The Effect of Sunlight Intensity on Flower Opening Time and Exposure Duration in Rice (*Oryza sativa* ssp. *indica*) Landraces

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Running Title : Sunlight intensity influences rice flowering time

Highlights

Low solar illuminance significantly delays flower-opening time in rice landraces. However, the effect of experimental shading on flower opening duration of the same landraces is indeterminate, likely due to blocking of sunlight.

Abstract

We recorded sunlight intensity at anthesis of 390 rice landraces flowering in the short-day season in a year, and find that flower opening time (FOT) is delayed but flower exposure duration (FED) is shortened on cloudy days (<30000 lux). These results construe a reversal of the effect previously reported from a larger set of 1114 landaces (including the 390 landraces in this study), recorded during short-day and long-day seasons over 3 years. To resolve this Simpson's paradox, we subsequently recorded solar illuminance at FOT of 25 landrace populations, half of which was fully exposed to the sun, compared to another half under shade. This experiment revealed that low sunlight intensity, mimicking cloudy days, significantly delays FOT, corroborating the pattern detected in our earlier findings. However, experimental shading has an indeterminate effect on FED of the same landraces. We hypothesize that the delayed FOT on overcast days is an adaptation in rice plants in anticipation of rain, for protection of the pollen from rainwash.

Keywords: Anthesis, Flower opening time, Landraces, Rice, Sunlight intensity, Sunlight to anthesis duration.

List of Abbreviations:

FCT : Flower closing time

FD: (First) flowering date

FED : Flower exposure duration

FOT : Flower opening time

IQR = Interquartile range

SAD : Sunrise-to-anthesis duration

1 Introduction

The preconditions of successful cross pollination between different rice (*Oryza sativa*) cultivars include short (<100 m) pollination distance (OGTR, 2005), proximity of flowering dates (FD), proximity (<30 min) of flower opening time (FOT) and the overlap of the duration of their respective flowers remaining open (= flower exposure duration, FED) (Deb and Bhattacharya, 2021; Deb et al., 2023). In spite the received wisdom that cross pollination frequency in rice rarely exceeds 2% (Moldenhauer and Gibbons, 2003; OGTR, 2005; GOI, 2011; and citations there), cross pollination is likely to occur at a high frequency between neighbouring cultivars with close proximity of FOT and a large temporal overlap of FED (Deb and Bhattacharya, 2021).

Our documentation of FOT in 1114 *indica* landraces, constituting the largest database (Deb, 2022) suggests that a majority of these landraces are likely to be cross pollinated due to considerable overlaps of FOT and FED among hundreds of landraces. However, the FOT and FED of the same cultivar may vary with seasonal photoperiod (short day vs long day), daylight condition (sunny vs cloudy) (Deb et al., 2023), and day temperature (Kobayasi et al., 2010; Cho et al., 2017; Deb et al., MS *under review*). The first study of the variability of FOT and FED with flowering season and daylight conditions (Deb et al., 2023) indicated that the adequate solar illuminance is likely as important to elicit FOT as day maximum temperature. This led us to explore the effect of incident sunlight intensity at the time of anthesis on both FOT and FED. The present study describes our attempt to find a relationship between sunlight intensity with FOT and FED, based on empirical observations of flowering times and durations of 390 rice landraces (420 populations), and on a comparison of 25 landraces subjected to full exposure to natural daylight and under shading (mimicking cloudy days) in a field experimental setup.

2 Materials and Methods

2.1 *Study site, cultivation season and materials*

We documented the FOT and FED of 390 landraces grown on Basudha conservation farm (http:// cintdis.org/basudha), located in Rayagada district of southern Odisha

(19°42′32.0″N, 83°28′8.4″E) during short-day (*Aman*) season of the year 2022. The procedure of recording of FOT, flower closing time (FCT), FED (the difference between FOT and FCT), and sunrise to anthesis duration (SAD) on the days of flowering of each of these landraces is given in detail in Deb et al. (2023).

