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Research Paper

Influence of Flag Leaf Characteristics on Pollen Sterility of Rice (*Oryza sativa* L.) under Heat Stress

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Abstract: Late flowering-affected spikelet sterility was found in dry zone of Sri Lanka. It was defined as a combination effect of air temperature and relative humidity at anthesis. It is considered important to develop mitigation options to avoid temperature stress. Therefore, the impact of some phenotypic markers on pollen sterility was studied. This paper highlights flag leaf characteristics regulate the direct sunlight penetration and augment the spikelet surface temperature. A field trial was conducted at the Field Crops Research and Development Institute, Mahailluppallama, Sri Lanka during 2013/14 and 2014/15 *Maha* seasons (main cultivating season; October to February). Due to water shortage, this experiment was not conducted in the *Yala* season (March to September). A split plot design was adopted with early and late planting as a main factor and two rice (*Oryza sativa* L) varieties (Bg358 and Bg366) and two rice lines (Bg3171 and Bw03-1198) as a sub factor, in a two factor factorial arrangement. Flag leaf characters at late booting stage, daily maximum air temperature and relative humidity during anthesis were recorded. Spikelet surface temperature and pollen sterility were recorded throughout the flowering period. Spikelet temperature changed with air temperature. While synchronizing high temperature in late flowering, the spikelet temperature increased. However, significant temperature changes were occurred only in 2014/15 *Maha* season. Pollen sterility was affected by late planting with genotypic variations. Flag leaf length and area correlated (R^2 = -0.79 and -0.78 for pollen fertility and -0.74 and -0.75 for spikelet temperature) with spikelet surface temperature and pollen sterility. The best combinations of flag leaf characters to obtain the maximum canopy cooling with lesser spikelet temperature in 85 cm^2 of flag area and 55.0 cm of flag leaf length. Therefore, it is better to select rice varieties having longer flag leaf than panicle with lesser leaf temperature for variety development programmes aimed at temperature stress.

Keywords: Canopy cooling, Flag leaf characters, Pollen sterility, Spikelet temperature

Introduction

Dry Zone is more vulnerable for climate change in Sri Lanka. The maximum temperature in Sri Lanka has increased annually at a rate of 0.026 °C (Eriyagama *et al.*, 2010). Literature explains that temperature more than 36 °C under high relative humidity (near 85 %) has an effect on evaporative cooling of spikelet and pollen sterility, and thus the subsequent yield losses (Abeysiriwardhana *et al.*, 2002; Weerakoon *et al.,* 2008). When temperature increases by 2 to 4 °C estimated yield of rice was reduced by 13.3 % and 23.3 % in India (Rani and Maragatham, 2013). Therefore, temperature and heat stress will have an impact on rice production in the future.

It is thus, important to find out mitigation options to reduce heat stress owing to expected high temperatures in Sri Lanka. Development of appropriate plant architecture of rice crop to reduce heat stress is an existing requirement. Many scientists were found phenotypic markers such as panicle surrounded by many leaves (Shah *et al.,* 2011), semi dwarf varieties (Wassmann *et al.,* 2009a) and early morning flowering (Ishimaru *et al.,* 2010, 2012) to reduce heat stress. However, literature on the influence of flag leaf characteristics and tiller angle on pollen sterility are meagre. Therefore, a study was conducted with the objective of assessing the influence of flag leaf characteristics and tiller angle on pollen sterility in direct seeded rice in Sri Lanka.

Materials and Methods

Location, seasons and experimental design

A field trial was conducted at the Field Crops Research and Development Institute, Mahailluppallama, Sri Lanka during 2013/14 and 2014/15 *Maha* seasons (main cultivating season; October to February). The *Maha* has higher water availability and high air temperature at late flowering. A two-factor factorial experiment was conducted in a split plot design with four replicates. Size of the sub plot was 5 m length x 3 m width. The main factor was early and late planting, to synchronize flowering of the crop at comparatively low and high maximum air temperatures separately. Sub factor was rice (*Oryza sativa* L) varieties namely Bg358, Bg366 and two promising rice lines selected from National Coordinated Rice Varietal Test (NCRVT) namely, Bg3171 and Bw03-1198. The selected varieties/lines were morphologically different in flag leaf characteristics. Flowering time was set to synchronize low and high maximum air temperatures by early and late planting, respectively, based on meteorological data at Mahailluppallama.

