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Effect of canopy thermal changes on pollen fertility and yield as influenced by time of planting and water stress imposed at reproductive phase of rice

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Abstract

Drought and heat stress at flowering stage affect rice yield. Hence, it is very important to understand the thermal changes of rice canopy during drought and heat stress conditions. Thus, objective of this study was to assess the thermal changes inside the canopy and its impact on pollen fertility and yield. A split plot design with three replicates was used for the field experiments at Field Crops Research and Development Institute, *Mahailluppallama* in three *Yala* seasons from *Yala* 2014 to *Yala* 2016. Main factor was the planting time (early and late planting) which synchronizes flowering at different temperature regimes. Sub-plots were Bg 358 flooded, Bg 358 drought stress, Bg 366 flooded and Bg 366 drought stress. Data were collected on ambient temperature, relative humidity, canopy temperature, volumetric moisture content of soil, pollen fertility and grain yield. The lowest canopy temperature was observed in early planting flood treatments. Maintaining volumetric moisture content at 27.5% with a canopy temperature of 30 ^{o}C resulted in 90% pollen fertility. Peak canopy temperature and canopy temperature at flower opening was highly correlated with grain yield. Peak canopy temperature and canopy temperature at 10:00 augmented the rice yield. The critical canopy temperature on yield was estimated as 34 ^{o}C. Maintenance of standing water at flowering stage is an important migratory option to avoid heat stress in rice.

Key words: Canopy temperature, Moisture stress, Planting time, Pollen fertility.

Introduction

Rice (*Oryza sativa* L.) is the staple food crop in Sri Lanka and two third of the cultivated extent is found in Dry and Intermediate zones. Air temperature is one of the important climatic factors that affects rice yield. Flowering stage of rice is highly sensitive to temperature stress (Satake and Yoshida, 1978; Jagadish *et al*., 2007). Increasing spikelet temperature, above 31^{o}C will gradually increase the pollen sterility and temperatures above 36 ^{o}C with high relative humidity (85 %) can completely sterile pollens of rice (Weerakoon *et al*., 2008). It was reported that temperature injuries occurred as temperature exceeded 35 ^{o}C during anthesis and reduced the size of the pollen and rice yield (Yoshida, 1981; Kumar *et al*., 2015). Further, the combined effect of temperature and water stress may be highly sensitive at flowering stage of rice. With the climate change, while temperature is increasing, irrigation water is also becoming a limiting factor. Under stress conditions canopy emits infrared waves and increases the canopy temperature (Fischer *et al*., 2003). Therefore, canopy temperature has been used as an indicator of moisture stress (Blum *et al*., 1989; Guentouz *et al*., 2012).

Normally air temperature in the dry zone increases beyond 35 ^{o}C from July to August (Weerakoon *et al*., 2009) and pollen sterility would be a common occurrence due to late planting. In open field, assessing of yield under heat stress is a difficult task. Canopy thermal data are highly precise than the weather station data (Fukuoka *et al*., 2012). A collaborative international research program was established to understand canopy temperature changes with heat stress in eight international locations (Yosihmoto *et al*., 2012) including Sri Lanka. Sri Lankan rice varieties were tested under heat and drought stress with the objective of realizing the thermal changes inside the canopy and its impact on pollen fertility and yield.

Materials and methods

Location

This study was conducted in 3 *Yala* seasons from 2014 to 2016 in the same field at the Field Crops Research and Development Institute (FCRDI), Mahailluppallama (8°06'13.73" N 80°27'55.33" E) located in the DL1b agro ecological region. Air temperature in Mahailluppallama area has been reported beyond 35 ^{o}C during late July to August (data not shown). Further, *Yala* season was found to be very dry in the particular location. Therefore, the location was highly suitable for heat and drought field experiments when flowering period is synchronized with critical temperature for anthesis of rice (35 ^{o}C) by adjusting planting time (to flower around 235 Julian days) and imposing drought by draining off irrigation water. Soil of the experimental site was Low Humic Gley soil associated with Reddish Brown Earth.

