J.Natn.Sci.Foundation Sri Lanka 2026 54 (2): 229 - 241
DOI: http://dx.doi.org/10.4038/jnsfsr.v54i2.12870

RESEARCH ARTICLE

Agriculture

Phenotypic characterization of flag leaf and pollen germination behavior in relation to grain yield in selected *Oryza nivara* populations in Sri Lanka

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Submitted: 24 November 2025; Revised: 04 March 2026; Accepted: 28 April 2026

Abstract: The flag leaf, the most important functional leaf in the rice plant, positively influences the photosynthetic process and yield components. Meanwhile, pollen germination is a critical indicator of species compatibility for successful seed setting. This study analyzed the correlation between flag leaf traits and panicle traits and pollen germination characteristics of six wild rice (*Oryza nivara*) populations. Plants from six populations (N1 to N6) were collected from different geographical locations and established in a common garden. Morphological traits of the flag leaf were evaluated shortly after heading and yield traits were measured at the maturity stage. Agro-morphological, reproductive, and physiological traits related to grain yield were recorded. Data were analyzed using one-way ANOVA, followed by Duncan's multiple range test ($p<0.05$), least significant difference (LSD) ($p<0.05$), and Pearson's correlation test ($p\geq0.05$ to $p<0.001$). The Kilinochchi (N6) population recorded the highest significant values for flag leaf length (26.58 ± 0.49), flag leaf area (23.04 ± 1.22), number of seeds per panicle (71.40 ± 3.52), panicle length (24.55 ± 0.38), 100-seed weight (1.76 ± 0.027), and pollen germination (65.74 ± 3.51) compared to other populations. The Anuradhapura (N1) population had the highest ratio of fertile seeds per panicle (0.75 ± 0.023). The Kurunegala (N3) population showed the lowest seed fertility (0.11 ± 0.012) after removal of the flag leaf, showing a 71.8% reduction in seed fertility compared to the intact flag leaf. According to Pearson's correlation analysis, the highest positive correlation was observed between flag leaf length ($r = 0.79$, $p < 0.001$) and panicle length, followed by chlorophyll content ($r = 0.26$, $p < 0.05$) and pollen germination ($r = 0.36$, $p < 0.01$) with the ratio of fertile seeds per panicle. Results suggested that flag leaf length, chlorophyll content, and pollen germination could be the best yield-attributing indicators for selecting *O. nivara* wild rice populations to adopt in rice pre-breeding studies.

Keywords: Chlorophyll content, flag leaf excision, *Oryza nivara*, pollen germination, seed fertility, wild rice

INTRODUCTION

The leaf is the primary organ that intercepts light energy and converts it into organic compounds through photosynthesis. In particular, the flag leaf characteristics of the Poaceae species act as a key determinant of the grain yield and biomass (Liu et al., 2015). As a cereal crop, the grain yield of rice directly relies on photosynthetic efficiency, with particular emphasis on the morphological characteristics of the flag leaf (Yue et al., 2006). The flag leaf is the uppermost leaf, subtending the panicle, directly associated with panicle development and grain filling. Reports have underscored the significance of the flag leaf in rice productivity, attributing up to 45% to grain filling (Abou Khalifa et al., 2008; Mahesh et al., 2022) due to its position and assimilation-related characteristics. Traits such as length, width, angle, and chlorophyll content of the flag leaf during the reproductive period have been

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positively correlated with rice yield (Rahman et al., 2014; Zhang et al., 2015). Further, the angle of the flag leaf directly affects light interception and significantly contributes to determining rice grain yield (Mahesh et al., 2022). Leaf chlorophyll content also has positive impacts on leaf photosynthesis (Takai et al., 2010; Rahman et al., 2014). Studies have shown that leaf photosynthesis and chlorophyll content are higher in the flag leaf of rice at the heading stage (Takai et al., 2006; Ohsumi et al., 2007; Takai et al., 2010), suggesting a strong correlation with yield characteristics. However, the flag leaf has been identified as one of the most variable phenotypic traits among populations of *Oryza nivara* (Sandamal et al., 2022).

Pollen viability is essential for successful fertilization, which is directly linked with seed fertility of rice. Thus, pollen viability is a critical indicator for evaluating reproductive success, as it directly influences grain yield (Dafni & Firmage, 2000; Rahman et al., 2014).

Wild relatives of rice serve as natural germplasm resources that play a crucial role in rice breeding by providing an alternative source of resistance or tolerance to abiotic and biotic stresses (Vaughan, 1994; Jena, 2010) with genomes AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ serving as an important reservoir of useful genes (Brar and Khush, 1997). Sri Lanka hosts five wild relatives of rice species, namely, *Oryza nivara*, *Oryza granulata*, *Oryza eichingeri*, *Oryza rufipogon*, and *Oryza rhyzomatis* (Vaughan, 1994). Among these, *O. nivara*, the main wild ancestor is closely related to cultivated rice (*O. sativa*) with the AA genome (Wang et al., 2017; Sandamal et al., 2018). This species has been extensively utilized in rice breeding programs (Bhasin et al., 2012; Yamagata et al., 2019; Zheng et al., 2024), strong potential for use in breeding programs. *O. nivara* populations from diverse geographic locations in Sri Lanka show significantly higher phenotypic diversity among populations than within populations (Sandamal et al., 2022; Sandamal et al., 2024). Additionally, *O. nivara* is known to possess fertility restoration (Rf) genes, and new and useful alleles that can improve grain yield, grain quality, and yield-contributing traits (Kaladhar et al., 2008; Gaikwad et al., 2014; Haritha et al., 2018).