We also recorded the intensity of incident sunlight on the panicles at FOT and FCT of each of the 420 landrace populations during short-day (*Aman*) season of 2022 (**Table 1**). For measuring incident sunlight illuminance, we used a cell phone application Lux Light Meter Pro version 031.2022.01.11 (Doggo Apps) and a Digital Illuminance Meter (http://drmeter.com/ products/lx1330b-digital-illuminance-light-meter, model No. LX1330B) held next to the panicle, without touching it, at the onset of florets opening and after the full closure of the last florets on the panicle. The solar illuminance on all overcast days at the rice plant level never exceeded 30000 lux, and therefore we decided on a threshold of "sunny" days to be 30000 lux.

2.2 *Field experiment*

During the long-day (*Boro*) season of 2023, we set up an experiment with 26 landraces in the same field to compare the FOT and FED of these landraces under natural illuminance in open sunlight and low illuminance under shade. Light intensity was measured during the FOT of the first florets and the FCT of the last florets of each landrace population in this experiment, by lux meters, as described above.

The design of the field experiment is shown in **Fig. 1**. An opaque polythene sheet was built over half of each landrace population, and kept in place from the booting stage until after the last FCT of the population. The shade allowed entry of only diffused light from the sides, with incident light intensity never exceeding 4560 lux, mimicking overcast and rainy days. The other half of the same population was left open under full sun, where the sunlight intensity exceeded 30000 lux throughout the study period.

2.3 *Statistical analyses*

Statistical correlation and regression analyses were made using Excel software on desktop. Normality tests of distribution was performed using Shapiro-Francia and Anderson-Darling tests online
(Georgiev, 2023). When the assumption of normal distribution was violated, we performed

nonparametric tests (Gibbons and Chakraborti, 2021) to compare the medians between two groups.

For pairwise comparison of the landrace populations, Wilcoxon-Mann-Whitney test was employed to estimate the significance of the difference in SAD and FED medians of the populations between sunny and shaded conditions. The hypotheses considered in this analyses are:

$$H0 : \delta = 0, H1 : \delta < 0$$

where δ is [median of SAD-S or FED-S] – [median of SAD-C or FED-C]. We chose the confidence interval at 99% for all statistical analyses. To control for multiple testing, we used Bonferroni corrections to draw valid conclusions. We also conducted a paired permutation test (Manly and Navarro Alberto, 2022) for location considering the same hypotheses as above, and conducted 10,000 iterations for the 25 paired data. All analyses were conducted using R statistical software 4.3.0.

3 Results

We describe the results in two sections, namely, the results of analyses of the associations of FOT, SAD and SED with different daylight conditions on the day of anthesis of all the landraces (459 observations) under section *3.1*, and the same analyses for the selected 25 landraces experimentally subjected to both open sunlight and under shade, under section

3.1 General field observations

The data of FOT and FED, as well as the day temperatures and the daylight conditions on the respective flowering dates of each of 420 landraces grown in 2022 are available on Harvard Dataverse (Deb, 2022). In addition to these data, the sunlight intensity at the respective FOT and FCT of each of the total of 420 landrace populations are deposited with Harvard Dataverse (Deb, 2023).

3.1.1 The effect of daylight conditions on SAD and FED

The landraces tend to open their florets slightly later (n = 300, median SAD = 266 min.,

IQR = 30) on sunny days (light intensity ≥30000 lux) than on cloudy days with sunlight intensity <30000 lux (n = 120, median SAD = 261.5 min., IQR = 31.25) during the same season. The difference between the median values on sunny and cloudy days is statistically not significant (Wilcoxon-Mann-Whitney z = 2.22, p = 0.0524). Permutation test of the same data (with 10000 iterations) for location reveals that the difference is not significant (p >0.4).

The median FED (64 min., IQR = 26) of the landraces on sunny days is significantly lesser than the mean FED (72 min, IQR = 39.25) on cloudy days, with Wilcoxon-Mann-Whitney z = – 3.11 (p = 0.003) (**Fig. 2**). Two-sample permutation test with 10000 iterations for location confirmed the significance of the difference at 99% confidence level.

3.1.2 The relationship between FOT/ SAD and FED on sunny and cloudy days

When the daylight conditions (S or C) exactly at FOT are examined as separate groups, FED shows a strong inverse relationship with FOT (**Fig. 3A**), and equivalently, with SAD (**Fig. 3B**). The coefficient of determination (R^2) for FED on both FOTd and SAD are almost identical, and indicates that delayed anthesis is strongly associated with shorter FED in landraces, and that the slope of regression is steeper on cloudy days.

