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Rapid and Nondestructive Methods to Capture the Flower Opening Time in Rice to Identify Donors for High Temperature Stress

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The time of day of flowering or the early morning flowering (EMF) trait has been demonstrated to significantly reduce heat stress damage by employing an escaping mechanism. Early flower opening is being one of the important traits that aids in the escape of rice flowers from spikelet sterility due to the high temperature stress. This strategy, which is less explored, can be used to breed cultivars that escape heat at flowering because of their early morning flowering (EMF) trait. With the advancement of instrumentation, the study was proposed to screen the genotypes using rapid and non-destructive methods like use of interval shot cameras to identify EMF traits. Once the early morning flowering trait is identified and studied for heat tolerance, it can be used as a potential donor in rice breeding programs to develop an early morning flowering variety for high temperature prone areas. The already available diverse rice germplasm collections having wide genetic diversity for FOT based on First Spikelet Opening Time (FSOT) at PBS, TNAU was used in this study. Nearly twenty one rice germplasms (landraces) were grown under natural ambient conditions (field) and interval shot cameras were used to capture the time of flower opening (Start, peak and end of anthesis) to identify the early morning flowering trait. These lines with EMF trait will be suitable donors to high temperature stress tolerance. Among them two lines namely, Mattikar and Panamara samba showed early morning flowering trait.

Key words: Rice, Early morning flowering, High temperatures stress, Donors

The impact of global warming on rice production resulted in yield reduction. High temperature stress is becoming a burning problem in the world scenario. Increase in mean temperature during sensitive stages may be harmful for rice production and can drastically reduce grain yield (Watanabe and Kume, 2009). In Tamil Nadu, districts of Dharmapuri, Madurai, Ramanathapuram and Tirunelveli which are solely involved in rice cultivation and experiences high temperature stress (HTS) which leads to severe yield loss. The period from booting to flowering is the most sensitive stage to high temperature stress in rice (Satake and Yoshida, 1978). If the temperature exceeds 38^0C it may lead to the poor spikelet fertility and reduced yield (Ishimaru *et al*., 2012). Recently, the time of day of flowering (Julia and Dingkuhn, 2012) or the early morning flowering (EMF) trait has been demonstrated to significantly reduce heat stress damage by employing an escaping mechanism (Ishimaru *et al.,* 2010; Hirabayashi *et al.,* 2014; Bheemanahalli *et al.,* 2017). The temperature prevailing during the flower opening time (FOT) of the day (anthesis 10.00 am-12.30 am) plays an important role in deciding the crop yield. Heat stress for 1 h induces spikelet sterility if it coincides with flowering (Jagadish *et al.*, 2007). One of the important traits that aids in the escape of rice flowers from spikelet sterility due to the high temperature stress is early flower opening. Again, morphological traits also show variation under high temperature stress. Oh-e *et al.* (2007) have observed that, plant height and number of tillers in rice increased with increase in temperature under temperature gradient chambers. Still, reports on correlating plant height and number of tillers with flower opening time is unclear. Ishimaru *et al*. (2010) attempted to develop near-isogenic lines (NILs) for EMF in the *indica*-type genetic background by exploiting the EMF locus from wild rice, *O. officinalis* which had positive results supporting the use of landraces as donors for early morning flowering trait. Hence, the present study was aimed (1) To identify the EMF trait in the landraces of rice through use of interval shot cameras (2) To check whether the EMF traits correlates with morphological traits such as plant height and tiller number.

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Material and Methods

Field experiments were carried out at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore during Kharif 2018. The already available diverse rice germplasm collections having wide genetic diversity for FOT based on First Spikelet Opening Time (FSOT) was used in this study. Among them twenty one landraces were selected for the study (Table 1). Nursery was raised at PBS, TNAU and the transplanting was done at an area of 2.0 acres. The experiment was laid out in randomized

block design. As the soil had enough phosphorous and potassium, a basal dose of 5 kg of nitrogen was alone given. Crop management and protection measures were taken as per the recommendations.

The morphological and growth characters like plant height and number of tillers were measured at the active tillering stage. Plant height was measured from the base of the shoot to the panicle tip and expressed in cm. For number of tillers, in each landrace, three plants were selected, labelled and counted manually. At heading stage one primary tiller (one per plant) was tagged with least disturbance. Flowering pattern observations such as first spikelet opening time (FSOT, the time when first spikelet lemma and palea opens on a given flowering day) and peak spikelet opening time (PSOT, the time when maximum number of spikelets opened) were captured from 7.30 to 13.00 (Indian Standard Time, IST) throughout the flowering period of the tagged primary panicle using interval shot cameras (RICOH W6-40) which is similar to the method opted by Kobayasi *et al*. (2012).

Digital photographs of the panicles were taken instead of physically inspecting plants. The panicles were photographed automatically at two minutes interval with water proof interval shot cameras (RICOH W6-40) to determine the FOT of each landrace. We used tripods for the camera and it had automatic built-in timer to control measurement intervals and number of shots to be taken.

Results and Discussion

Morphological and growth characters

The plant height increased with the rise of temperature within the range of 30–35 °C (Krishnan *et al.,* 2011). During the active tillering stage of the crop, the plant height was measured for all the twenty one landraces taken for the study. It showed a linear trend. Their plant height ranged between 74.9 and 103.3cm. Among them, Rascadam showed medium flower opening time and minimum plant height of 74.9cm and Thatan samba which showed extra early morning flowering had the maximum plant height of 103.3cm (Fig 1).