Cultural practices, data collection and analysis

Basal fertilizer was added at the final land preparation as per recommendation of the Department of Agriculture (DOA). Seed rate was adjusted based on 1000 grain weight of the varieties and pre-germinated seeds were direct seeded on well prepared plots. Weed control was done by application of Bispyribac sodium at the recommended rate using a 16 litre calibrated Knapsack sprayer. Top dressing of urea and muriate of potash were applied as per the DOA recommendations. Crop was maintained without any water, pest and disease stresses.

The maximum air temperature and maximum relative humidity (RH) of the day, relative humidity (RH) at the maximum temperature, ambient temperature and RH at pollen collection were obtained from the automatic weather station (Watch Dog model ET 2900®), which was located 500 m away from the experimental site. All experimental data were collected from five samples in each treatment per replicate. The flag leaf characteristics and tiller characteristics recorded at late booting or heading stage were; tiller angle (angle from main culm to primary tiller), flag leaf angle (angle between leaf blade and main axis of the panicle at initial heading), flag leaf length (cm), flag leaf width (mm) and flag leaf area (cm^2) and panicle length (cm). The surface temperature (°C) of the flag leaf was also recorded at 13:00 h at two days intervals using an infrared thermometer (Extech®, China). The infrared thermometer was placed opposite to the direction of the sun at a 30° angle from the horizontal plane and 25 cm distance from the spikelet/leaves.

Spikelets were collected at two-day intervals for 6 days during flowering in a 70 % ethanol solution. Pollen grains were stained in KI (Smith *et al*., 2001) and pollen sterility % was recorded. Each plot was harvested at the physiological maturity and seeds were air dried to 13 % moisture content. Yield was measured and converted to t/ha. The percentage values of pollen sterility were analyzed after arcsine transformation. All quantitative data including flag leaf characteristics of 2014/15 *Maha* (because a significant temperature difference occurred in 2014/15 *Maha*) and tiller angle were analyzed using SAS statistical software, and mean separation was done using DMRT. Correlations were analyzed between flag leaf characteristics, tiller angle, spikelet surface temperature, leaf surface temperature and pollen sterility in late planting treatment.

Results and Discussion

<u>Change of ambient temperature and RH</u>

The maximum ambient temperature at day before pollen collection of early flowering and late flowering in 2013/14 *Maha* was marginally different but higher than 32 °C (Table 1). However, the maximum air temperature day before pollen collection during late flowering of 2014/15 *Maha* was higher. The difference of temperature between the early and late flowering was 2.7 °C. The maximum relative humidity (%) at day before pollen collection was always higher in both *Maha* seasons. However, at the time of maximum temperature, the relative humidity was low; around 40 to 50 %. Further, the ambient temperature difference between early flowering and late flowering at the time of pollen collection was 2.2 °C in 2014/15 *Maha* season but closer to30 °C. The RH % was moderate at the time of pollen collection in early and late flowering of both *Maha* seasons (Table 1).

Table 1. The maximum ambient temperature day before flowering, ambient temperature at pollen collection, the maximum relative humidity at day before flowering and relative humidity at pollen collection of 2013/14 *Maha* and 2014/15 *Maha* seasons.

Parameter	2013/14 *Maha*		2014/15 *Maha*	
	Early flowering	**Late flowering**	**Early flowering**	**Late flowering**
Maximum ambient temperature day before flowering (°C)	33.8	33.6	31.4	34.1
Relative humidity (%) at the maximum temperature	39.9	42.1	48.2	43.3
Maximum relative humidity (%) day before flowering	98.6	94.1	98.4	97
Ambient temperature at pollen collection (°C)	28.2	29.3	27.4	29.6
Relative Humidity (%) at pollen collection	63.3	59.3	60.1	61.7

The clear relationship between the ambient temperature and pollen fertility has been recorded (Yoshida, 1981). High temperature stress during anthesis was the most detrimental (Satake and Yoshida, 1978). The optimum temperature range for normal growth of rice is 22 to 32 °C (Yoshida, 1973) and the threshold temperature of rice during anthesis is 33.7 °C (Jagadish *et al.,* 2007). Further, under open field condition, pollen sterility has been recorded at 31 ± 0.8 °C (Weerakoon *et al.,* 2009).

Under moderate humidity (around 60 %), water vapor move from spikelet to environment and reducing the temperature stress due to transpiration cooling of spikelet thus increasing the pollen viability (Weerakoon *et al.,* 2008). Moderate to high RH help producing the required pressure for the split of septum and increase pollen deposition on stigma (Weerakoon *et al.,* 2008). During very low RH (35-40 %), loss of pollen viability or reduction in pollen germination could occur due to desiccation of the stigma surface (Abeysiwardena *et al*., 2002).