3.1.3 The effect of sunlight intensity at FOT, combined with that at FCT

To examine if the inverse relationship between FOT and FED holds for these landraces closing their flowers at different sunlight exposures, we classified the phenological observations into four sunlight exposure categories (**Table 2**), namely, flowers opening and closing at sunny hours (SS, 224 landrace populations); opening at sunny but closing at a cloudy hour (SC, 76 populations); opening and closing both at cloudy hours (CC, 66 populations); and opening at cloudy but closing when it was sunny (CS, 54 populations).

The relationship of FED with FOTd for the populations under SS, SC, CS and CC conditions is inverse, and all are significantly (p <0.01) different from zero (**Fig. 4**). This pattern of relationship is corroborative of the strongly inverse (p < 0.0001) relationship of FED with FOTd of the same populations, when they are examined at S and C conditions at

FOT alone (**Fig. 3**).

All the above analyses show the relationship of FED with FOT and SAD of different landraces flowering during short-day season of 2022. The trend of SAD and FED appears to have an inverse association, as corroborated in **Fig. 3**.

3.2 Results of the experiment

The records of FOT, FCT, SAD and the sunlight intensity of each of 25 landraces exposed both to direct sunlight (S) and screened from sunlight under shade (mimicking cloudy daylight condition, C) are presented in **Supplementary Table S1**.

3.2.1 The effect of sunlight and shade on SAD and FED

Our experiment with the 25 populations exposed to sunlight (S) showed a median SAD of 276 min. (IQR = 41) and 308 min. (IQR = 72) under shade (mimicking cloudy condition, C). The distributions of SAD values in the two subpopulations are significantly different (Wilcoxon-Mann-Whitney $z = -2.62$, $p < 0.01$).

Conversely, the median FED of the S group cwas 71 min (IQR = 16), while that of the C group was 75 min. (IQR = 31.5). Wilcoxon-Mann-Whitney test cannot detect any difference between the groups ($z = 0.12$, $p = 1$). Permutation test with 10000 iterations confirms this finding, as summarized in **Table 3**.

The difference in the distribution of SAD in two different illumination regimes becomes visually prominent when the SAD of the C subpopulation is plotted against that of the S subpopulation of the same landraces (**Fig. 5A**). The SAD shows consistent lengthening when grown under shade, at <5000 lux. Conversely, the FED of the same landraces flowering in full sunlight and under shade shows no definitive trend (**Fig. 5B**).

3.2.*2 The relationship between SAD and FED in open sunlight and under shade*

Our experiment with a set of landraces exposed simultaneously to full sunlight (denoted hereafter as S) and under constant shade (denoted as C) afforded the possibility of finding a definitive relationship of FOT and FED with sunlight intensity. The rice panicles of the 'population C' received only diffused light from two sides, so the range of sunlight intensity

under shade (simulating the cloudy condition, C) was at least 2 orders of magnitude lower (min. 110 lux, max. 4568 lux) than the intensity of direct sunlight incident on the 'population S' (min. 35.7K lux, max. 347.6K lux) of each landrace examined. The C group was continually under shade, so the florets of this group opened as well as closed under shade. Thus, the comparison between these two groups essentially compares the effect of SS in the 'population S' on SAD and FED with that of CC condition of the 'population C' under shade.

The regression of FED on SAD for the S populations shows a strong (p <0.0005) inverse relationship (**Fig. 6A**), but the relationship between FED and SAD for the C populations (**Fig. 6B**) is not statistically significant. Furthermore, the extent of change in the FED is not proportionate to the change in SAD. The % difference in SAD between S and C of the same landraces does not show any unimodal correspondence with the respective difference (%) in FED (**Fig. 7**) of the landraces, nor is the relationship significantly different from zero (R^2 = 0.03, t = 1.17, p >0.1).

4. Discussion and Conclusions

Some generalizations may be drawn on the above findings, as described below.