Experimental design and treatments

A Split plot design with 3 replications was adopted for the study in all three seasons. Main factor was planting time (early planting and late planting). Late planting was done by adjusting planting date to flowering synchronization around 235 Julian days and early planting was done 3 weeks before late planting. It was assumed that flowering stage of rice varieties could be exposed to a temperature regime, which is lower than the critical temperature by early planting and to equal or above critical temperature by late planting. Sub factor was two different varieties (Bg 358 and Bg 366), exposed to a two water stress levels (Flooded and drought stress). Drought stress was imposed by draining water out of plots at 50 days after planting (DAP) by observing the plant response according to IRRI, (1980) and soil dryness (Moisture level) in the soil was maintained beyond aerobic soil conditions (i.e. The volumetric moisture content was around 30 %). However, it was decided to further increase the stress up to a severe stress (volumetric moisture content was around 20 %) in *Yala* 2015 and 2016 by draining off water at 40 days after planting.

Land preparation and cultural practices

Research field was ploughed, harrowed and leveled as it is done for wetland rice cultivation. Size of the main plot was 26.5 m in length and width. Size of a sub plot was 5.5 m in width and 5.5 m in length. All sub plots were separated by 1.5 m cultivation free area and bunds were covered with 700 gauge polythene to prevent surface water movement across plots. Rice nursery was maintained as a wet bed nursery and transplanted at 15 days of age with 20 cm x 15 cm spacing. However, date of planting was adjusted to synchronize flowering period approximately on 215 and 235 Julian days as per the treatment.

Weed control was done with the application of Bispiribac Sodium (Nominee®). Urea was applied in four splits at two weeks interval as a top dressing at the rate of 225 kg/ha. Triple super phosphate (55 kg/ha) was applied as a basal dressing and Muriate of potash (60 kg/ha) was applied in 2 splits at 4^{th} and 6^{th} weeks after seed sowing. Experimental plots were maintained without pest and disease infections by applying recommended pesticides. All experimental plots were maintained under flooded condition up to 50 days after planting with few exceptions where water was drained out in the drought stress treatment at 40 days in *Yala* 2014.

Data collection

Air temperature and relative humidity data were collected from the nearby weather station at Mahailluppallama (500 m away from the experimental site) in 1 hour intervals within 24 hour cycle. Wind speed and direction did not consider for the study. During flowering period, temperature and humidity data were averaged in one hour intervals and the standard deviation was calculated. From late booting to end of flowering of the crop, canopy level temperature was recorded only in *Yala* 2014 and 2015 by using a Micrometeorological Instrument for Near Canopy Environment of Rice (MINCER), manufactured by National Institute of Agro Environmental Sciences, Japan. It was coupled with the Logistic control® V 0.09 data logger (Monota RO Co., LTD, Japan) in 3 minute intervals. These instruments were placed within the canopy in the middle of the plots. Recorded daily MINCER data during flowering were averaged in one hour intervals.

Soil moisture content of each treatment was monitored from panicle initiation to the end of stress period in 3 days intervals. When plants became stressed the monitoring interval was reduced up to 1 day interval (data at monitoring period were not shown). Soil samples were taken at a depth of 20 cm and oven dried at 105 ^{o}C for 48 hours up to a constant weight to measure gravimetric soil moisture content and converted to volumetric moisture content (VMC) by using bulk density.

Date of flower synchronization among in each variety was recorded for each planting time and stress condition. After flower synchronization, pollen fertility was recorded. When the crop reached a maximum drought stress, pollen samples were collected from previously selected panicles, which emerged on the same date. While collecting pollens, spikelets that were opened on the previous day as well as immature ones were removed. Remaining spikelets containing matured pollens were put into a 70%

ethanol solution. Pollen grains were stained with 1% Potassium Iodide and observed through the optical microscope (Smith *et al*., 2001). Number of sterile pollens and fertile pollens were counted and pollen fertility % was calculated.

Crop was manually harvested at physiological maturity where 85% of the spikelets had become golden brown from each treatment separately. Filled grains were separated and air dried up to 13% moisture and grain yield was recorded.