There is a significant research gap particularly in Sri Lanka, concerning the yield potential associated with flag leaf characteristics in wild rice germplasm. This study aims to evaluate the flag leaf characteristics and yield traits of *O. nivara* populations across different geographical locations in Sri Lanka. Additionally, we assessed correlations of chlorophyll content, pollen viability, and analyzed spikelet fertility after flag leaf removal in selected superior *O. nivara* populations to assess their yield potentials (Zheng et al., 2024). This study highlights how flag leaf characteristics, chlorophyll content, and pollen viability can influence yield traits in *O. nivara* populations.

MATERIALS AND METHODS

Sample collection and field establishment

Mature seeds were collected from six natural habitats of *O. nivara* across different geographical locations in Sri Lanka, including both the intermediate and dry zones, showcasing a wide diversity of typical environments (Sandamal et al., 2022). Each population consisted of 20 individuals, ensuring a minimum distance of 5 m between individuals to avoid the multiple sampling of the same genet. The six *O. nivara* populations (N1-N6) were established in a common garden at the Faculty of Agriculture, University of Ruhuna (latitude: 06.060337°N, longitude: 80.5681455°E) in Sri Lanka, using one plant per cement pot (16”x16”x18”) arranged as a single experiment unit in a completely randomized design (Table 1). The garden conditions included an annual average air temperature of 29.16 ± 0.44 °C and a relative humidity level of 83.08 ± 1.21 % in an open field (Data source: Faculty meteorology unit, Faculty of Agriculture, University of Ruhuna). The established plants were allowed to grow for one year, from 2024 to 2025. Flag leaves of ten randomly selected plants were excised at the panicle insertion stage, and the remaining ten were kept with the flag leaf as a set of control treatment for each population. Morphological descriptors (both qualitative and quantitative) such as flag leaf traits, pollen viability, and yield were assessed in all populations, as described by the guidelines of the International Rice Research Institute (IRRI) (Brewbaker & Kwack,1963; Rahman et al., 2014).

Phenotypic characterization of the flag leaf and panicle

Phenotypic characterization of the flag leaf and panicle for wild rice was performed following the guidelines set by the IRRI descriptors. The measurements included flag leaf length, flag leaf width, flag leaf area, flag leaf angle, number of spikelets per panicle, ratio of fertile spikelets per panicle, panicle length, and 100-seed weight detailed in Table 2.

Table 1: Geographical and ecological information of six *Oryza nivara* populations collected from different locations and subsequently established in a common garden for flag leaf characterization.

Label	Location	GPS coordinates		Agro-ecological zone*	Annual mean temperature* of the district	Natural habitat description
		Latitude	Longitude			
N1	Anuradhapura	8.135274	80.553759	DL_{1b}	26.5°C	Shallow water lake
N2	Kataragama	6.443126	81.309686	DL_5	26.8°C	Roadside, waterlogged, marshy area
N3	Kurunegala	7.704122	80.163938	IL_3	26.4°C	Roadside, waterlogged, marshy area
N4	Batticaloa	7.623656	81.716753	DL_{2b}	27.5°C	Shallow water lake
N5	Mannar	9.016355	80.066568	DL_4	29.3°C	Shallow water lake
N6	Kilinochchi	9.341323	80.409326	DL_3	29.0°C	Shallow water lake

*Source: Department of Meteorology in Sri Lanka

Table 2: Methods for measuring traits and corresponding code details

Traits	Code	Method of trait measurement
Flag leaf length (cm)	FLL	Measured from tip to leaf base
Flag leaf width (cm)	FLW	Measured at the widest part of the leaf
Flag leaf area (cm^2)	FLA	$= k \times FLL \times FLW$ (k; 0.75) ...(01) (Yoshida et al., 1976) (FLL - Flag leaf length, FLW - Flag leaf width)
Flag leaf angle (°)	FLAN	Measured at the collar as the angle of attachment between the flag leaf blade and the main panicle axis using the Angulus (version 5.1.0) mobile application (Porkodi et al., 2023).
Number of spikelets per panicle	NSP	Count of the individual seeds in the panicle
Ratio of fertile spikelets per panicle	RFSP	Count of the fertile spikelets by pressing the spikelets with fingers and noting those that do not have grains at the mature stage. $= \frac{Number\ of\ fertile\ seeds\ per\ panicle}{Total\ number\ of\ seeds\ per\ panicle}$ ...(02)
Panicle length (cm)	PL	Measured panicle base to tip
100 seed weight (g)*	STW	Weight of 100 well-developed whole grains, dried to 13% moisture content, and weighed on a precision balance.
Chlorophyll content (SPAD value)	CC	Measured using the CCM 200 plus SPAD meter at the heading stage of the flag leaf
Pollen germination (%)	PG	Recorded using the in vitro pollen germination method $Pollen\ germination\ (\%) = \frac{Number\ of\ germinated\ pollen\ grains}{Total\ number\ of\ pollen\ grains\ on\ slide} \times 100$ ...(03)

*100-seed weight was used instead of 1000-seed weight due to biological constraints (low seed fertility, and high seed shattering) in wild rice populations.

Excision of the flag leaf

At the heading stage (panicle insertion stage), the flag leaves were carefully removed and the number of spikelets per panicle was counted at the fully mature stage (after 21 days) to determine the ratio of fertile spikelets to the total number of spikelets per panicle (Rahman et al., 2014; Das et al., 2018).