According to the climate data recorded during the experimental period, high temperature differences at anthesis has occurred between early and late flowering in 2014/15 *Maha* season. Therefore, pollen sterility, spikelet temperature, grain yield, flag leaf characteristics including flag leaf surface temperature, tiller angle of 2014/15 *Maha* season will be further discussed in this paper.

Pollen sterility % and spikelet surface temperature

Late planting was significantly increased the pollen sterility and spikelet surface temperature. Interaction of planting time on varieties was significantly different (Table 2).

Table 2. Effect of planting time and varieties on pollen sterility spikelet surface temperature and grain yield of rice.

Treatment	Pollen sterility (%)	Spikelet surface temperature (°C)	Grain yield (t/ha)
Time of Planting			
Early planting	2.6 b	29.4 b	5.1 a
Late planting	27.0 a	33.4 a	4.2 b
Variety			
Bg358	25.0 a	32.7 a	3.8 b
Bw03-1198	21.2 a	32.0 b	4.0 b
Bg366	7.3 b	31.0 c	5.7 a
Bg3171	0.4 c	29.7 d	5.0 a
Time of planting *x* Variety	*	*	*
CV %	7.8	1.5	10.7

Means followed by the same letters are not significantly different at p=0.05. * = significantly difference at p=0.05

Spikelet surface temperature of rice plants in the late planting treatment was higher ($p<0.05$) than in the early planting plots. However, an increasing trend of spikelet temperature was the same in Bg358 and Bw03-1198 and the highest was recorded in Bg358. There was a significant interaction between Bg366 and Bg3171 and the planting time. The lowest spikelet surface temperature was recorded in Bg3171 in both planting times (Figure 1).

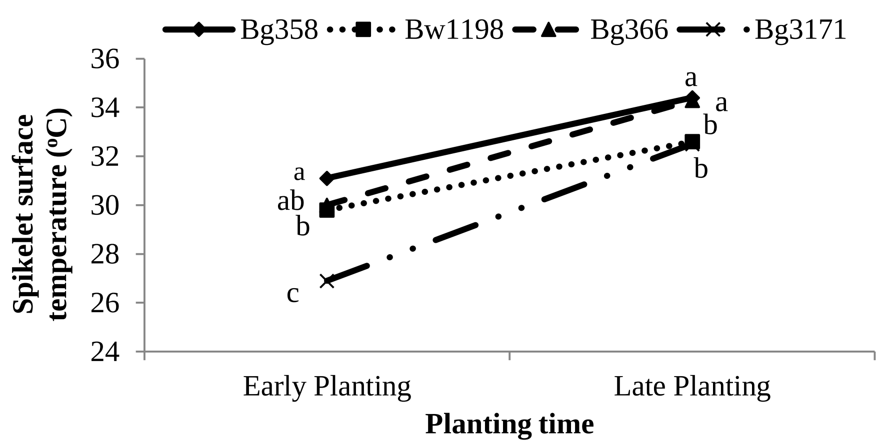

Figure 1. Influence of early planting and late planting on spikelet surface temperature in the 2014/15 *Maha* season. (within a planting time, symbols with same letters are not significantly different at p=0.05)

Further, pollen sterility increased in all tested varieties. However, the rate of increase in pollen sterility varied among the varieties. The pollen sterility of rice varieties Bg366 and Bg3171 increased at a decreasing rate. While that in Bg358 and Bw03-1198 increased at an increasing rate with the interaction with planting time. The highest pollen sterility was recorded in the rice variety Bg358 (Figure 2).