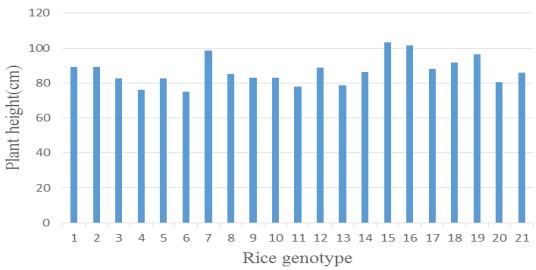

Fig.1. Plant height (cm) at active tillering stage of twenty one landraces of rice

Number of tillers increases with rising temperature in the range of 15-33 °C (Krishnan *et al.,* 2011). During active tillering stage of the crop, the number of tillers was measured for all the twenty one landraces taken for the study. It showed a linear trend. The number of tillers ranged between 16.3 and 42.3. Among them, Chivapu chithiraikar showed that medium flower opening time and minimum number of tillers (16.3) and Thooyamali recorded extra early morning flowering had the maximum number of tillers (42.3) which is presented in Fig 2.

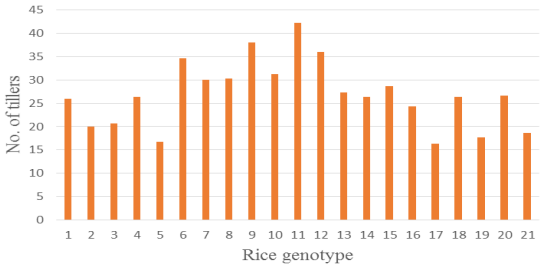

Fig.2. Number of tillers at active tillering stage of twenty one landraces of rice

Flower opening time

The flower opening time of all twenty one landraces were observed between the range of 9.00 to 11.30 (Indian Standard Time, IST) as previously reported by Kobayasi *et al*. (2012).

Table 1. Rice genotypes used in the study

Rice genotype No.	Name of landrace
1	Mapillai samba
2	Seakar
3	Kuruvai kalanjiyam
4	Vellai chithiraikar
5	Chivapu chithiraikar
6	Rascadam
7	Kattikar
8	Sornavari
9	Aarkadu kichilli
10	Karuthakar
11	Thooyamali
12	Jeeraga samba
13	Mattikar
14	Norungan
15	Thatan samba
16	Karungan
17	Panamara samba
18	Thilainayagam
19	Kodai
20	Vadakathi samba
21	Nootri pathu

Among them, Thatan samba, Panamara samba, Thooyamali, Norungan, Mattikar, Sornavari and Seakar showed flowering in between 9.30 and 10.00 IST which is grouped as extra early flowering. Vellai chithiraikar, Nootri pathu, Kodai, Karungan and Thilainayagam showed flowering in between 10.01 and 10.30 IST which is grouped as early flowering. Kuruvai kalanjiyam, Vadakathi samba, Rascadam, Chivapu chithiraikar, Mapillai samba and Jeeraga samba showed flowering in between 10.31 and 11.00 IST which is grouped as medium flowering. Kattikar,

Karuthakar and Aarkadu kichili showed flowering in between 11.01 and 11.30 IST which is grouped as late flowering.

Table 2. Grouping of 21 landraces of rice according to flower opening time

Name of landrace	Flower opening time (am)	Group
Thatan samba	9.31*	Extra early flowering (Flower opening between 9.30 to 10.00 IST)
Panamara samba	9.39*	
Thooyamali	9.45	
Norungan	9.51	
Mattikar	9.54	
Sornavari	9.55	
Seakar	10	
Vellai chithiraikar	10.02	Early flowering (Flower opening between 10.01 to 10.30 IST)
Nootri pathu	10.06	
Kodai	10.22	
Karungan	10.28	
Thilainayagam	10.3	
Kuruvai kalanjiyam	10.35	Medium flowering (Flower opening between 10.31 to 11.00 IST)
Vadakathi samba	10.35	
Rascadam	10.42	
Chivapu chithiraikar	10.44	
Mapillai samba	10.45	
Jeeraga samba	11	
Kattikar	11.06	Late flowering (Flower opening between 11.01 to 11.30 IST)
Karuthakar	11.16	
Aarkadu kichilli	11.31	

*Early morning flowering lines

The flower opening time observed is similar to the results of Ashwin *et al.* (2018). The lines Mattikar and Panamara samba showing early morning flowering trait can be used as a source for identification of potential QTLs/markers governing accelerated flowering under high temperature stress which can be used in marker assisted breeding programmes. Thus, these lines serve as potential donors for mitigating damage caused due to high temperature stress. In line with the above findings, Bheemanahalli *et al*. (2016) also observed EMF traits in Mattikar and Panamara samba.

Correlation between plant height and flower opening time

Correlation study was conducted between plant height and flower opening time of all twenty one landraces. A positive correlation of 0.45% was detected between plant height and flower opening time (Table 3) (Ranawake *et al*., 2014; Weng *et al.,* 2014). Hence, it can be concluded that the plant height is related to flower opening time to some extent.

Correlation between number of tillers and flower opening time

Correlation study was conducted between number of tillers and flower opening time of all twenty one landraces. A positive correlation of 22.9 % was detected between number of tillers and flower opening time

Table 3. Correlation between plant height, number of tillers and FOT

	Plant height	Number of tillers	FOT
Plant height	1		
Number of tillers	-0.25685	1	
FOT	0.004969	0.234781	1

Similar results were observed by Mohanan and Mini (2008) in rice. Hence, it can be concluded that the number of tillers is related to a comparatively greater extent than plant height to flower opening time. However, the plant height and number of tillers are negatively correlated at 25.7%.

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