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RESEARCH NOTE

Evaluation of Selected Philippine Germplasm for Resistance to Rice Stem Borer (*Scirpophaga* spp.) Using Seven Morphological Traits

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Plant morphological structures are one of the primary factors that deter insect pest infestations. In this study, stem borer (*Scirpophaga* spp.) resistance-related morphological traits of six selected Philippine germplasm were quantified, compared to the resistance check TKM6, and analyzed in three seasons 2018 WS (wet season) 2019 DS (dry season), and 2019 WS using analysis of variance with Tukey's test ($p > 0.05$) and hierarchical cluster analysis based on Ward's method algorithm. These analyses were conducted to support the selection in pre-breeding activities using seven identified morphological traits such as leaf color, flag leaf length, flag leaf width, leaf width, stem diameter, culm length, and length of the 4th internode. Inarciaga resulted with the highest number of statistically comparable structures to TKM6, with six traits, during 2018 WS. Inarciaga, Mukol, and C-4 Dinorado demonstrated two statistically comparable traits to TKM6 during 2019 DS. In 2019 WS, Mukol, and the susceptible TN1 both showed six traits, the highest among the selected germplasm, statistically comparable to TKM6. Thus, cumulative morphological traits similarity to TKM6 revealed that Inarciaga, Mukol, and C-4 Dinorado were good candidate genotypes for morphological phenotype selection. Based on hierarchical cluster analysis, three clusters were reported: Cluster I (Dinorado and C-4 Dinorado), Cluster II (TKM6, Hinumay, and Mukol), and Cluster III (Inarciaga, Red 18, and TN1). Inarciaga and Red 18 were delineated among other germplasm based on the highest number of desirable morphological traits against stemborer such as lesser leaf surface area, narrower stem diameter, and shorter height.

Keywords: ANOVA, germplasm, morphological traits, stem borer, Ward's method algorithm

Rice, a staple food for billions of people, faces significant yield losses due to insect pests, particularly stem borers (Iqbal 2020; Mohankumar *et al.* 2003; Litsinger *et al.* 2011). While pesticide use remains common, it poses environmental and health risks (Kaur and Garg 2014). Therefore, developing rice varieties with inherent resistance to stem borers has become a crucial strategy for sustainable agriculture.

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Host plant resistance, influenced by morphological and biochemical factors, can significantly reduce stem borer damage (Hossain 1997). However, limited research has focused on utilizing Philippine germplasm to develop resistant varieties against yellow stem borer and white stem borer, two major pests in the country (Litsinger *et al.* 2011).

This study aimed to evaluate the morphological traits of selected Philippine rice germplasm associated with stem borer resistance, compare these traits to the known

resistant check, TKM6, and analyze the variation in morphological traits across three growing seasons – namely 2018 WS (wet season) 2019 DS (dry season), and 2019 WS.

By identifying germplasm with desirable morphological features such as leaf color, flag leaf length, flag leaf width, leaf width, stem diameter, culm length, and length of the 4th internode (Hosseini *et al.* 2011; Pathak 1969; Rubia-Sanchez *et al.* 1998; Pathak and Patanakamjorn 1967; Chaudhary *et al.* 1983; Hosseini *et al.* 2010; El-Adl *et al.* 2011; Ntanos 2000), this study contributes to the development of more resilient rice varieties.

The plant materials were sown and transplanted after 25 d in the pots in a controlled environment at the screen house for three seasons – namely 2018 WS (July– December), 2019 dry season (January–June), and 2019 WS (July–December) – establishment at Philippine Rice Research Institute–Central Experiment Station, Maligaya, Science City of Muñoz, Nueva Ecija, the Philippines.

Table 1. Selected Philippine germplasm with field resistance to rice stemborer.

Accession name	Accession no.	Collection no.
Dinorado		12798-A
Hinumay		12799-B
Inarciaga	PRRI003164	3920
Red 18		13806
C-4 Dinorado	PRRI002274	1246
Mukol		12393
TN1 (S – check)*		
TKM6 (R – check)*		

*Note: [S] susceptible; [R] resistant

During the early maturity stage, the six Philippine germplasm and two checks were measured with three replicates per entry. Seven morphological features or traits were quantified per replicate. SPAD 502 chlorophyll meter was used to measure the flag leaf color when the sunlight intensity was the highest (11:00 AM–02:00 PM). Flag leaf width and leaf width were measured using a ruler; flag leaf length, culm length, and length of the 4th internode were quantified using a meter stick, and stem diameter was determined using a digital caliper.