Data analysis

Percentage of pollen fertility (transformed into arc sine), volumetric moisture content and grain yield were analyzed by following analysis of variance procedure (ANOVA) and mean separation was done using Duncan's Multiple Range Test of SAS statistical software (V 9.1). Correlation and multiple regression analysis was performed to quantify the impact of soil moisture (VMC) and average canopy temperature during flowering period (from 09:00 to 14:00 in hourly intervals, because spikelet opening occurred during this period) on pollen fertility and grain yield. Based on the regression analysis a model was developed and the critical canopy temperature for yield of rice was estimated.

Results and discussion

Effect of temperature and humidity at flowering

Synchronized flowering among the varieties is important to compare the canopy temperature changes. According to Table 1, flowering was slightly delayed under stress condition compared to the flooded condition in 2015 and 2016 *Yala* seasons. Because of that, average temperature of flowering period could have slightly fluctuated whereas a short duration change would not have an impact on comparison of stress conditions.

Prasad *et al*. (2006) reported that, anthesis of rice occurs from 10:00 to 12:00 hours. However, there was a time difference of flower opening between Bg 358 and Bg 366 (field observation) but time of peak opening occurred within 10:00 to 12:00 hours.

Majority of the flowers opened simultaneously among the tested varieties. Early planting drought stress and flooded treatments were subjected to an ambient temperature higher than 32 ^{o}C (Figure 1) and a relative humidity of 52 to 70% (Figure 2) for both varieties. Maximum ambient temperature at early planting was 33.6, 34.0 and 34.7 °C in *Yala* 2014, 2015 and 2016, respectively. Maximum ambient temperature at late planting was 32.7, 35.1 and 33.4 °C in *Yala* 2014, 2015 and 2016, respectively (Figure 1).

Table 1. Flowering synchronization by planting time and soil moisture status

Season	Planting time	Soil moisture status	Synchronizing date (Julian days)		
			From	To	Number of days
Yala 2014	Early	flood	218	225	7
		stress	218	225	7
	Late	flood	232	238	6
		stress	232	238	6
Yala 2015	Early	flood	217	223	6
		stress	221	225	4
	Late	flood	241	245	4
		stress	243	247	4
Yala 2016	Early	flood	215	220	5
		stress	219	224	5
	Late	flood	234	240	6
		stress	237	243	6

Maximum temperature (35 °C) was higher than the critical temperature (Yoshida, 1981) in 2015 *Yala* season. Relative humidity at maximum temperature varied from 47 to 52 % in both planting times in all seasons (Figure 2). This humidity level could be considered as a low humidity level (Weerakoon *et al*., 2008, 2009), which would adversely affect on stigma receptivity, pollen germination and fertility.

According to the average ambient temperature during flowering period, temperature changing pattern at early planting and late planting was similar during 2014 and 2016 *Yala* seasons (Figure 1). Ambient temperature at early planting was higher than that of late planting, but late planting temperature was higher than early planting in 2015 *Yala* season. Wani *et al*. (2013) reported that increase in temperature greater than 2 °C beyond the critical level, reduced the rice yield by 0.75 t/ha. Increased air temperature from 0.1 to 0.5 °C, beyond the critical level, reduced the rice yield by 1-5 % in Sri Lanka (Vidanagae and Abeygunawardana, 1994). According to Figure 1, average temperature at the time of flower opening in early planting was higher than late planting by 0.7 and

0.9 ^{o}C in 2014 and 2016 *Yala* seasons, respectively. According to the ambient temperature data, it was concluded that considerable amount of ambient temperature differences occurred between early and late plant flowering.

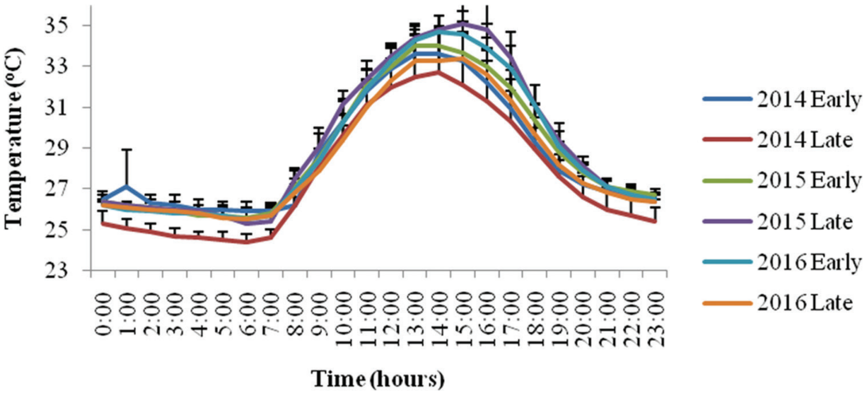

Figure 1. Ambient temperature changing pattern within a day of the whole flowering period of early and late planting during 2014 to 2016 *Yala* seasons (Error bar indicated by standard deviation)