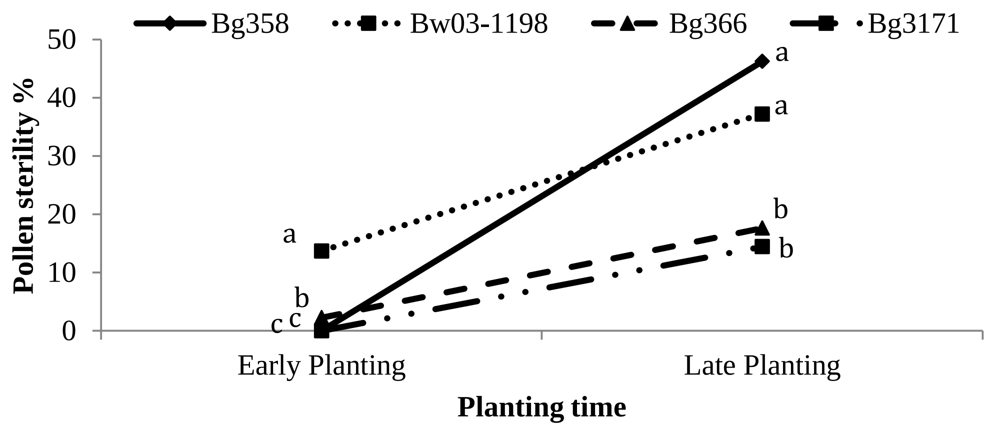

Figure 2. Influence of early planting and late planting on pollen sterility in the 2014/15 *Maha* season (Within a planting time, symbols with same letters are not significantly different at p=0.05).

Late planting made the rice plants exposed to an increased maximum ambient temperature compared to that of early planting. Relative humidity was low (Table 1) at the time of maximum temperature in day before pollen collection. According to Abeysiriwardena *et al.* (2002) and Weerakoon *et al.* (2008, 2009), the effect of increasing temperature and moderate relative humidity may vary among the genotype, but the ultimate result was different rate of transpiration from spikelet surface. Further, a low RH may result in loss of pollen viability and pollen germination due to desiccation of stigma surface (Abeysiriwardena *et al.*, 2002).

Low humidity of the surrounding environment and rate of transpiration of the variety may decide the spikelet temperature. In field condition, the maximum air temperature and low RH may govern the spikelet temperature. However, the ambient temperature at pollen collection (just before anthesis) increased as a result of late planting but the value was lower than the critical level with a moderate RH. Therefore, results of this study suggested that, at day before pollen collection, the maximum temperature and low RH at a particular hour may have an effect on pollen sterility in both planting times. However, a significantly higher temperature experienced due to late planting of rice has increased the pollen sterility.

In future, a high maximum air temperature and high temperature at the time of pollen collection will further aggravate the pollen sterility due to increasing spikelet surface temperature. Moreover, moderate relative humidity at pollen collection could also increase the spikelet cooling and reduce the degree of pollen sterility.

<u>Grain yield</u>

Late planting significantly decreased the grain yield of rice. Interaction of planting time and varieties were significantly different (Table 2), and the degree of interaction was variety dependent. The grain yield of Bg366 did not change with the planting time. Grain yield reduction of Bg3171 was low compared to Bg358 and Bw03-1198 (Figure 3). However, varietal response of yield on late planting was proportionate with pollen sterility. Results revealed that, amount of pollen sterility significantly impact on grain yield in existing environment conditions depending on the variety.

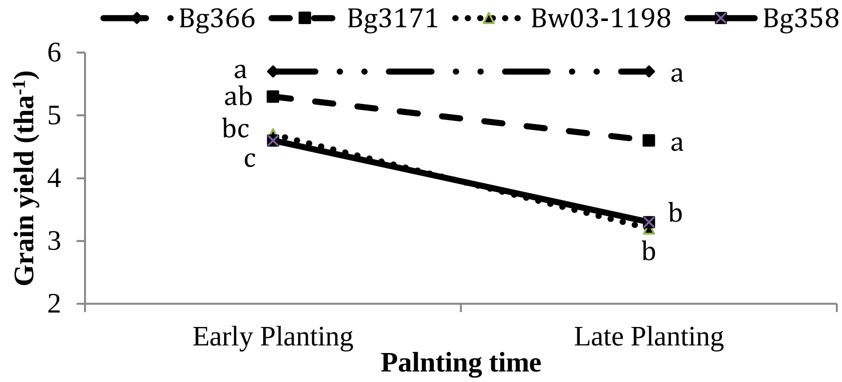

Figure 3. Influence of early planting and late planting on grain yield of rice in the 2014/15 *Maha* season Within a planting time, symbols with same letters are not significantly different at p=0.05).

Flag leaf characteristics of rice varieties

Planting time significantly affected the flag leaf surface temperature, tiller angle, flag leaf angle, flag leaf length and flag leaf width. The tiller angle, flag leaf surface temperature and flag leaf width increased ($p<0.05$) with increased temperature in the late planting conditions. High temperature experienced by rice plants due to late planting decreased ($p<0.05$) the flag leaf angle and flag leaf length. However, flag leaf area was not affected due to time of planting ($p>0.05$). Further, interaction of planting time on varieties affected ($p<0.05$) the tiller angle, flag leaf angle, flag leaf length, flag leaf temperature and flag leaf area (Table 3).