The statistical analyses included an analysis of variance with Tukey's honestly significant difference (HSD) at $p > 0.05$, performed using XLSTAT (version 2020.1.3). Hierarchical cluster analysis was conducted using Ward's method algorithm in Euclidean distance with RStudio (version 1.3.959). Pearson's correlation coefficient was calculated using Microsoft Excel (version 16.85) to assess the relationships among the seven morphological traits (leaf color, flag leaf length, flag leaf width, leaf width, stem diameter, culm length, and length of the 4th internode) across three seasons (2018 WS, 2019 DS, and 2019 WS).

To compare the variation across two clustered seasons (WS 2018 and 2019; DS 2019), Table 2 presented the summarized mean values of seven morphological traits for six germplasm accessions. The highest 'greenness' leaf color was consistently observed in Dinorado, with values of 45.45 in WS and 54.17 in DS, whereas the lowest was recorded for C4-Dinorado (33.82) in WS and TKM6 (32.03) in DS. C4-Dinorado exhibited the longest flag leaf at 53.57 cm in WS, whereas Dinorado had the highest in DS at 35.56 cm. The shortest flag leaf was observed in TN1 with 26.44 cm in DS. Hinumay demonstrated the broadest measurements for both flag leaf width and leaf width at 21.28 and 17.17 mm, respectively, in WS, whereas TKM6 had the narrowest flag leaf and leaf width measuring 7.11 and 6.78 mm in DS, respectively. The widest stem diameter was found in Dinorado during the DS at 8.40 mm, whereas the narrowest was noted in TKM6 with 4.53 mm. Hinumay had the longest culm length at 142.11 cm in WS, with the shortest in TN1 at 62.17 cm in DS. Finally, Dinorado also had the longest 4th internode in WS with 184.34 mm, whereas the shortest was recorded in Red 18 during DS at 35.33 mm.

Across three seasons (2018 WS, 2019 DS, and 2019 WS), mean values of morphological traits with Tukey's HSD were shown in Figure 1. For LC, results showed that Red 18, C-4 Dinorado, and TN1 had no significant difference compared to TKM6. All germplasm had no significant difference from TKM6 based on FLL. C-4 Dinorado and TN1 were not significantly different from TKM6 based on FLW and LW. For SD, Inarciaga and TN1 had no significant difference compared to TKM6. Dinorado, Hinumay, Inarciaga, C-4 Dinorado, and Mukol were not significantly different based on CL and L4I.

Based on seven morphological traits gathered across three seasons (2018 WS, 2019 DS, and 2019 WS), Figure 2 presented a hierarchical cluster dendrogram using Ward's method algorithm in the Euclidean similarity index. The dendrogram showed three clusters – namely Cluster I (Dinorado and C-4 Dinorado), Cluster II (TKM6, Hinumay, and Mukol), and Cluster III (Inarciaga, Red 18, and TN1).

Morphological traits are primary factors influencing host-plant resistance to insects, including the rice stem borer (Heinrichs 1992; Nwilene *et al.* 2009; Oyetunji *et al.* 2014). This study evaluated the morphological traits of stem borer-resistant rice germplasm.

C-4 Dinorado consistently exhibited the lowest "greenness" trait, potentially reducing its attractiveness to adult moths for

Table 2. Summarized mean values of the morphological traits of six germplasm and two checks across two clustered seasons (WS 2018 and 2019; DS 2019).

Accession names	Leaf color (SPAD)	Flag leaf length (cm)	Flag leaf width (mm)	Leaf width (mm)	Stem diameter (mm)	Culm length (cm)	Length of the 4th internode (mm)
WS 2018 and 2019							
Dinorado	45.45	45.45	18.61	14.89	7.40	129.00	184.34
Hinumay	44.28	39.60	21.28	17.17	6.43	142.11	155.95
Inarciaga	41.10	41.06	15.95	12.39	5.67	100.78	114.06
Red 18	39.06	36.81	15.06	13.61	7.09	77.73	81.61
C4-Dinorado	33.82	53.57	14.28	11.39	7.20	137.45	177.00
Mukol	41.16	46.47	15.67	13.23	6.35	139.06	148.32
TKM6 (R-check)	36.59	45.84	11.11	9.00	5.09	119.23	132.06
TN1 (S-check)	35.60	33.89	13.34	11.67	5.68	68.28	72.66
DS 2019							
Dinorado	54.17	35.56	16.44	15.11	8.40	101.83	120.33
Hinumay	45.23	32.78	15.33	13.89	7.50	113.67	137.00
Inarciaga	48.67	30.44	12.22	9.00	5.97	65.33	75.00
Red 18	48.93	30.06	11.78	9.56	5.17	50.67	35.33
C4-Dinorado	45.07	40.11	10.11	7.56	5.97	106.33	131.00
Mukol	48.93	34.56	10.33	8.78	6.63	113.33	112.67
TKM6 (R-check)	32.03	21.05	7.11	6.78	4.53	91.33	154.33
TN1 (S-check)	41.30	26.44	10.44	8.89	5.73	62.17	71.00