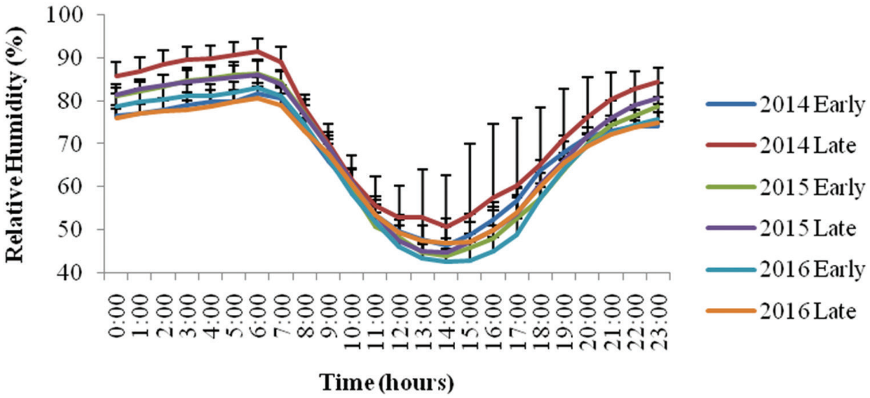

Figure 2. Relative humidity changing pattern within a day of the whole flowering period of early and late planting during 2014 to 2016 *Yala* seasons (Error bar indicated by standard deviation)

Effect of soil moisture during flowering

Soil moisture content under the flooded condition during 2014, 2015 and 2016 *Yala* varied from 36.2% to 48.1% (Table 2). This amount was higher than that of the stress treatments in 2014, 2015 and 2016 *Yala*. Therefore, plants were not undergone stress in flooded condition. In the stress treatment, soil moisture conditions of both varieties were below saturated to aerobic condition (mild stress) in 2014 *Yala* season because field capacity of the soil was around 21%. However, in 2015 and 2016 *Yala* seasons, both varieties in the stress treatments were under severe stress condition where VMC was 14 to 19% (Table 2). This was indicated by rice leaves, which were rolled up to "O" shape (O'Toole *et al*., 1979). Thus, it was evident that a severe stress condition prevailed in *Yala* 2015 and 2016.

Table 2. Soil moisture content during flowering period of Bg 358 and Bg 366 rice varieties under flooded and stress conditions

Season	Treatment	Volumetric moisture content of the soil (%)		
		Maximum	Minimum	SD
Yala 2014	Early Flood Bg358	43.4	40.2	±1.2
	Early Flood Bg366	42.0	37.4	±2.7
	Early Stress Bg358	30.2	28.1	±1.2
	Early Stress Bg366	29.2	26.1	±1.5
	Late Flood Bg358	44.2	39.0	±2.2
	Late Flood Bg366	42.1	38.8	±1.4
	Late Stress Bg358	32.5	28.9	±1.2
	Late Stress Bg366	31.9	28.2	±1.9
Yala 2015	Early Flood Bg358	36.2	33.6	±1.5
	Early Flood Bg366	38.5	37.1	±0.6
	Early Stress Bg358	19.0	10.0	±3.8
	Early Stress Bg366	18.0	8.0	±2.8
	Late Flood Bg358	36.2	32.5	±1.7
	Late Flood Bg366	48.1	45.6	±1.3
	Late Stress Bg358	14.5	10.0	±2.2
	Late Stress Bg366	15.6	7.5	±1.6
Yala 2016	Early Flood Bg358	42.2	37.2	±2.1
	Early Flood Bg366	43.5	39.3	±2.4
	Early Stress Bg358	14.4	14.3	±0.0
	Early Stress Bg366	19.2	16.4	±1.6
	Late Flood Bg358	41.2	39.7	±0.9
	Late Flood Bg366	42.8	39.8	±2.1
	Late Stress Bg358	17.4	14.5	±1.5
	Late Stress Bg366	17.3	15.0	±1.3