The optimum temperature for normal development of rice is 27 to 32 °C (Yin *et al*., 1996). Further, effect of temperature stress vary with cultivar and temperature injuries may occur at more than 35 °C (Yoshida, 1981). Late planting in the present study coincided with environmental temperature exceeding the optimum for normal development, but was lower than 35 °C. Our results suggested that there is a significant impact of temperature changes on tested flag leaf characteristics except flag leaf area, due to different planting times in all rice varieties tested.

Table 3. Influence of planting time on tiller angle, flag leaf angle, flag leaf length, flag leaf width and flag leaf area of tested varieties in the 2014/15 *Maha* season

Treatment	Leaf temperature (°C)	Tiller angle º	Flag leaf angle º	Flag leaf length (cm)	Flag leaf width (mm)	Flag leaf area (cm^2)
Time of Planting						
Early planting	30.4 b	7.1 b	5.7 a	29.3 a	17.3 b	38.3 a
Late planting	32.2 a	10.1 a	4.7 b	27.3 b	18.0 a	37.3 a
Variety						
Bg358	27.6 c	10.0 a	5.3 b	26.6 b	16.9 b	34.1 b
Bg366	32.8 a	7.3 c	6.3 a	33.3 a	19.2 a	47.9 a
Bg3171	33.3 a	7.0 bc	4.2 c	29.1 b	17.3 b	38.1 b
Bw03-1198	31.5 b	9.2 ab	5.2 b	26.2 b	17.5 b	34.7 b
Time of Planting *x* Variety	*	*	*	*	ns	*
CV %	2.7	12.2	7.0	7.0	4.3	8.9

Means followed by the same letters are not significantly different at p=0.05. * = significantly difference at p=0.05, ns = non-significant (p=0.05)

Spikelet surface temperature positively correlated with pollen sterility. However, flag leaf length, flag leaf temperature and flag leaf area were negatively correlated with pollen sterility (Table 4). Thus, among the tested characteristics, flag leaf area and flag leaf length negatively affected on spikelet surface temperature. Spikelet temperature was the leading factor for the pollen sterility. Therefore, it is important to discuss interaction effect of planting time on flag leaf length, flag leaf temperature and flag leaf area.

Therefore, increased flag leaf length and area under high temperature due to late planting was observed in Bg3171. The Bg366, Bg358 and Bw03-1198 showed decreased flag leaf length (Figure 4) and flag leaf area (Figure 5) under increased temperature owing to late planting.

Table 4. Correlation matrix at late planting.

	TA	FLAng	FLL	FLW	FLA	PSt	SST	LT
TA	1	-0.25 ns	-0.17 ns	-0.26ns	-0.23 ns	0.19 ns	0.33 ns	0.17 ns
FLAng		1	0.09 ns	0.14 ns	0.13 ns	0.11 ns	-0.02 ns	-0.23 ns
FLL			1	0.53 ns	0.96 **	-0.79 *	-0.74*	0.06 ns
FLW				1	0.72 *	-0.48 ns	-0.52 ns	0.18 ns
FLA					1	-0.78 *	-0.75 *	0.09 ns
PSt						1	0.83 **	-0.61 *
SST							1	-0.32 ns
LT								1

* = significantly different at p=0.05, ** = significantly different at p=0.005. ns = non-significant (p=0.05); TA = tiller angle. FLAng = flag leaf angle, FLL = lag leaf length, FLW = flag leaf width, FLA = flag leaf area, PSt = pollen sterility, SST = spikelet surface temperature; LT = leaf temperature.

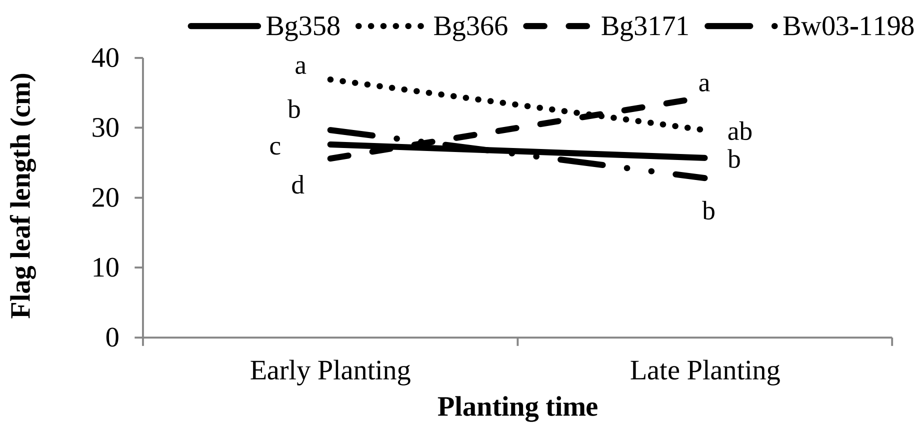

Figure 4. Influence of early and late planting on flag leaf length of tested rice varieties season (Within a planting time, symbols with same letters are not significantly different at p=0.05).