Total chlorophyll content

Total chlorophyll content was measured using the CCM 200 plus SPAD meter at the heading stage of the flag leaf, as a higher photosynthesis rate can be observed during this time, which is related to yield (Takai et al., 2006; Ohsumi et al., 2007; Takai et al., 2010).

Pollen viability

Pollen viability was recorded using the *in vitro* pollen germination method described by Brewbaker and Kwack (Brewbaker & Kwack,1963). Briefly, the protocol included the following steps: Brewbaker and Kwack medium was prepared using 10% sucrose, 100 mg/L boric acid, 300 mg/L calcium nitrate, 200 mg/L magnesium sulfate, and 100 mg/L potassium nitrate. Pollen grains were placed on a slide covered with one to two drops of Brewbaker and Kwack medium using a fine brush. The slide was then placed on a wet filter paper in a watch glass to maintain high humidity and kept at room temperature for 40 minutes. Finally, the number of germinated pollen grains was observed using a light microscope (Jayaprakash, 2018).

Data analysis

The data were analyzed using SPSS version 25 and R Studio version 4.4.2. An initial ANOVA was conducted to analyze all variables, including flag leaf and yield traits. Additionally, the least significant difference (LSD) test was used to identify statistically significant differences between means at a 5% level among populations. Pearson's correlation was performed for correlation analysis ($p<0.05$) using the R programming language. The correlation matrices for flag leaf traits and yield components were visualized using a heatmap created in R Studio (Turney, 2024; Howlader et al., 2025). Pearson's coefficient of correlation (r) was calculated using the formula given below (Turney, 2024).

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}} \quad ...(04)$$

Here, r = Pearson coefficient of correlation, n = sample size, x = each x-variable value, and y = each y-variable value.

Table 3: Variation of flag leaf morphological traits, yield attributed traits, and pollen germination characteristics of six *Oryza nivara* populations.

Variable	Anuradhapura	Kataragama	Kurunegala	Batticaloa	Mannar	Kilinochchi	LSD*
FLL (cm)	21.76 ± 0.52^{b}	10.63 ± 0.27^{a}	21.27 ± 0.38^{b}	10.84 ± 0.39^{a}	22.05 ± 0.58^{b}	26.58 ± 0.49^{c}	1.27
FLW (cm)	0.85 ± 0.022^{c}	0.72 ± 0.025^{b}	0.85 ± 0.022^{c}	0.61 ± 0.028^{a}	1.15 ± 0.043^{e}	1.05 ± 0.034^{d}	0.085
FLA (cm^2)	13.92 ± 0.62^{b}	5.78 ± 0.34^{a}	13.61 ± 0.56^{b}	5.03 ± 0.39^{a}	17.41 ± 0.84^{c}	23.04 ± 1.22^{d}	2.053
FLAN (^{O})	37.28 ± 4.58 ^{a}	27.38 ± 3.51^{a}	50.16 ± 5.62^{b}	69.69 ± 3.93^{c}	34.38 ± 3.88^{a}	39.82 ±1.63^{ab}	11.46
NSP	48.70 ±2.74^{d}	23.40 ± 1.09^{b}	35.00 ± 1.89^{c}	16.60 ± 1.14^{a}	42.90 ± 2.62^{d}	71.40 ± 3.52^{e}	6.63
RFSP – IFL	0.75 ± 0.023^{d}	0.48 ± 0.029^{b}	0.39 ± 0.028^{a}	0.64 ± 0.027^{c}	0.50 ± 0.035^{b}	0.51 ± 0.019^{b}	0.080
RFSP – FLE	0.17 ± 0.026^{b}	0.21 ± 0.009^{b}	0.11 ± 0.012^{a}	0.30 ± 0.031^{c}	0.23 ± 0.024^{b}	0.44 ± 0.027^{d}	0.063
PL (cm)	21.44 ± 0.30^{d}	16.80 ± 0.27^{b}	16.66 ± 0.20^{b}	12.33 ± 0.25^{a}	19.13 ± 0.39^{c}	24.55 ± 0.38^{e}	0.87
100 -STW (g)	1.70 ± 0.031^{b}	1.46 ± 0.038^{a}	1.70 ± 0.042^{b}	1.48 ± 0.044^{a}	1.75 ± 0.028^{b}	1.76 ± 0.027^{b}	0.102
CC (SPAD value)	11.00 ± 0.35^{b}	7.32 ± 0.25^{a}	8.51 ± 0.32^{a}	12.89 ± 0.44^{c}	14.79 ± 0.86^{d}	13.42 ± 0.40^{c}	1.36
PG (%)	42.50 ± 3.38^{b}	20.62 ± 2.02^{a}	16.31 ± 1.40^{a}	37.55 ± 3.98^{b}	24.72 ± 1.71^{a}	65.74 ± 3.51^{c}	8.06

Note: FLL: flag leaf length, FLW: flag leaf width, FLAN: flag leaf angle, NSP: number of seeds per panicle, RFSP – IFL: ratio of fertile seeds per panicle with an intact flag leaf, RFSP-FLE: ratio of fertile seeds per panicle, flag leaf excised, PL: panicle length, 100-STW: 100 seed weight, CC: chlorophyll content, and PG: pollen germination. Within a row, means followed by the same letters are not significantly different by Duncan's multiple range test (DMRT) at $p > 0.05$.