Note: [R] resistant; [S] susceptible; [WS] wet season; [DS] dry season

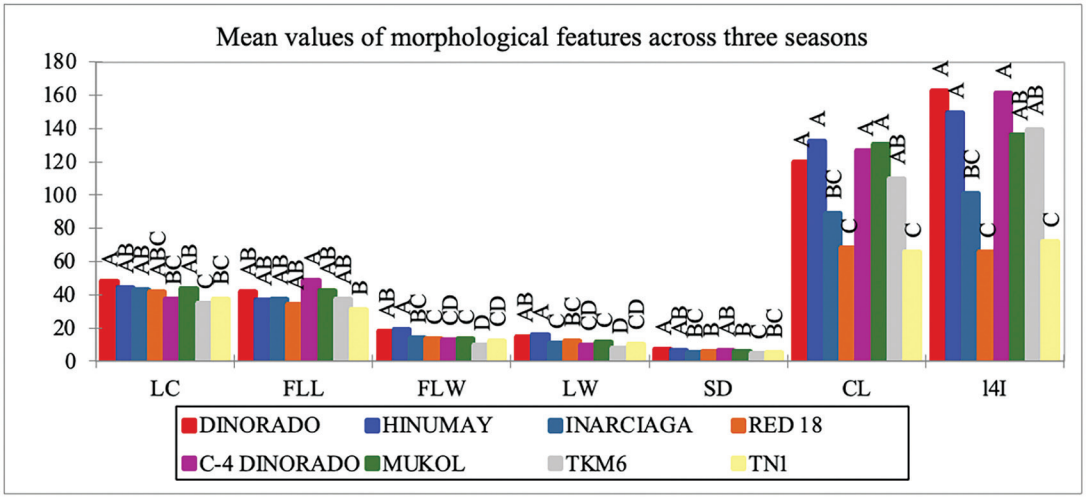

Figure 1. Mean values of the morphological traits of six germplasm and two checks with Tukey's HSD across three seasons (2018 WS 2019 DS, and 2019 WS). Note: mean values followed by the same letter within a column are not significantly different at $p > 0.05$ based on Tukey's HSD.

oviposition (Hosseini *et al.* 2011). Red 18 and C-4 Dinorado displayed reduced flag leaf length and width, decreasing the surface area available for egg mass deposition (Pathak 1969). Additionally, C-4 Dinorado's narrower leaves have been linked to reduced stem borer infestation (Hosseini *et al.* 2010; Rubia-Sanchez *et al.* 1998).

Inarciaga and Red 18 exhibited narrower stem diameters, which can hinder larval movement and development within the stem (Pathak and Patanakamjorn 1967; Pathak 1969; Pathak *et al.* 1998; Chaudhary *et al.* 1983; Hosseini

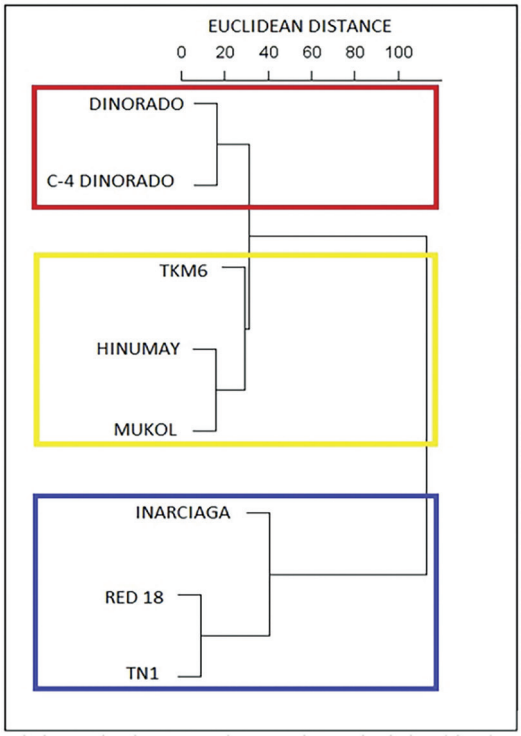

Figure 2. Hierarchical cluster dendrogram using Ward's method algorithm in Euclidean similarity index as revealed by seven morphological traits across three seasons (2018 WS, 2019 DS, and 2019 WS).

et al. 2010; Ntanos *et al.* 2010; El-Adl *et al.* 2011). Shorter culm length and fourth internode length, as observed in Red 18, can reduce the plant's attractiveness to ovipositing moths (Ntanos *et al.* 2010; Hosseini *et al.* 2011).