4.1 In the present study of estimates of day light illuminance in a single season (*Aman* 2022), rice florets were found to open later on sunny days than in cloudy condition (**Fig. 2A**), although the difference between S and C conditions is not statistically significant (p > 0.8). This is in contrast with our previous study (Deb et al., 2023) showing a strong lengthening trend of SAD and shortening duration of FED among 1660 populations. The present study of *Aman* 2022 examines 120 C populations and 300 S populations, which constitute a subset of our previous study of 1660 populations in both long-day and short-day seasons over 3 years (Deb, 2022); hence, the paradoxical reversal of the association between S and C conditions for both SAD and FED in this present study is an example of Simpson's paradox, in which the association of variables in a data set may be reversed in one or more subsets (Sprenger & Weinberger, 2021). Moreover, except a small proportion of repetitions, the populations examined in S and C conditions do not belong to the same landraces. An examination of the same set of landrace populations in both S and C conditions would therefore be more conclusive. Our experimental study of two subpopulations of 25 landraces exposed to full sun

and under shade at flowering period corroborates our earlier generalization that SAD tends to significantly lengthen on overcast days (when solar illuminance is considerably low.

4.2 The FED is inversely related with FOT and SAD on both sunny and cloudy days, and this relationship is strong at $p < 0.0001$ (**Fig. 3**), corroborating our previous finding (Deb et al., 2023) using 1114 landraces, including those reported in the present study.

4.3 The inverse relationships of FOT and SAD with FED hold unaltered, irrespective of the sunlight intensity at FCT (**Fig. 4**), indicating that the sunlight intensity at the time of FCT is not crucial as it is at FOT. However, when the same landraces are examined in open sun and experimentally under shade, the association appears weak ($p > 0.05$) for the shaded populations (**Fig. 6B**).

4.4 The difference of the mean (and median) of SAD between the 25 experimental populations in open sunlight and under shade is strongly significant, whereas the difference of the means of FED between sunny and shaded conditions is null (**Table 3**).

4.5 The % change in FED (min) from S to C condition is not proportionate with that of SAD from S to C conditions of the same landrace populations (**Fig. 7**), as reflected in a weak regression ($p > 0.5$) of FED on SAD in the experimental populations (**Fig. 6B**). This weak association of SAD and FED under shade appears to confirm that a drastic experimental reduction in illumination on florets of the same landraces does not show any significant effect on FED (**Fig. 5B**).

4.6 The present findings enable us to present two following explanatory hypotheses.

4.6.1 The disappearance of the inverse relationship between SAD and FED under shaded condition (**Fig. 6B**) may be a result of the blocking of solar illuminance to which rice florets are sensitive. When unshaded, rice florets can receive polarized solar radiation even on cloudy days, which may elicit an appropriate floret closure response, so that a delayed FOT is associated with a shorter FED (**Fig. 3**; also see Deb et al., 2023). In contrast, under experimental shade, the open rice florets are deprived of direct solar cues, resulting in an indeterminate effect of shading on FED.

4.6.2 The unequivocal delay in FOT (or, lengthening of SAD) in deficient daylight condition (**Fig. 5A**) is likely an adaptation of the rice plant in anticipation of rainfall, for protection of the pollen from rainwash. This adaptive response of the rice floral florets to rainfall is further indicated by the facts that (a) rice florets hurry to close immediately after the first raindrop falls on the panicle (pers. observation), and that (b) on a rainy day, FOT is delayed till afternoon (Kobayasi et al., 2010; pers. Observation), even to the next morning (pers. observation). In fact, evidence from a large number of angiosperms confirms that "during the rainy season temporary floral closure would provide protection of the pollen against moisture due to rain, fog, or dew" (van Doorn and Kamdee, 2014: 5751). After a sufficient delay, only in the absence of rain, do the rice florets begin to open.

The published literature do not yet provide experimental proof for the hypothesis that FCT is likely induced by optimization of the dispersal of viable pollen. We can assert here, paraphrasing van Doorn and Kamdee (2014), that "the data do provide suggestions for further experimental work," which we have presemtly undertaken.

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Author Contributions

DD conceived and designed the study, conducted the experiment, analysed data, and prepared the MS. SD conducted major statistical analyses, and edited the MS.