RESULTS AND DISCUSSION

Variation of flag leaf and yield traits of *Oryza nivara* populations

Results indicated significant differences among the populations for the assessed quantitative traits of flag leaf and yield (Table 3).

Morphological traits of the flag leaf such as flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA), flag leaf angle (FLAN), and chlorophyll content (CC) were significantly different among the populations. In terms of yield traits, the number of seeds per panicle (NSP), ratio of fertile seeds per panicle with an intact flag leaf (RFSP - IFL), ratio of fertile seeds per panicle to flag leaf excised (RFSP-FLE), panicle length (PL), 100-seed weight (STW), and pollen germination percentage (PG) were distinct parameters in differentiating populations.

Within the populations studied, the Kilinochchi population had the longest flag leaf (FLL - 26.58±0.49), highest flag leaf area (FLA -23.04 ± 1.22) and longest panicle length (PL - 24.55±0.38), showing significant differences compared to the other populations ($p < 0.05$). On the other hand, the Kataragama (FLL-10.63 ± 0.27; FLA - 5.78 ± 0.34) and Batticaloa (FLL-10.84 ± 0.39; FLA - 5.03 ± 0.39) populations had the shortest flag leaves among the populations studied.

The Mannar population showed an intermediate flag leaf length of 22.05 ± 0.58; however, it had the highest flag leaf width at 1.15 ± 0.043, which was significantly different from other populations. Conversely, the Batticaloa population had the lowest flag leaf width at 0.61 ± 0.028, which was also significantly different from other populations.

The characteristics of the flag leaf are modified by genetic and environmental factors. It has been reported that the size of the flag leaf is controlled by several genes (Zhang et al., 2015). According to Zhao et al. (2011), the observed SNPs at 31 Mb on chromosome 4 are significantly associated with flag leaf width in 413 diverse accessions. Additionally, the NARROW LEAF1 (*NAL1*) gene is linked to flag leaf size (Taguchi-Shiobara et al., 2015). Among environmental factors low light intensity has been found to enhance flag leaf area and width (Evans & Poorter, 2001; Fabre et al., 2016).

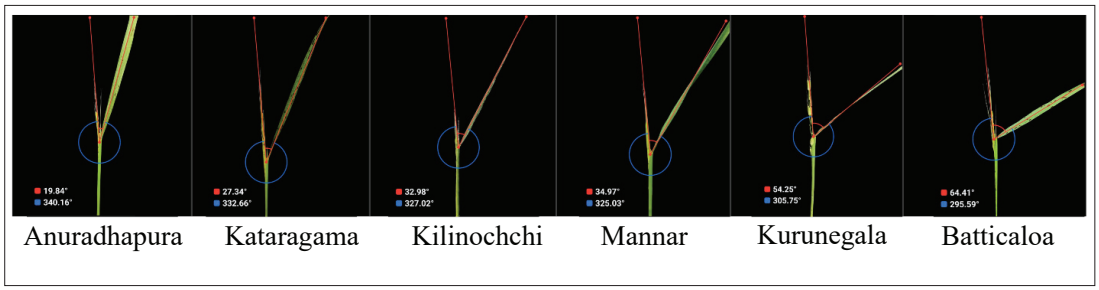

Figure 1: Variation in flag leaf angle of six *Oryza nivara* populations studied.

Significant variation in flag leaf angle was observed among the six *O. nivara* populations, as shown in Figure 1. The Flag leaf angle has been classified into four groups based on the IRRI standard descriptors (Chang & Bardenas, 1965; Mahesh et al., 2022): erect (0° – 30°), intermediate (31° – 60°), horizontal (61° – 90°), and descending (≥ 91°). The Batticaloa population exhibited a horizontal flag leaf angle (69.69 ± 3.93), while the Kataragama population showed the lowest flag leaf angle (27.38 ± 3.51), indicating an erect flag leaf angle. The Anuradhapura (37.28 ± 4.58) and Mannar populations (34.38 ± 3.88) showed an intermediate flag leaf angle (Figure 1). The genetic basis of flag leaf angle largely relies on *qFLANF.1A*, which is a major QTL associated with flag leaf angle and consistently shows a strong signal on chromosome 1A that explain 9 - 13 % of the variation across different environments (Mantilla-Perez & Salas Fernandez., 2017; Kumar et al., 2024). Moreover, similar variations in the flag leaf angle have been observed in different wheat accessions (Kumar et al., 2024). These studies have identified three stable genomic regions (MTAs) on chromosomes 1A, 5A, and 5B that regulate flag leaf angle, and remain stable across various environments in wheat plants (Kumar et al., 2024).

The Kilinochchi population had significantly higher values for the number of seeds per panicle and panicle length, with averages of 71.40 ± 3.52 and 24.55 ± 0.38, respectively. In contrast, the Batticaloa population had the lowest values, with an average of 16.60 ± 1.14 for the number of seeds per panicle and 12.33 ± 0.25 for panicle length. *qTSN4* is involved in both flag leaf and panicle traits, including flag leaf size and the number of spikelets per panicle (Fujita et al., 2013; Takai et al., 2013). According to Panda et al. (2018), the *Gn1a* gene is associated with an increase in the number of spikelets, while a loss-of-function mutation in the *LOG* gene results in several floral defects, such as a reduction in panicle size and the number of spikelets. The influence of environmental factors on flag leaf and panicle traits is well studied (Evans & Poorter, 2001; Fabre et al., 2016). For instance, plant grain production can be affected by environmental factors, particularly light levels, which affect panicle size (Fabre et al., 2016). These findings clearly demonstrate that the morphological traits of flag leaves and panicle characteristics vary according to environmental and genetic factors. In this study, *O. nivara* collected from six different geographical locations in Sri Lanka clearly showed significant differences in flag leaf traits and panicle morphology among populations, indicating adaptive phenotypic plasticity in various geographic locations.

