



## Characterization of Temperature Sensitive Genic Male sterile (TGMS) Lines for Morphological and Floral Traits in rice (*Oryza sativa* L.)

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### ABSTRACT

The investigation was carried out for characterization of 119 rice Temperature Sensitive Genic Male Sterile (TGMS) lines for morphological, yield traits at fertility favoring environment; sterility and floral traits at sterility favoring environment. The analysis of variances exhibited highly significant differences for all the character studied except sterile lemma length. The TGMS lines viz., TNAU 115S and TNAU 139S was found to be earlier for days to 50% flowering in both environments. Based on floral traits viz., pollen fertility per cent, pollen sterility per cent, panicle exertion per cent, anther length, stigma breadth, glume opening angle, the following TGMS lines namely TNAU 147S, TNAU 103S, TNAU 14S, TNAU 19S, TNAU 45S and TNAU 100S were identified as promising TGMS lines. These lines can be used as male sterile parents for development of two line rice hybrids,

**Keywords:** Characterization, Rice, TGMS, Morphological, Floral traits

### INTRODUCTION

Rice is an ancient grain crop and a staple food for people due to it being high in carbohydrates, low in fat and rich in proteins, vitamins and minerals. It has been used as a major food for over ten thousand years. The area under rice in India has decreased drastically in the recent years. With increasing population, demand for increased production of rice must be met from less land with limited resources. The hybrid rice technology is the best option to increase the rice production in tropics as reported by Ikehashi et al. (1995).

Hybrid rice varieties increase rice production by 15-20% over the commercial cultivars (Virmani, et al., 1982), (Wa et al., 2008). Cytoplasmic male sterility (CMS) system is the most widely used male sterility system in India for development of rice hybrids. Though this system is viable, it possess difficulty in identification of restorer and hybrid seed production. Another important tool to develop hybrid seed production in rice is environmental sensitive genetic male sterility system, the male sterility is governed by temperature, day length or both.

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In countries like India, where significant temperature differences exist in different altitude or different season, TGMS system may be used for development of hybrid (Borkakati & Virmani, 1996), (Virmani & Ilyas, 2001), (Kumar & Nautiyal, 2016). TGMS lines have complete pollen sterility at a day and night temperature ( $>30\text{ }^{\circ}\text{C}/ >24\text{ }^{\circ}\text{C}$ ) and revert back to partial to normal pollen fertility at a lower temperature ( $<24\text{ }^{\circ}\text{C}/ >16\text{ }^{\circ}\text{C}$ ). The advantage of TGMS system over the CGMS, there is no need of maintainer and restorer line for seed multiplication. The seed production is simple and cost effective. Rice is the self pollinated crop, out crossing is mainly influenced by its floral traits which play a major role in hybrid seed production. Characterization for floral traits in TGMS lines is an important one for selection of appropriate parent for hybrid breeding program. Morphological characterization is important for distinguishing the cultivars. With this aim, the present investigation was carried out for characterization of 119 rice TGMS lines for eight morphological and ten floral traits.

#### MATERIALS AND METHODS

One hundred and nineteen rice temperature sensitive genetic male sterile (TGMS) lines were evaluated at two locations *viz.*, (i). Hybrid Rice Evaluation Centre, Gudalur during *Khari* 2017 for fertility reversion studies. It is located at  $11.5^{\circ}\text{ N}$  latitude and  $76.5^{\circ}\text{ E}$  longitude with an elevation of 1500 m above mean sea level. (ii). Department of Rice, TNAU, Coimbatore during *Rabi* 2017-18 for evaluation of sterility and floral traits. This station is located at  $11^{\circ}\text{ N}$  latitude and  $77^{\circ}\text{ E}$  longitude with an elevation of 426.7 m above mean sea level. All the lines were planted in a Randomized Block Design with three replications adapting a spacing of 20 x 20 cm in one row of 4 m each. Normal cultural practices were followed as per standard recommendations to get a good crop stand. The observations for agro morphological and floral traits were recorded in five randomly selected plants in a genotype and mean values are arrived for statistical analysis. The traits

*viz.*, days to 50% flowering, plant height, panicle length, panicle exertion, flag leaf length, numbers of productive tillers were observed at both environments. The floral traits namely anther length, anther width, stigma length, stigma width, glume opening angle, sterile lemma length and pollen sterility were recorded in sterility favoring environments, whereas pollen fertility, spikelet fertility, number of productive tillers and single plant yield recorded at fertility favoring environments. Among the floral characters anther length, anther breadth, stigma length, stigma breadth, stigma color, pollen fertility, pollen sterility were measured by using a digital microscope with help of imaging software tool SCOPE IMAGE V 9.1 build 90225p. Simple statistics was calculated to know the level of variation in the experimental material.