Flag leaf surface temperature increased in Bg358 and Bw03-1198 with increased ambient temperature owing to late planting. However, flag leaf surface temperature of Bg366 and Bg3171 did not significantly change with increased temperature. The lowest flag leaf temperature was always recorded in Bg358 (Figure 6). Leaf temperature is a transpiration related measurement (Belko *et al.*, 1978). An inverse relationship between leaf surface temperature and leaf transpiration was recorded in rice (Hirayama *et al.*, 2006).

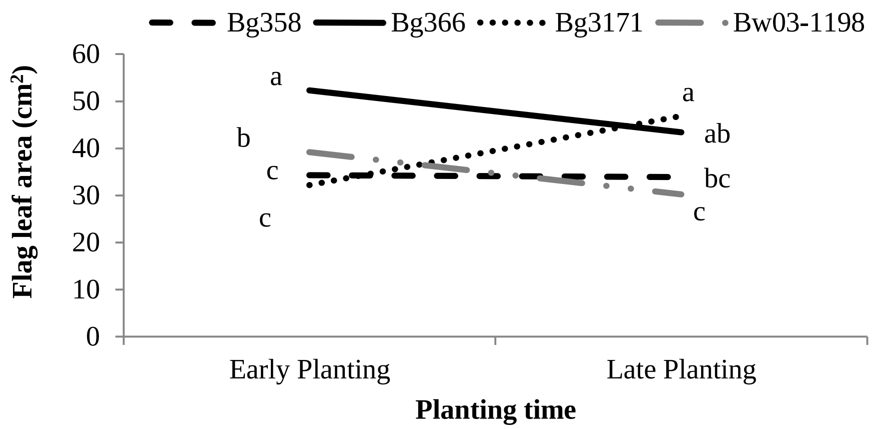

Figure 5. Influence of early and late planting on flag leaf area of tested varieties (within a planting time, symbols with same letters are not significantly different at p=0.05).

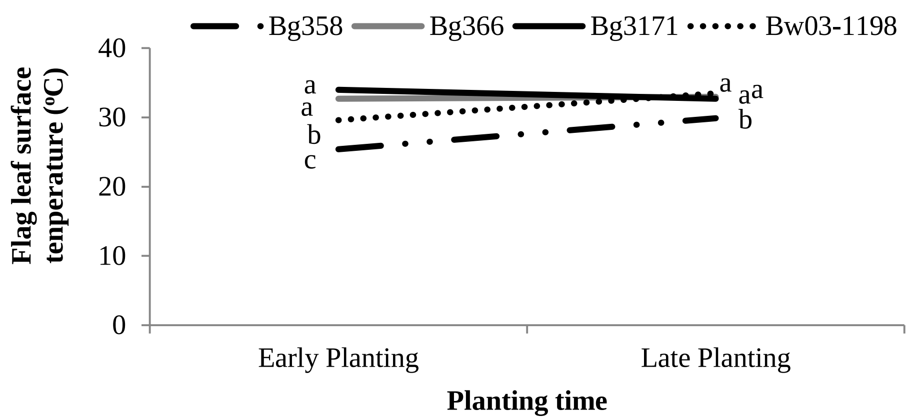

Figure 5. Influence of early and late planting on flag leaf temperature of tested varieties (within a planting time, symbols with same letters are not significantly different at p=0.05).

The results revealed that Bg358 transpired more water vapour than other tested varieties. Further, with increasing ambient temperature in late planting treatment, the leaf temperature of Bg358 and Bw03-1198 increased and thus, showed decreased leaf transpiration. The low canopy cooling of Bg358 and Bw03-1198 resulted in the highest pollen sterility at late planting. However, spikelet surface temperature of Bg358 and Bg366 were the highest in late planted plots. Therefore, under higher spikelet temperature, Bg366 may be avoiding the temperature stress. According to Weerakoon *et al*. (2008) Bg366 would have some spikelet characteristics to avoid temperature stress.