Based on morphological trait analysis, Inarciaga, Mukol, and C-4 Dinorado emerged as promising candidates for stem borer resistance breeding. However, the susceptible TN1 also shared several morphological traits with the resistant check TKM6, highlighting the complex nature of stem borer resistance.

Future research should explore the interplay between morphological, physiological, and biochemical factors to gain a comprehensive understanding of stem borer resistance mechanisms. Microscopic traits such as trichome density and orientation (Viz and Pacada 2022) and biochemical components such as total phenolic content (Abilgos-Ramos *et al.* 2020) may also contribute to resistance. By integrating these factors into breeding programs, it is possible to develop rice cultivars with enhanced and durable resistance to stem borers.

To summarize, cumulative morphological trait similarity across three seasons (2018 WS, 2019 DS, and 2019 WS) showed that Inarciaga, Mukol, and C-4 Dinorado were a possible good candidate germplasm for morphological phenotype selection. Based on morphological traits alone, hierarchical cluster analysis based on Ward's method algorithm revealed that Inarciaga and Red 18 were the best candidate genotypes against stemborer by having lesser leaf surface area, narrower stem diameter, and shorter height. Aside from morphological features characterization, physiological, biochemical, and genetic parameters must be considered to fully establish the dynamics of plant-insect interactions of rice and stem borer.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no potential conflict of interest related to this publication.

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APPENDICES

Table I. Mean values of the morphological traits of six germplasm and two checks across three seasons (2018 WS 2019 DS, and 2019 WS).

Accession names	Leaf color (SPAD)	Flag leaf length (cm)	Flag leaf width (mm)	Leaf width (mm)	Stem diameter (mm)	Culm length (cm)	Length of the 4th internode (mm)
2018 WS							
Dinorado	43.30	42.56	20.89	16.67	7.60	135.67	186.00
Hinumay	47.03	39.67	21.89	17.78	6.63	146.00	180.00
Inarciaga	41.93	36.89	17.11	12.78	5.60	119.00	119.00
Red 18	41.03	40.83	17.22	14.00	7.87	76.67	95.67
C4-Dinorado	31.90	47.44	15.11	11.22	6.40	140.67	220.67
Mukol	41.47	41.61	16.89	14.78	6.60	153.33	154.33
TKM6 (R-check)	41.10	34.89	11.11	8.78	4.73	123.67	130.67
TN1 (S-check)	38.07	40.06	14.78	12.33	6.20	69.33	60.00
2019 DS							
Dinorado	54.17	35.56	16.44	15.11	8.40	101.83	120.33
Hinumay	45.23	32.78	15.33	13.89	7.50	113.67	137.00
Inarciaga	48.67	30.44	12.22	9.00	5.97	65.33	75.00
Red 18	48.93	30.06	11.78	9.56	5.17	50.67	35.33
C4-Dinorado	45.07	40.11	10.11	7.56	5.97	106.33	131.00
Mukol	48.93	34.56	10.33	8.78	6.63	113.33	112.67
TKM6 (R-check)	32.03	21.05	7.11	6.78	4.53	91.33	154.33
TN1 (S-check)	41.30	26.44	10.44	8.89	5.73	62.17	71.00
2019 WS							
Dinorado	47.59	48.33	16.33	13.11	7.20	122.33	182.67
Hinumay	41.53	39.52	20.67	16.56	6.23	138.22	131.89
Inarciaga	40.27	45.22	14.78	12.00	5.73	82.56	109.11
Red 18	37.09	32.78	12.89	13.22	6.30	78.78	67.54
C4-Dinorado	35.730	59.70	13.44	11.56	7.99	134.22	133.33
Mukol	40.840	51.33	14.44	11.67	6.09	124.78	142.31
TKM6 (R-check)	32.08	56.78	11.11	9.22	5.44	114.78	133.44
TN1 (S-check)	33.13	27.72	11.89	11.00	5.16	67.22	85.32