#### RESULTS AND DISCUSSION

In the present study, analysis of variance (Table 1 & 2) showed highly significant differences for all morphological and floral characters studied except sterile lemma length at sterility favouring environments revealed that large variation found in the experimental materials taken for the study.

##### Morphological traits

The descriptive statistics of TGMS lines for morphological and quantitative traits in fertility and sterility favoring environment are presented in Table 3. The range of days to 50% flowering was observed from 57 to 91 days with a mean of 76 days in fertility favouring environment where as in sterility favouring environment, the TGMS lines had a range from 57 to 94 days with a mean of 78 days. In both environments, TNAU 115S and TNAU 139S were found to be identified as early flowering type. Chakraborty and Hajarika (1972) reported significant variation for days to 50% flowering in rice. The shortest and tallest plant was recorded by GDR 5S (69.8cm) and TNAU 145S (97.5cm) at fertile environment, where as the line GT 48/14 and TNAU 1S observed minimum (47.3cm) and maximum (94.2cm) height in sterile phase

respectively. The TGMS lines identified as dwarf and semi dwarf types in this study are ideal trait formale sterile line in development of hybrid rice.

Forty six lines were recorded longer panicle length than the grand mean with a range of 17.7 cm (GT48/14) to 26.3cm (GDR 24S) at fertile phase. The longest panicle length was observed in GDR 39S (27.3cm), 64 lines with high panicle length than the grand mean at sterility favouring environment. The TGMS lines viz., GDR 24S, GDR 27S, GDR 39S were found to have long panicles (> 25 cm) on both the environments. These genotypes can be utilized as male sterile parent in two line breeding program. The maximum panicle exertion was noted by TNAU 104S with 100% and 97.6% exertion in fertile and sterile environments respectively. A high panicle exertion in male sterile lines would explore more number of spikelets for out crossing than lines with incomplete panicle exertion. Abeysekara et al. (2003) and Virmani (1994) reported that high panicle exertion may associated with high out crossing rate. The line with higher panicle exertion percent coupled with high seed set and higher spikelet fertility percent can be exploited well for development of hybrid rice which has a greater influence on out crossing ability (Arasakesary et al. 2015).

Flag leaf plays an important role in grain yield of rice as reported by Wan and Shong (1981). In fertile phase, the flag leaf length was observed between 23.7cm (GDR 74S) and 34.5cm (GDR 53S), 59 lines noted with longer flag leaf length than the grand mean. In sterile phase, the high flag leaf length (36.4cm) was noted in TNAU 118S and shorter length of 12.6cm in TNAU 59 S-2 and 38 lines with higher flag leaf length than the grand mean. Many TGMS lines in this study observed semi erect flag leaf. The semi erect dwarf flag leaf in *cms* lines will give good yield potential (Pandey et al., 1998).

The line TNAU 4S – 1 (23 nos.) had more number of productive tillers per plant and 65 lines had more tillers than the grand mean at fertile phase where as in sterile phase, GDR 3S (26.9 nos.) has been observed with

more productive tillers per plant, 74 lines with maximum number of productive tillers than the grand mean. The TGMS lines with more productive tillers can be utilized for the development of two line hybrid in rice. Number of grains per panicle ranged from 34 (TNAU 112) to 326 (TNAU 114S) in fertile environment. The highest single plant yield was observed in GDR 44S (67.7g), which can be utilized for higher yield and can be inherited to other lines where the seed production of 'A' line is necessary in two line hybrid rice production.