Conflict of Interest

Authors declare that there is no conflict of interest.

Funding

This study received no institutional funding.

Data availability

The primary data pertaining to FOT, FED and SAD of 1660 landrace populations cultivated from 2020 to 2022 are openly available from Harvard Dataverse (Deb, 2023).
The records of illuminance during anthesis of 390 rice landraces examined here are available from Harvard Dataverse at (Deb, 2023). The phenological data of 25 landraces exposed to open sun and under shade are given in **Supplementary Table S1**.

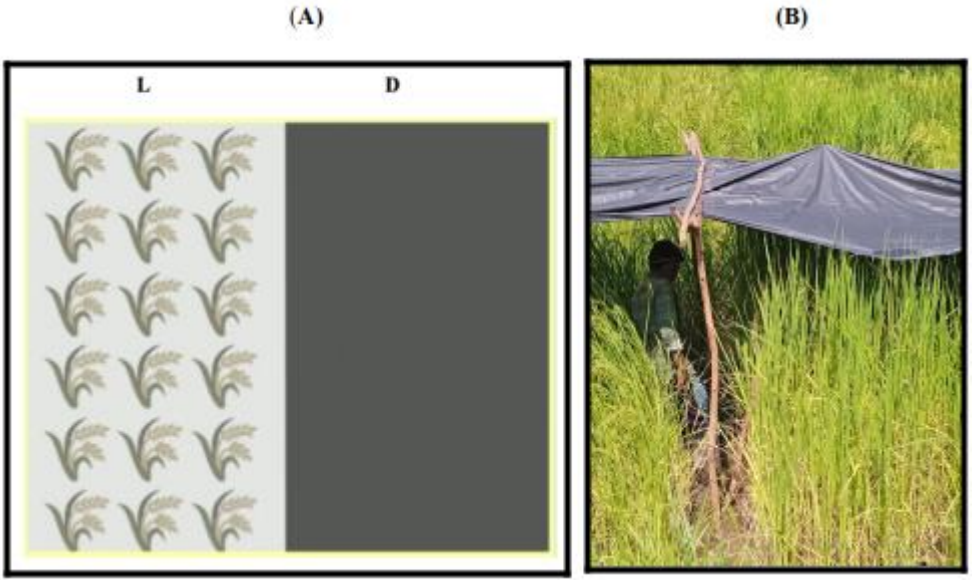

Fig. 1. (A) The Experiment with Illuminance. Half of each illustrative landrace population was exposed to direct sunlight (L), while the other half was kept in the dark (D) under opaque polythene sheet. (B) Reading lux meter next to a flowering panicle under the shade.

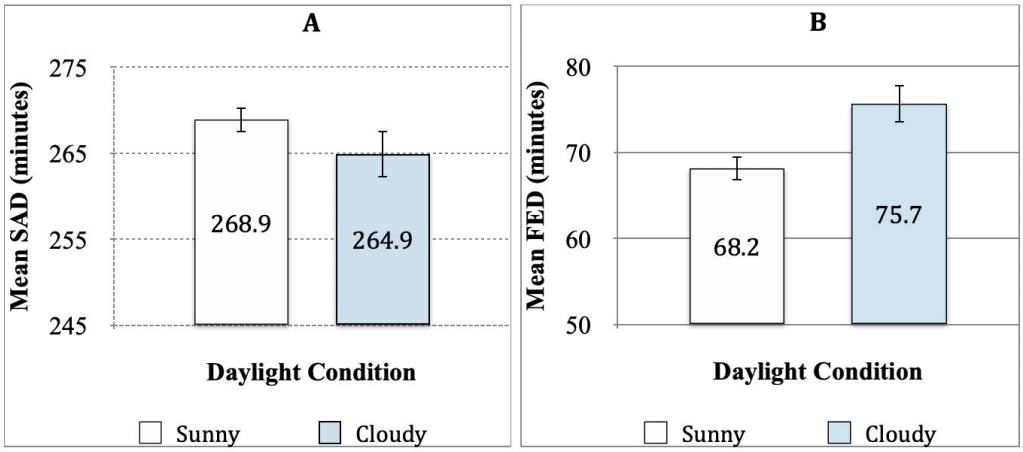

Fig. 2. Mean and standard error of **(A)** SAD (minutes) and **(B)** FED (minutes) on Sunny (*n* = 300) and Cloudy Days (*n* = 120).