At the time of maximum temperature and low RH, canopy of Bg358 and Bw03-1198 may be comparatively dry than Bg3171 and Bg366. Thus resulting in higher pollen sterility in the former. This zenith angle (angle of the sun with vertical plane of the earth) of the sun was comparatively low during the experimental period. Thus, sunlight may be directly received by the panicle. In such situation, if flag leaf is above the panicle in a crop, it could create a shading effect on the panicle. Further, with increasing temperature, Bg366 and Bg3171 maintained a constant transpiration rate (Figure 6) resulting in some canopy cooling at low RH under maximum temperature compared to that of Bg358 and Bw03-1198 at the panicle level. Therefore, the rate of increasing of spikelet surface temperature could be mainly controlled by the flag leaf transpiration and that of leaves surrounding the flag leaf, and by the shading effect.

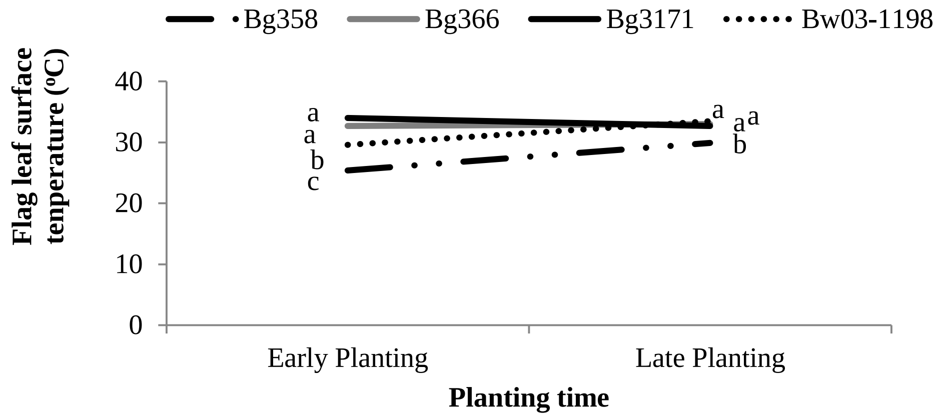

Figure 6. Influence of early and late planting on flag leaf temperature of tested varieties (within a planting time, symbols with same letters are not significantly different at p=0.05).

The cumulative effect of shading and canopy cooling has resulted in a higher spikelet cooling, maintaining low levels of pollen sterility in Bg366 and Bg3171. However, the RH was moderate during anthesis leading to spikelet cooling. During anthesis, a long large flag leaf may further reduce the spikelet temperature in rice. According to the equations generated from Figures 7 and 8, about 85 cm^2 of flag leaf area was assumed and/or at least 55 cm flag leaf length could be reduced if the spikelet temperature is below 30 °C, which is the nascent temperature for pollen sterility (Weerakoon *et al.*, 2008).

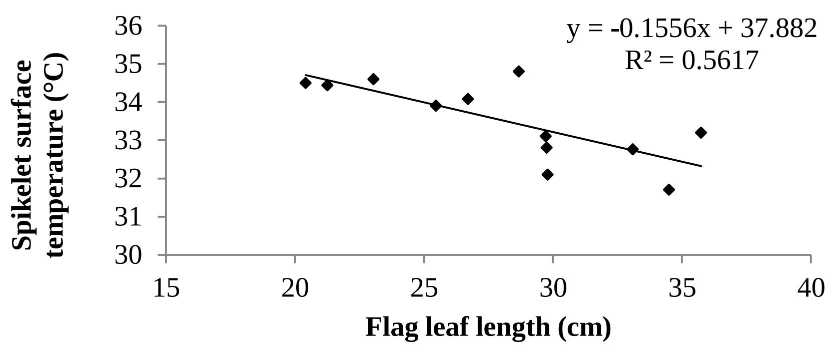

Figure 7. Change of spikelet surface temperature with increasing flag leaf length of tested varieties at late planting

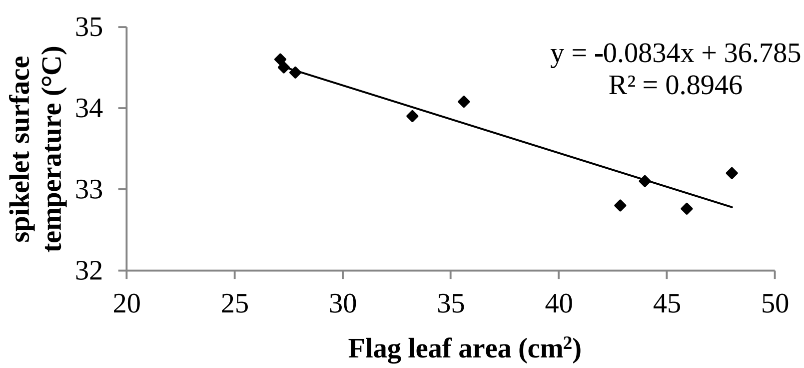

Figure 8. Change of spikelet surface temperature with increasing flag leaf area of tested varieties at late planting