Note: SD - standard deviation

Effect of canopy temperature dynamics at panicle level

In general, as air temperature increased to a peak in the mid of the day, canopy temperature also reached a peak in both varieties (Figure 3A and 3B). Peak canopy temperature at panicle level had increased under heat and drought. Flooded condition recorded the lowest canopy temperature in both varieties. It was suggested that ambient temperature, humidity and soil moisture would be important environment factors affecting the degree of canopy temperature. Therefore, correct time of planting and ensuring standing water would be an important mitigatory option for maintaining canopy temperature at a lower level.

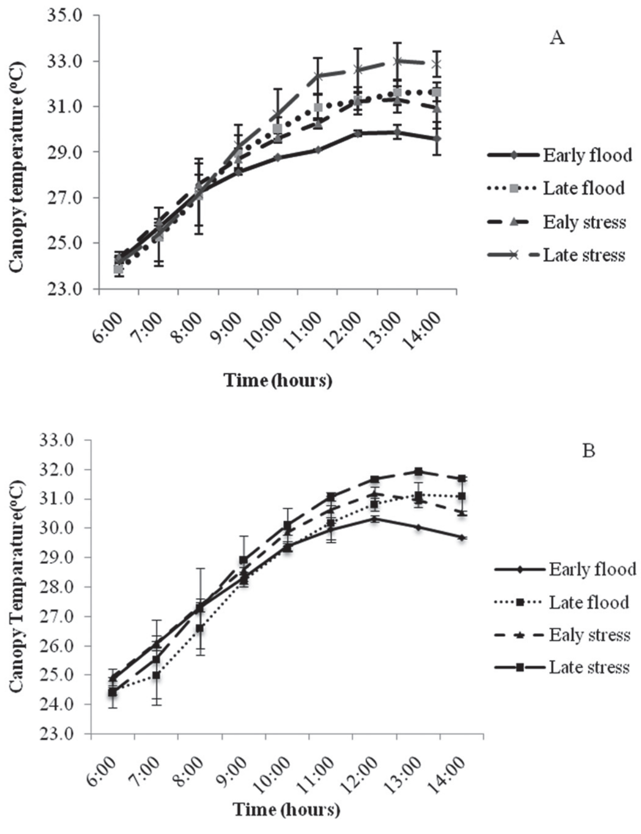

Figure 3. Canopy temperature of Bg358 (A) and Bg366 (B) under different treatments from 09:00 to 14:00 hours across seasons (error bars represent standard error)

Late planting, soil moisture and canopy temperature on Pollen fertility

Temperature and humidity data explained that, 32 ^{o}C ambient temperature and humidity around 70 % at flowering should have negative effect on pollen fertility. According to Weerakoon *et al*., (2008), pollen sterility had been recorded at 31.8 ^{o}C and it may probably be due to the spikelet cooling effect which, can be occurred due to the removal of water vapor from spikelet at moderate humidity. However, under water deficit conditions, this cooling mechanism would not work. Average pollen fertility percentage was significantly affected by late planting in 2014, 2015 and 2016 *Yala* seasons (Table 3). Degree of fertility was lower in 2015 *Yala* season because air temperature at the time of floret opening was comparatively higher (33.4 ^{o}C) and the maximum temperature (35.1 ^{o}C) was also higher than the critical temperature.

Table 3. Effect of planting time, varieties and moisture stress on pollen fertility % in different seasons.