Excision of the flag leaf affects the ratio of fertile seeds per panicle

The effect of flag leaf excision on panicle development varied significantly among the six *O. nivara* populations, as shown in Figure 2.

The impact of flag leaf removal on seed fertility differed significantly across populations, ranging from pronounced decreases to negligible effects (Figure 3). The Anuradhapura population exhibited the most severe reduction in seed fertility (77.3 %), indicating that the flag leaf (FL) contributes substantially to final yield in this population. In contrast, the Kilinochchi population displayed the highest seed fertility (0.44 ± 0.027) in the flag leaf excised treatment, with only a 13.73 % reduction compared to the treatment with an intact flag leaf, suggesting that the FL made the least contribution to grain yield. Additionally, the Kurunegala population exhibited the lowest seed fertility (0.11 ± 0.012) in the flag leaf excised treatment, with a 71.8 % reduction in seed fertility compared to the seed fertility of the treatment with an intact flag leaf treatment. According to Rahman et al. (2014), yield was reduced in rice cultivars after flag leaf excision, with varying effects among different cultivars. Furthermore, removing the flag leaf at the booting stage can decrease the yield capacity of wheat plants by more than 25%. The relationship between flag leaf and panicle characteristics depends on the variety of the plant and the environmental conditions (Racz et al., 2022).

The chlorophyll content index (CCI value) reflected by SPAD values showed significant variation among different populations. The Mannar population had the highest chlorophyll content (14.79 ± 0.86), followed by Kilinochchi (13.42 ± 0.40) and Batticaloa populations (12.89 ± 0.44). In contrast, the Kataragama population had the lowest chlorophyll content (7.32 ± 0.25), followed by the Kurunegala population (8.51 ± 0.32), with no significant difference between them. Over 900 quantitative trait loci (QTL) for chlorophyll content have been identified in rice, which include at least eight stay-green-related genes such as *DYE, SGR*, and *EF8* (Feng et al., 2014; Yamatani et al., 2018). Additionally, QTL linkage mapping has been used to explore the genetic architecture of natural variation in chlorophyll content in rice (Wang et al., 2015). Moreover, different levels of phosphorus can affect chlorophyll content, with phosphorus deficiencies potentially reducing chlorophyll levels in the flag leaf (Yang et al., 2022). Populations with higher chlorophyll contents (Mannar, Kilinochchi, Batticaloa, and Anuradhapura populations) recorded a greater number of fertile seeds compared to populations with lower chlorophyll contents (Kataragama, Kurunegala), indicating a strong link between chlorophyll levels in the flag leaf and the number of fertile seeds.

The Kilinochchi population had the highest 100-seed weight (1.76 ± 0.027) among all populations. In contrast, there were no significant differences in 100-seed weights among the Mannar (1.75 ± 0.028), Anuradhapura (1.70 ± 0.031), and Kurunegala populations (1.70 ± 0.042). The lowest 100-seed weights were found in the Batticaloa population (1.48 ± 0.044) and the Kataragama population (1.46 ± 0.038).

