WR accessions. This study aimed to fill that gap characterizing the different agronomic traits of Philippine WR accessions and identifying key traits that contribute to the observed variations. Our findings show that the traits that contribute most to the total variance are 100-seed weight, days to flowering, days to panicle initiation, days to maturity, PH, CL, leaf length, and PC. The key traits were identified from five principal dimensions, collectively explaining 77.59% of the total variation in the phenotypic data. Dimension 1, strongly associated with phenological traits, account for 23.01% of the variation, while Dimension 2, linked to structural traits, explains 19.5%. Dimension 3, related to both seed morphology and vegetative traits, contribute 13.6%, and Dimension 4, focused on seed morphological traits, explains 12.2%. Lastly, Dimension 5, associated with tillering ability, accounts for 9.4% of the observed variation. Furthermore, the Philippine WR accessions were grouped into six clusters, reflecting their similarities in growth cycles (shorter vs. longer growth periods), tillering ability (total tiller and number of productive tillers), plant size (PH, PL, CL, leaf width), and seed characteristics (presence of awn, PC, AL, grain weight).

These results offer valuable insights into the distinct agronomic traits that may contribute to the observed competitive ability of Philippine WR accessions. Interestingly, the traits observed in Philippine WR accessions align with those reported in WR populations from other countries such as red and dark purple PC, long awns, longer culms and taller PH, and shorter days to

maturity. Hierarchical clustering revealed which Philippine WR accessions share similarities with cultivated variety, IR64, and identified agronomic traits that contribute to these similarities including as grain width, CL, PL, and PH. The results of both FAMD and hierarchical clustering provide deeper understanding of the important traits of Philippine WR, its potential impacts to the yield and quality of harvested CR, and its relevance in designing effective control strategies.

Author's contributions

All authors read and agreed to the published version of the manuscript. JKM and AHMR: conceptualization of the manuscript and development of the methodology. JKM: data collection and curation. JKM: data analysis. JKM and AHMR: data interpretation. AHMR: supervision. JKM: writing the original draft of the manuscript. JKM and AHMR: writing, review and editing.

Data Availability Statement

The data for this article can be accessed by sending an email to the senior author. It is protected under privacy protection.

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