Treatment	*Yala* 2014 Average ± SE	*Yala* 2015 Average ± SE	*Yala* 2016 Average ± SE
Main plots			
Early planting	92.0 ± 1.7 a	79.5 ± 1.0 a	88.3 ± 2.7 a
Late planting	84.6 ± 2.4 b	75.8± 1.91 b	81.3 ± 2.1 b
Pr>0.05	0.007	0.03	0.69
Sub plots			
Flood Bg358	87.8 ± 2.7 a	88.1 ± 1.4 b	91.6 ± 3.0 a
Stress Bg358	82.3 ± 2.7 b	62.7 ± 1.6 c	72.4 ± 3.8 b
Flood Bg366	92.6 ± 2.7 a	92.3± 1.6 a	94.5 ± 3.0 a
Stress Bg366	87.6 ± 2.7 b	67.5 ± 1.4 c	72.8 ± 3.8 b
Pr>0.05	0.08	<0.0001	<0.0001
Pr>0.05 (planting time x treatment)	0.79	<0.0001	0.19
CV (%)	11.7	15.4	12.4

Note: means within a column with same letters are not significantly different at 5 % probability, (SE - standard error)

Impact of drought stress on pollen fertility was significantly different in three seasons (Table 3). Interaction effect of time into treatment was significant only in 2015 *Yala* season and therefore, it was not considered in interpreting data. In general, intensity of incidence radiation at 09:00 hour increased comparatively and spikelet opening also occurred at the same time. Temperature at spikelet opening as well as up to three hours from spikelet opening is important for high temperature stress induced spikelet sterility

(Satake and Yoshida, 1978). Therefore, canopy temperature at 09:00 to 13:00 (peak temperature) hours were critical. Relationship between volumetric moisture content and pollen fertility of Bg 358 and Bg 366 was significantly correlated at p>0.0001 (r^2 = 0.60 and 0.74, respectively). Change of pollen fertility with increasing soil moisture content clearly showed the curvilinear relationship. Results of regression analysis on soil moisture given in Table 2, canopy temperature and pollen fertility explained that maintaining the soil moisture at 27.5% (VMC) and canopy temperature less than 30 ^{o}C could be ample to obtain a pollen fertility of more than 90 % in both varieties.

Late planting, soil moisture and canopy temperature on grain yield

Grain yield significantly decreased by late planting in 2014, 2015 and 2016 *Yala* seasons (Table 4). Further, soil moisture condition significantly affected the yield in both varieties. Interaction effect on time into treatment was significant (Table 4). However, according to Table 5, pattern of canopy temperature change among the stress conditions could have inverse relationship with grain yield changes.

Table 4. Grain yield of tested varieties as affected by time of planting moisture stress

Treatment	*Yala* 2014 Average ± SE	*Yala* 2015 Average ± SE	*Yala* 2016 Average ± SE
Main plots			
Early planting	3.1±0.1a	3.8± 0.07a	4.2± 0.0.2a
Late planting	1.6 ± 0.1b	2.4 ±0.07b	2.9± 0.2b
Pr>f (0.05)	<0.0001	<0.0001	0.0001
Sub plots			
Flood Bg358	3.0±0.1a	4.0± 0.2 a	5.1 ±0.2a
Stress Bg358	2.4±0.1b	3.0 ±0.2b	2.2 ±0.3b
Flood Bg366	2.1±0.1 a	2.8± 0.2c	5.1±0.1 a
Stress Bg366	1.9 ±0.1c	2.3± 0.2d	2.0 ± 0.3 b
Pr>f (0.05)	0.0001	<0.0001	<0.0001
Pr>f (planting time x treatment)	0.009	0.0001	0.002
CV (%)	15.1	12.5	14.8

Note: means within a column with same letters are not significantly different at 5 % probability. (SE - standard error)

Late planting significantly decreased the grain yield of both varieties in different rates (Table 4). Both time of planting (the temperature effect) and soil moisture stress significantly decreased yield of all varieties (Figure 4). However, the presence of a significant interaction effect confirmed that, the proportion of grain yield decreased with the time of planting among the moisture stress and the tested varieties. Grain yield of 2014 and 2015 *Yala* seasons were comparatively lower than that of 2016 *Yala* season. However, yield reduction pattern among the treatments are same as the 2016 *Yala* season in both rice varieties.