Conclusion

Long and large flag leaf will help manipulating the spikelet surface temperature lower than threshold temperature and negatively impact on pollen sterility. The best combinations of flag leaf characters to obtain the maximum canopy cooling are 85 cm^2 of flag area and 55 cm of flag leaf length. Therefore, it is better to select rice varieties having longer flag leaf than the panicle with lesser leaf surface temperature for varietal development programs aiming at temperature stress.

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References

Abeysiriwardena D.S. de Z., Ohba K. and Maruyama A. (2002): Influence of temperature and relative humidity on grain sterility in rice. J. Nat. Sci. Found. Sri Lanka 30: 33-41.

Belko N., Zaman-Allah M., Diop N., Cisse N., Zombre G., Ehlers J. and Vadez V. (2012): Restriction of transpiration rate under high vapour pressure deficit and non-limiting water conditions is important for terminal drought tolerance in cowpea. Plant Biol. 15: 304–316.

Eriyagama, N., Smakhtin, Chnadrapala L. and K. Fernando. (2010): Impacts of Climate Change on Water Resources and Agriculture in Sri Lanka: A Review of Preliminary Vulnerability Mapping, Research Report 135, International Water Management Institute, Colombo

Hiruyama M., Wada Y. and Nemoto H. (2006): Estimation of drought tolerance based on leaf temperature in upland rice breeding. Breeding Sci. 56: 47-54.

Ishimaru T., Hirabayashi H., Ida M., Takai T., Yumiko A. S., Yoshinaga S., Anda I., Ogawa T. and Kondo M. (2010): A genetic resource for early - morning flowering trait of wild rice *Oryza officinalis* for mitigate high temperature - induced spikelet sterility at anthesis. Annals Bot. 106: 515-520.

Ishimaru T., Hirabayashi H., Kuwagata T., Ogawa T. and Kondo M. (2012): The early morning flowering traits of rice reduces spikelet sterility under windy and elevated temperature conditions at anthesis. Plant Prod. Sci. 15 (1): 19-22.

Jadadish S.V.K., Sumfleth K., Howel G., Redona E., Wassmann R. and Heuer S. (2007): Temperature effect on rice: Significance and possible adaptation. Climaadapt.Org. jagadish2007riceimpact.pdf (accessed on 26.02.2016)

Rani B.A. and Maragatham N. (2013): Effect of elevated temperature on rice phenology and yield. Indian J. Sci. Technol. 6(8): 5095-5097.

Satake T. and Yoshida S. (1978): High temperature induced sterility in Indica rice at flowering. Japan J. Crop Sci. 47 (1): 6-17.

Shah F., Huang J., Kui K., Nie L., Shah T., Chen C. and Wang K. (2011): Impact of high temperature stress on rice plant and its traits related to tolerance. J. Agric. Sci. 149(5): 545-556.

Smith M.B., Horner H.T. and Palimer R.G. (2001): Temperature and photoperiod effect on sterility in cytoplasmic male-sterile soybean. Crop Sci. 41: 702-704.

Wassmann R., Jagadesh S.V.K., Heuer S., Ismail A., Redona E., Serraj R., Sing R.K., Howell G., Pathak H. and Sumfleth K. (2009): Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. Adv. Agron. 101: 59-122.

Weerakoon W.M.W., Abeywickrama T., De Costa W.A.J.M. and Maruyama A. (2009): Out–crossing of heat stress affected spikelets of low land rice in the sub humid zone of Sri Lanka and its long term implications. WWW.niaes.affrc.go.jp./macro. (accessed on 24th May 2018)

Weerakoon W.M.W., Maruyama A. and Ohba K. (2008): Impact of humidity on temperature induced grain sterility in rice. Journal of Agronomy and Crop Science 194: 135-140.

Yoshida S. (1981): Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Laguna, Philippines.

Yoshida S. (1973): Effect of temperature on growth of rice plant (*Oryza sativa* L.) in controlled environment. Soil Sci. Plant Nutr. 19(4): 299-310.