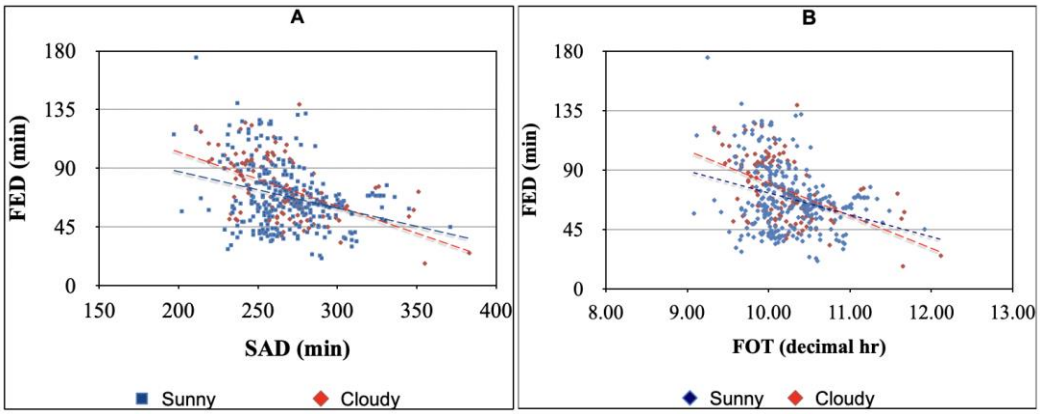

Fig. 3. The regression of FED **(A)** on SAD (for Sunny days, Slope $b = -0.28$, $R^2 = 0.09$, $t = 5.31$; for Cloudy days, $b = -0.42$, $R^2 = 0.27$, $t = 6.16$), and **(B)** on FOTd (on Sunny days, $df = 300$, Slope $b = -16.62$, $R^2 = 0.09$, $t = 5.28$; on Cloudy days, $df = 119$, Slope $b = -24.65$, $R^2 = 0.26$, $t = 5.96$).

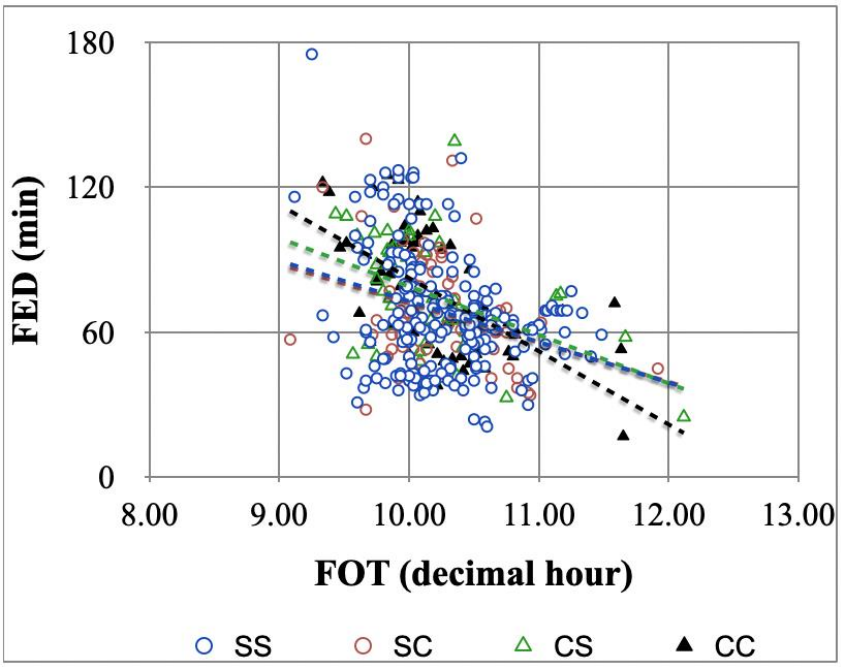

Fig. 4. Relationship of FED (minutes) with FOTd at different sunlight exposures during flower opening and successively on closing time (see text). The regression slopes of SS (n =224), SC (n = 76), CS (n = 54) and CC (n = 66) populations are : b = –16.8, R^2 = 0.09 (t = 4.53); b = – 16.2, R^2 = 0.09 (t = 2.63); *b* = – 20.0, R^2 = 0.19 (t = 3.39); and *b* = – 30.3, R^2 = 0.35 (t = 5.28), respectively.