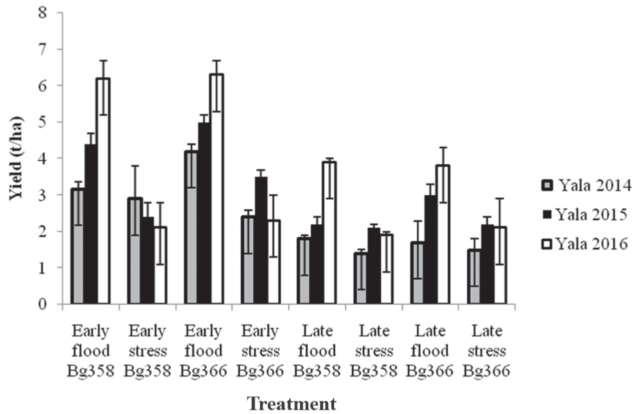

Figure 04. Change of grain yield in different treatments in early and late planting, (error bars indicated by standard error)

According to Figure 4, grain yield of early planting stress was lower than the yield of early planting flooded Bg 358 and Bg 366. However, yield reduction due to late planting with flooding compared to early planting with flooding was comparatively low in both Bg 358 and Bg 366. However, yield reduction of Bg 358 was greater than Bg 366. It could be suggested that the impact of soil moisture stress was greater than the temperature stress in the variety Bg 358 in comparison to Bg 366 because yield under early planting stress was higher than late planting under flooding in Bg 358.

Impact of canopy temperature on grain yield

The relationship between average canopy temperature dynamics of flowering period (Figure 3) and grain yield was estimated using correlation and multiple regression analysis. It was revealed that rice yield was positively correlated with canopy temperature at 10:00, 11:00 and 12:00 (correlation coefficient was 0.65, 0.81 and 0.93, respectively at P= 0.007, 0.0002 and <0.0001) whereas it was negatively correlated with the peak canopy temperature (r= -0.80, P= 0.003). Canopy temperature is a relative indicator of canopy transpiration. When canopy temperature reaches a maximum, transpiration may reach a minimum due to stomatal closure. Consequently, photosynthesis would also decrease. Further, canopy temperature indicates the level of stress of the plant (Guendouz *et al*., 2012). When canopy temperature becomes maximum, the grain yield is negatively affected. Thus, canopy temperature at spikelet opening has a strong impact on yield of rice. Multiple regression analysis revealed that among the canopy temperatures (CT) at 10:00, 11:00, 12:00 and peak canopy temperature (CT_{13pm}), there was a significant negative relationship on CT_{13pm} and positive relationship on canopy temperature at 10:00 (CT_{10am}) with a model r^2 of 0.81 (Table 5). Further, it was suggested that the increase in canopy temperature during 10:00 hour up to 30 ^{O}C would increase rice yield. Therefore, the following model (Formula 1) was worked out from multiple regression analysis.

$$Yield = -1.45CT_{peak} + 0.87CT_{10} + 23.2 \quad (1)$$

Table 5. Parameter estimate and significance of hourly canopy temperature at flowering and peak canopy temperature using multiple regression analysis.

Canopy temperature	Parameter estimate	F value	Pr>f
CT_{13pm}	-1.45	49.5	<0.0001
$CT10_{am}$	0.87	10.7	0.006
Intercept	23.3	15.1	0.002

Using peak canopy temperature data (Figure 3), regression analysis estimated that no rice yield could be obtained at a canopy temperature of 34 ^{o}C. This analysis showed that, the slope of the curve was -1.009 and intercept was 34.45 at a regression coefficient of 0.65. Therefore, it could be suggested that, critical canopy temperature for rice yield would be 34.2 ^{o}C. Therefore, it is important to maintain canopy temperature below 34.2 ^{o}C during 12:00 to 14:00 hours and it is important to find out mitigatory options to maintain the canopy temperature below 34.2 ^{o}C. However, within canopy humidity condition and canopy transpiration rate were not considered in this study but they should be studied in future experiments.

Conclusion

Canopy temperature is a combination effect of ambient temperature, relative humidity, soil moisture and/or standing water. Late planting water stress increased the canopy temperature. It has a negative impact on pollen fertility and grain yield. Maintaining soil moisture at 27.5% of volumetric moisture content and canopy temperature less than 30 ^{o}C could be sufficient to obtain a pollen fertility of more than 90%. Further, canopy temperature during 10:00 and canopy temperature at maximum temperature (13:00 hour) highly influenced rice yield. Critical canopy temperature for yield was estimated at 34.0 ^{o}C. It was suggested that maintaining standing water during flowering and early planting is needed to maintain the canopy temperature below the critical level.

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