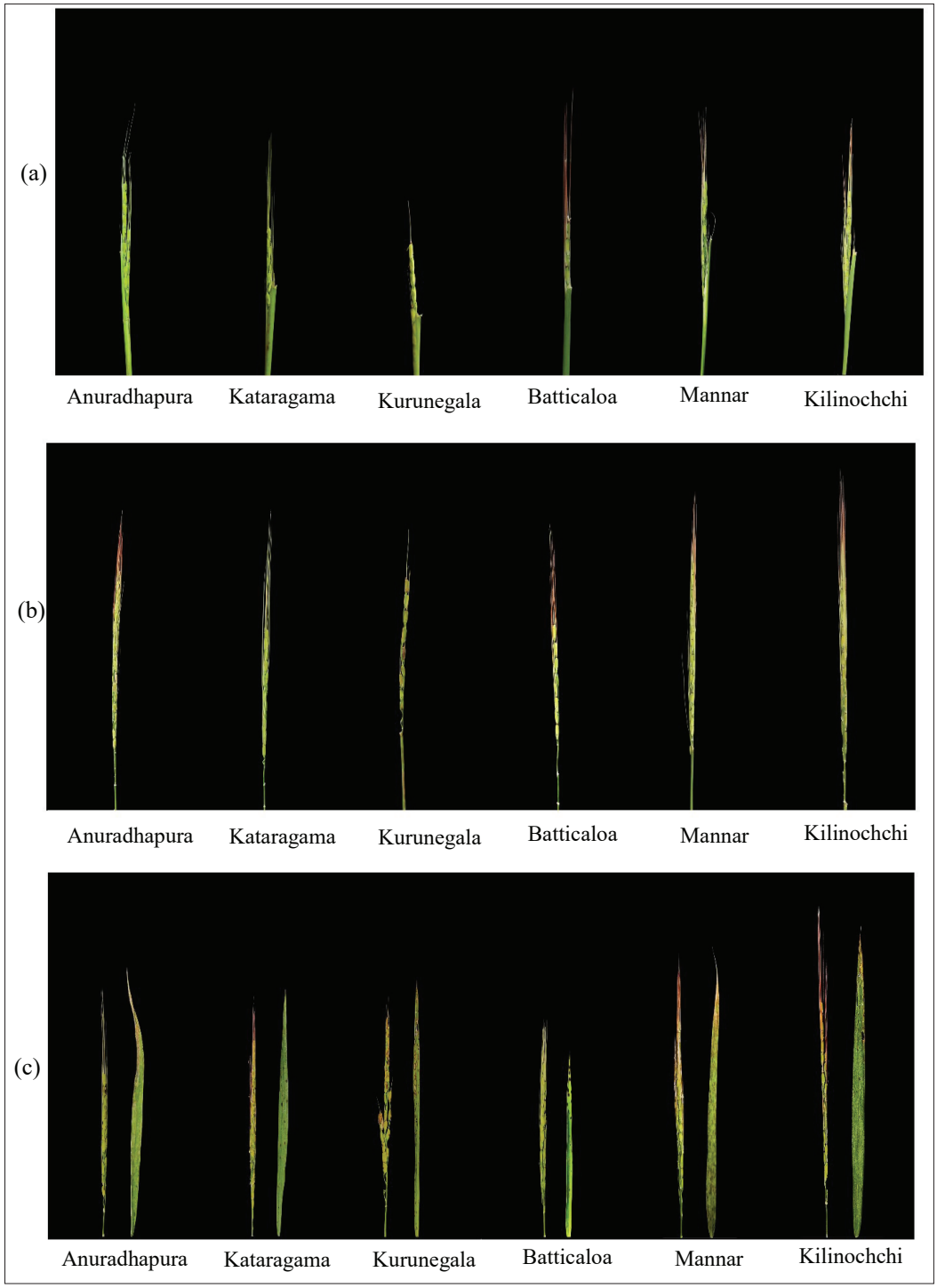

Figure 2: Effect of flag leaf excision on panicle development of six *Oryza nivara* populations. (a). Excised flag leaf at the booting stage (panicle insertion stage), (b). Panicle development after flag leaf excision. (c). Variations in flag leaf length and panicle length of six *O. nivara* populations in Sri Lanka.

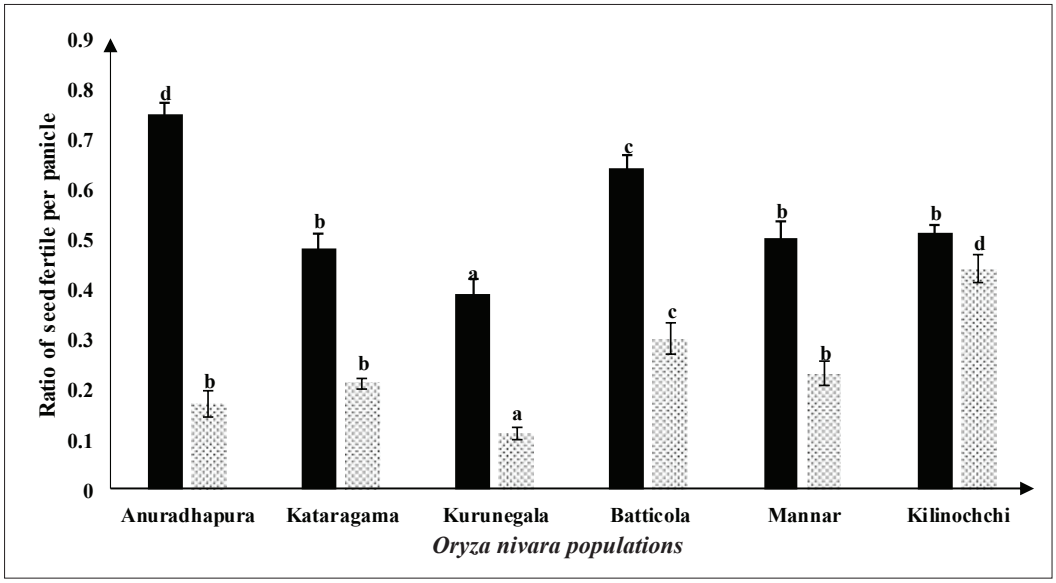

Figure 3: Comparison of the ratio of fertile seeds per panicle with an intact flag leaf; RFSP–IFL (black filled) and with the flag leaf excised; RFSP-FLE (dotted).