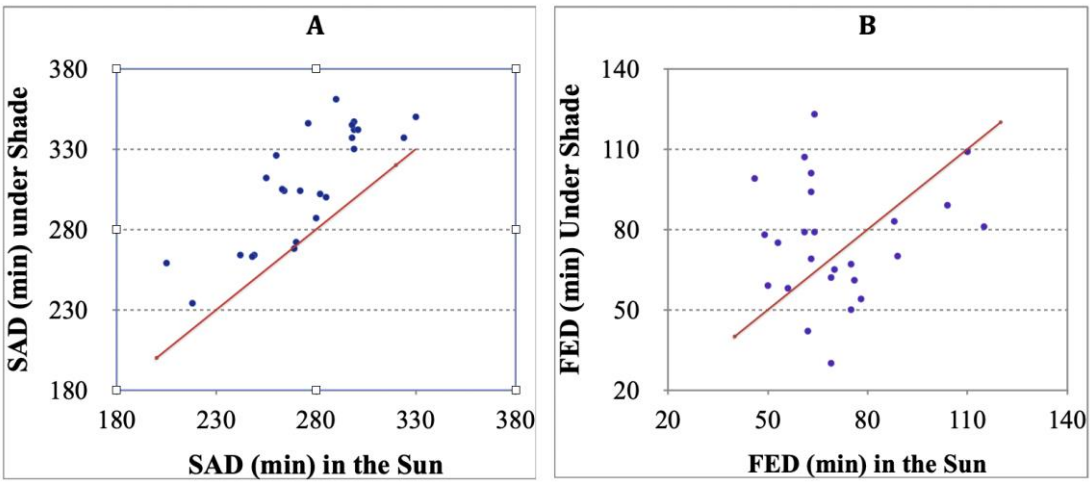

Fig. 5: A Comparison of (**A**) SAD and of **(B)** FED of 25 landraces flowering in open sunlight and under shade. The red line in each panel is the isocline.

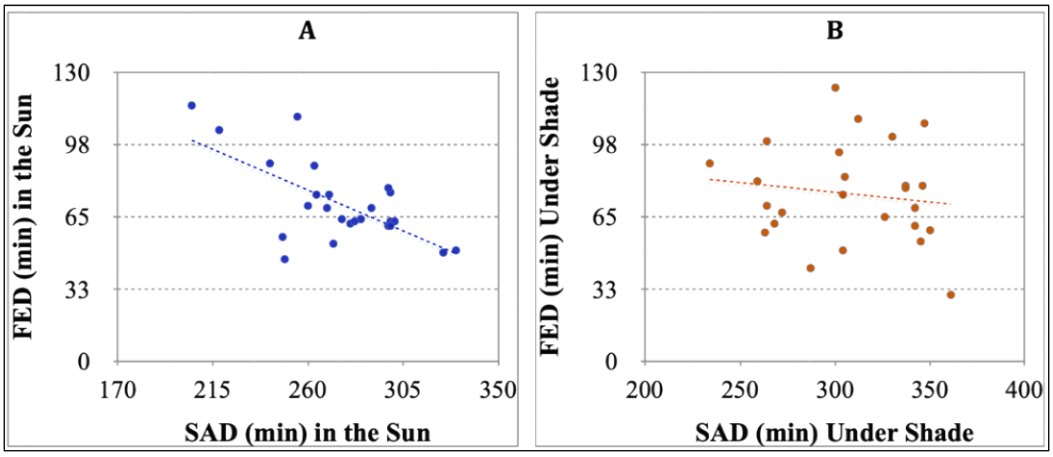

Fig. 6. Regression of FED on SAD of (**A**) sun-exposed (S) as well as (**B**) shaded (C) populations of 25 rice landraces. For S, $R^2 = 0.43$, $t = 3.73$, $p < 0.001$; and for C, $R^2 = 0.02$, $t = 0.69$, $p > 0.1$).