Note: [R] resistant; [S] susceptible; [WS] wet season; [DS] dry season

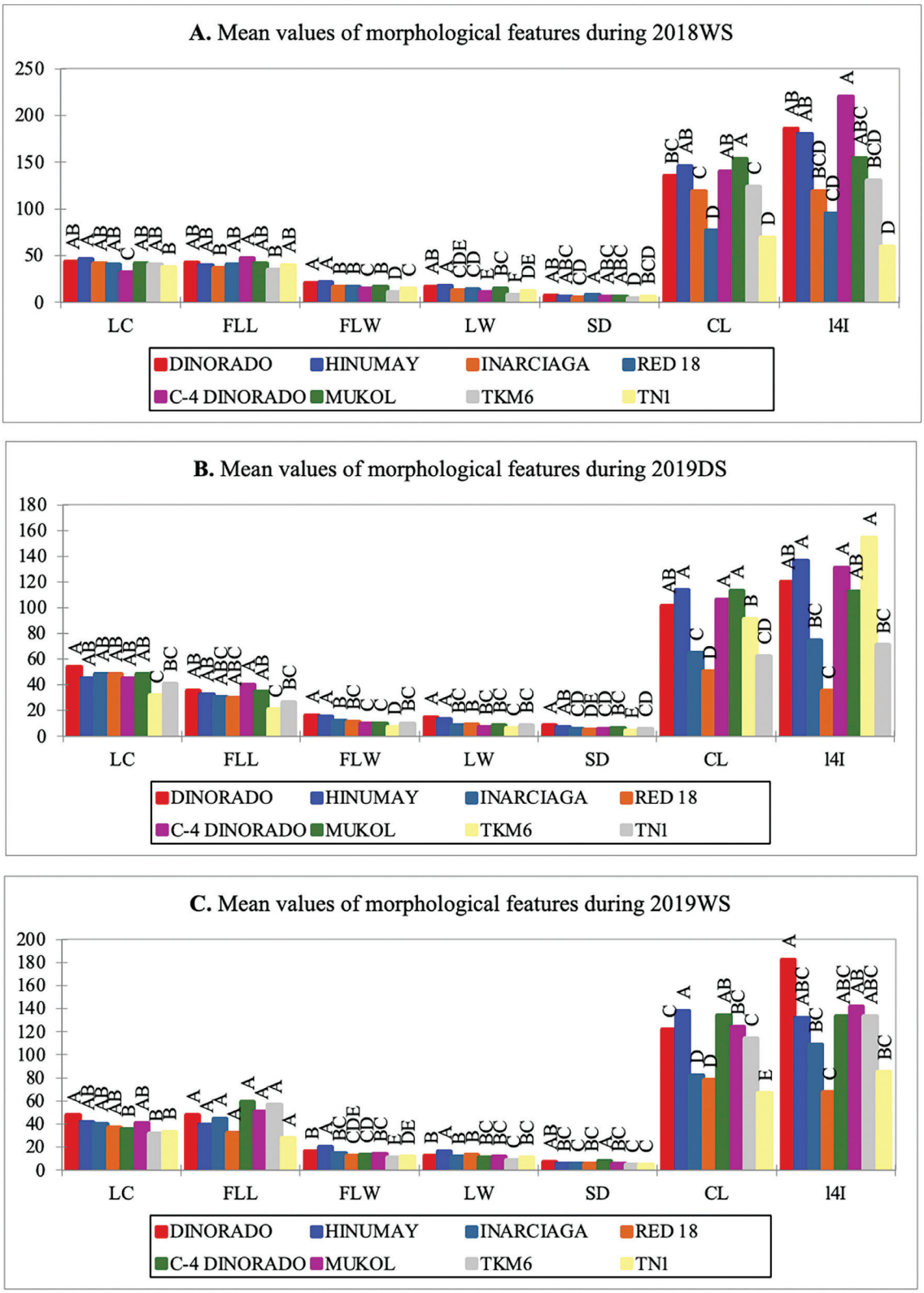

Figure I. Mean values of the morphological traits of six germplasm and two checks with Tukey's HSD during 2018 WS (A) 2019 DS (B), and 2019 WS (C). Note: mean values followed by the same letter within a column are not significantly different at $p > 0.05$ based on Tukey's HSD.

Table II. Correlation analysis among seven identified morphological traits across three seasons (2018 WS, 2019 DS, and 2019 WS).

	Leaf color (SPAD)	**Flag leaf length**	**Flag leaf width**	**Leaf width**	**Stem diameter**	**Culm length**	**Length of the 4th internode**
Leaf color (SPAD)	1						
Flag leaf length	–0.20	1					
Flag leaf width	0.23	0.25	1				
Leaf width	0.21	0.19	**0.92****	1			
stem Diameter	0.35	0.30	**0.52***	**0.55***	1		
Culm length	–0.01	0.42	**0.50***	0.43	0.31	1	
Length of the 4th internode	–0.09	0.35	0.35	0.30	0.27	**0.79****	1

Note: [**] strong positive correlation; [*] moderate positive correlation