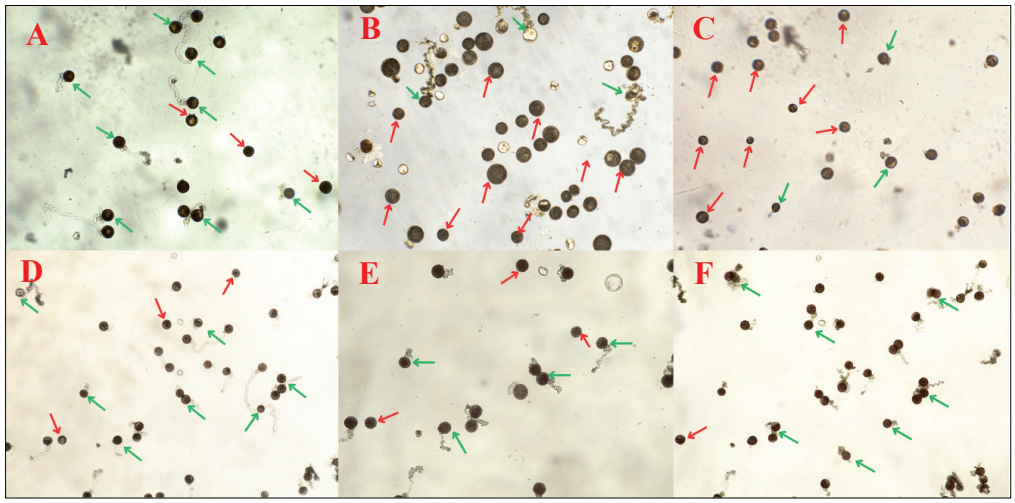

Figure 4: Pollen germination of six *Oryza nivara* populations in Sri Lanka. (A) Anuradhapura population. (B) Kataragama population. (C) Kurunegala population. (D) Batticaloa population. (E) Mannar population. (F) Kilinochchi population. The green and red arrows indicate germinated and non-germinated of pollen grains, respectively.

Variation in Pollen Germination among Six *O. nivara* Populations

Pollen germination percentages were observed across the six populations of *O. nivara*, as illustrated in Figure 4. The highest pollen germination was observed in the Kilinochchi population (65.74 ± 3.51), followed by the Anuradhapura population (42.50 ± 3.38) and the Batticaloa population (37.55 ± 3.98), both of which were not significantly different from each other. The lowest

pollen germination was observed in the Kurunegala population (16.31 ± 1.40), which was not significantly different from the Kataragama (20.62 ± 2.02) and Mannar (24.72 ± 1.71) populations. Environmental conditions significantly influence pollen development and germination, with temperature being a key factor (Coast et al., 2016). High temperatures during anthesis can reduce both pollen viability and germination (Tran Loc Thuy et al., 2020). However, some studies suggest that certain rice cultivars exhibit strong pollen germination even under high temperatures (Zakaria et al., 2022). Additionally, research has shown that both environmental and genetic factors affect pollen germination (Hu et al., 2025). Genetic factors such as the *OsSTA*, *OsAP65*, *Cdi*, and *MGP2* genes may play a role in pollen germination (Ling et al., 2015; Huang et al., 2013; Li et al., 2012). In this study, all populations were established in a common field providing equal conditions. All collected populations were from the dry zone and intermediate zone of Sri Lanka where high temperatures prevail for most of the year. Therefore, considering both environmental and genetic factors, it is likely that genetic factors have influenced pollen germination across the six populations.

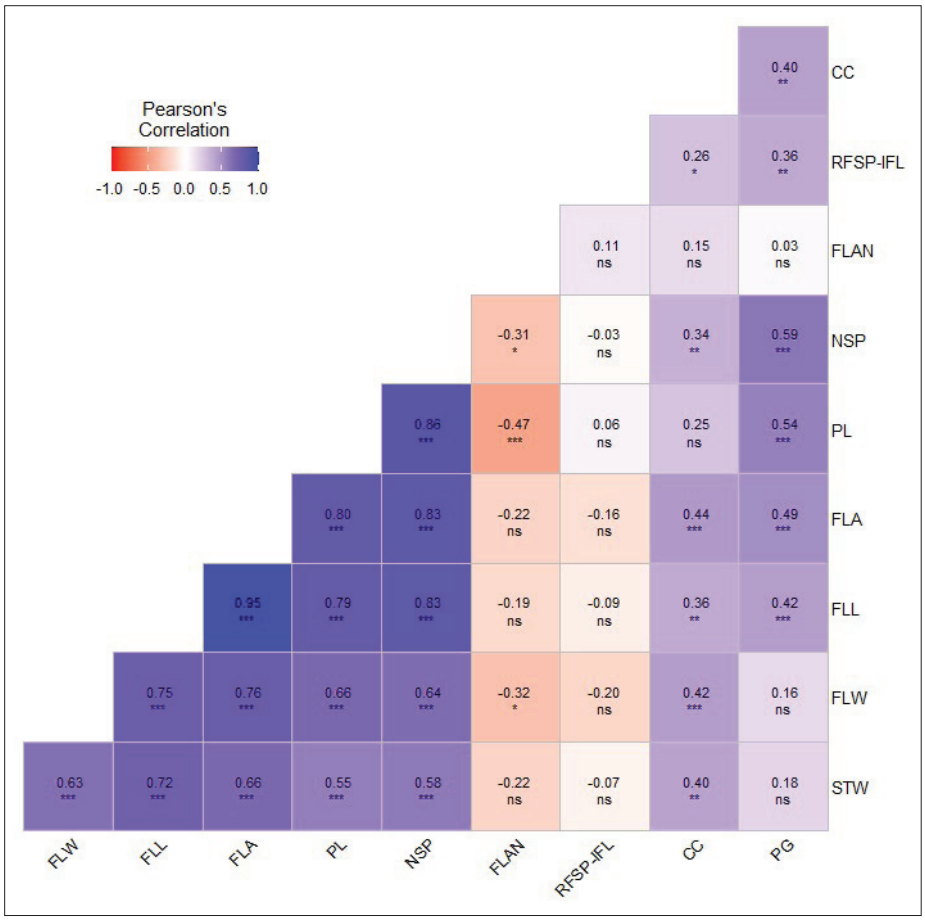

Figure 5: Correlations between flag leaf related traits and yield related traits. The number in the middle of the cell is the correlation coefficient; 'ns', '*', '**', and '***' indicate levels of significance (p>= 0.05, p<0.05, p<0.01, and p<0.001). FLW, flag leaf width; FLL, flag leaf length; FLA, flag leaf area; PL, panicle length; NSP, number of seeds per panicle; FLAN, flag leaf angle; RFSP-IFL, ratio of fertile seeds per panicle with an intact flag leaf; CC, chlorophyll content; PG, pollen germination; STW, seed test weight.