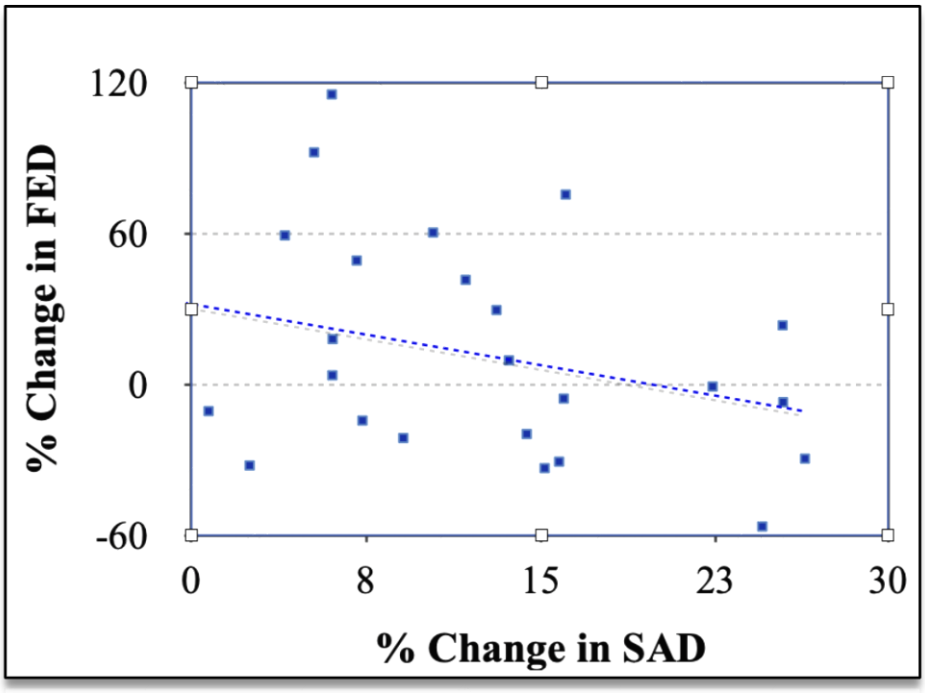

Fig. 7. Regression of % change in FED on % change in SAD of 25 landraces (with repetitions) between S and C conditions of daylight at FOT (*b* = – 1.62, R2 = 0.09, t = 1.49, p >0.07)

Table 1. Cultivation Seasons and Phenological Stages of 389 Landraces Examined.

Season	No. of Landraces (excl. repeats)	Sowing Dates	Flowering Dates (FD)	Dates of Harvest	No. of Populations (incl. repeats)
Aman 2022	390	21 Jun - 06 Jul	31 Aug - 27 Nov	25 Sep - 07 Feb	420

Table 2. Schema of recording of incident light intensity at FOT and FCT of 414 landraces with repeated observations.

FOT	No. of Observatios (No. of Landraces)	FCT	Category	No. of Observatios (No. of Landraces)
At Sunny hours (>20000 lux)	300 (273)	*At Sunny hour*	SS	224 (205)
		At Cloudy hour	SC	76 (68)
At Cloudy hour (≤20000 lux)	120 (117)	*At Sunny hour*	CS	54 (54)
		At Cloudy hour	CC	66 (63)
Total	420 (390)			420 (390)

Table 3. Statistical Tests of Significance between 25 pairs of Landraces Under Sunny and Shaded Conditions.

Variable	Test	*N*	Statistic	*p*	Bonferroni adjusted *p*
SAD-S vs SAD-C	Wlcoxon-Mann-Whitney test	25	$z = 3.24$	0.001	0.002
	Paired Permutation Test	10000	$\delta^{\wedge} = -825$*	<0.0001	<0.0001
FED-S vs FED-C	Wilcoxon-Mann-Whitney test	25	$z = 0.932$	0.357	0.714
	Paired Permutation Test	10000	$\delta^{\wedge} = 111$*	0.433	0.866

* $\delta^{\wedge}$ is the difference between the median values in sunny and cloudy conditions (see Methods)