Correlations among the flag leaf traits with yield

The correlation between flag leaf related and yield related traits are given in Figure 5. The FLL significantly and highly positively correlated with FLA ($r = 0.95$, $p < 0.001$). Additionally, FLL is also significantly and highly positively correlated with panicle length ($r = 0.79$, $p < 0.001$). This positive correlation between FLL and panicle elongation is consistent with findings in rice (Rahman et al., 2014). Furthermore, FLA ($r = 0.83$, $p < 0.001$), FLL ($r = 0.83$, $p < 0.001$), and FLW ($r = 0.64$, $p < 0.001$) are significantly and positively correlated with the number of seeds per panicle. According to Wang et al. (2022), FLL, FLW, and FLA showed highly significant positive correlations with spikelet number per spike in wheat. When FLL, FLW and FLA increase, the number of seeds per panicle also increases. Additionally, FLW is positively correlated with FLL ($r = 0.75$, $p < 0.001$), FLA ($r = 0.76$, $p < 0.001$), PL ($r = 0.66$, $p < 0.001$), and chlorophyll content ($r = 0.42$, $p < 0.001$). In wheat, FLL showed a strong positive correlation with FLW, FLA, and FLAN, indicating comparable patterns in other *Poaceae* species. In our study, we found that FLL was strongly positively correlated with FLW ($r = 0.75$, $p < 0.001$) and FLA ($r = 0.95$, $p < 0.001$), but it exhibited a negative correlation with FLAN (Liu et al., 2018; Wang et al., 2022). The ratio of fertile seeds per panicle is significantly positively correlated with pollen germination ($r = 0.36$, $p < 0.01$) and chlorophyll content ($r = 0.26$, $p < 0.05$), although the r values are relatively low. Furthermore, the number of seeds per panicle is positively correlated with chlorophyll content ($r = 0.34$, $p < 0.01$). Moreover, several studies showed a strong correlation between leaf chlorophyll content and grain yield (Reynolds et al., 2000; Rosyara et al., 2010; Lopes et al.,2012). The 100-seed weight was positively correlated with FLW ($r = 0.63$, $p < 0.001$), FLL ($r = 0.72$, $p < 0.001$), FLA ($r = 0.68$, $p < 0.001$), PL ($r = 0.55$, $p < 0.001$), and the number of seeds per panicle ($r = 0.58$, $p < 0.001$).

Furthermore, PL was negatively correlated with FLAN ($r = -0.47$, $p < 0.001$). Meanwhile, FLW ($r = -0.20$, $p >= 0.05$), FLL ($r = -0.09$, $p >= 0.05$), and FLA ($r = -0.16$, $p >= 0.05$) were negatively correlated with the ratio of fertile seeds per panicle. The number of seeds per panicle ($r = -0.03$, $p >= 0.05$) was also negatively correlated with the ratio of fertile seeds per panicle. This implies that as the number of seeds per panicle increases, the ratio of fertile seeds per panicle tends to decrease. This may occur due to a low pollen germination percentage in the wild relatives of rice (Hu et al., 2025). Additionally, the ratio of fertile seeds per panicle with an intact flag leaf (RFSP–IFL) negatively correlated with 100-seed weight (STW) ($r = -0.07$, $p >= 0.05$). Furthermore, according to Khaliq et al. (2008), the association between fertile grains per panicle and 1000-grain weight is negatively correlated and highly significant. In particular, flag leaf traits are often positively correlated with yield traits in many cereal crops, including rice. Additionally, chlorophyll content varies among populations, varieties, and cultivars; however, it shows a positive correlation with yield traits (Rahman et al., 2014).

CONCLUSION

This work presents the first investigation into how yield-related flag leaf traits and pollen germination correlate with seed setting in wild rice. The flag leaf traits and yield characteristics significantly varied among six populations of *O. nivara,* showing phenotypic adaptation to diverse environments. Key traits, including flag leaf length, flag leaf area, flag leaf width, the number of seeds per panicle, the ratio of fertile seeds per panicle, and panicle length, were highly different among the populations and could be utilized as key indicators in selecting wild geographic locations in future studies. Conversely, traits such as flag leaf angle, 100-seed weight, chlorophyll content, and pollen germination showed minimal variation across populations, making them unsuitable for selection criteria. Variation in seed fertility across populations points to either differential dependency on flag leaf photosynthesis or the activation of population-specific compensatory mechanisms, governed by underlying genetic and physiological plasticity. Consequently, further research is required to elucidate the underlying physiological and genetic mechanisms. These findings provide phenotypically promising *O. nivara* populations serving as candidate populations for future pre-breeding studies.

Habitat-specific phenotyping of wild rice is recommended to capture the significant trait diversity of *O. nivara* populations across Sri Lanka. This approach is essential for developing targeted conservation strategies and promoting the sustainable management. Further research should focus on linking morphological traits to adaptive significance, rather than relying solely on descriptive trait variation.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China [Grant Number 32130008]. The authors acknowledge the assistance of Grammarly AI for English language editing, grammar correction, and improving the clarity of this manuscript.

All scientific content, data, analysis, interpretation, and conclusions were done by the authors.

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