#### Floral traits

Tejbir Singh (2016) reported that success of hybrid seed production in rice depends on well developed floral traits. The extent of out crossing in *cms* lines are further influenced by its floral traits namely stigma size, stigma exertion, anther size, filament length and duration of floret opening (Virmani et al., 1994). The mean performance of TGMS lines were evaluated for floral traits, anther length, anther breadth, stigma length, stigma breadth, glume opening angle, stigma exertion, sterile lemma length, pollen sterility at sterility favouring environment and pollen fertility, spikelet fertility at fertility favouring environment are presented in Table 4. The TGMS lines viz., TNAU 111S (2.59mm), TNAU 100S (2.45mm), GDR 21S (2.28mm), TNAU 60S (2.27mm), TNAU 147S (2.26mm) had higher anther length at sterile phase. Longer anthers are helpful in developing lines with higher outcrossing in a hybrid rice development and also for improving lines with abiotic tolerance which needs an optimum length of anther reported by Tanno et al. (1999), and also Matsui et al. (1997b), stated that narrow range of anther length may have over emphasized the importance of number of cell layers in high temperature tolerance. Despite the importance of anther length, anther breadth is also considered to be of equal importance. Higher anther breadth was observed in TNAU 124 S (1.22mm) and TNAU 53S (1.11mm) respectively.

At sterility favouring environment, the stigma length was ranged from 0.51mm

(GDR 16S) to 2.48mm (TNAU 113S), where as GDR 15S was found to have high stigma breadth (0.66mm). Wide range of variability was observed in stigma length and stigma breadth, the significant lines can be used for the hybrid rice production. Suzuki (1981) reported that there was no positive correlation between stigma length and anther length. The Japanese cultivars with shorter length have a disadvantage in  $F_1$  seed production in hybrid rice varieties as compared with other ecospecies with longer stigma length. In case of glume opening, the maximum angle was observed in GDR 27S (33.3), where as it is minimum in TNAU 135S (10.0) and 57 lines had angle more than the grand mean (20.2). The *cms* lines with wider glume angle will influence the greater the outcrossing in male sterile lines used for hybrid seed production. Greater the glume opening angle and more stigma exertion that leads to high seed setting as reported by Mahalingam et al. (2013). GDR 41 (100%), TNAU 92S (84.6%), TNAU 19S (70.8%) had high stigma exertion which is a desirable traits for male sterile lines. The

pollen sterility was ranged from 6.7% (TNAU 129S) to 100% (TNAU 14S, TNAU 19S, TNAU 45S, TNAU 100S, TNAU 147S) with a grand mean of 91.1% pollen sterility.

At fertility favouring environment, the TNAU 95S (98.6%) had higher pollen fertility percent. The lines with higher pollen fertility percent can be used a better parents for hybrid seed production. The spikelet fertility was ranged from 13.1% (TNAU 104S) to 97.0% (GDR 10S). The genotype possessing more spikelet fertility will be of greater importance in hybrid rice development. The sterile lemma length was ranged from 1.67mm (GDR 39S) to 2.5mm (GDR 38S).

In the present investigation, it was concluded that the TGMS lines identified (Table 5) based on important floral traits *viz.*, pollen fertility per cent, pollen sterility per cent, panicle exertion per cent, anther length, stigma breadth, glume opening angle were TNAU 147S, TNAU 103S, TNAU 14S, TNAU 19S, TNAU 45S and TNAU 100S. These TGMS lines can be utilized as female parent for development of two line hybrids.

**Table 1: Analysis of variance for morphological traits at fertility favoring environments in rice TGMS lines**

Sources of variation	df	Days to 50% flowering	Plant height (cm)	No. of productive tillers	Panicle length (cm)	Panicle exertion (%)	Flag leaf length (cm)	Pollen fertility (%)	Spikelet fertility (%)	No. of grains per panicle	Single plant yield (g)
Replication	2	25.62	49.188	0.252	22.693	30.39	31.82	2.65	0.573	14.04	23.86
Treatment	118	206.17*	100.51*	25.538*	11.142*	96.32*	17.32*	425.35*	245.36*	6817.69*	322.91*
Error	236	9.218	15.276	0.170	2.06	6.81	9.413	2.66	2.160	7.14	7.05

\*Significant at 5 % level

**Table 2: Analysis of variance for morphological and floral traits at sterility favoring environments in rice TGMS lines**

Sources of variation	df	Days to 50% flowering	Plant height (cm)	No. of productive tillers	Panicle length (cm)	Panicle exertion (%)	Flag leaf length (cm)	Anther length (mm)	Anther breadth (mm)	Anther breadth (mm)	Stigma breadth (mm)	Glume opening angle (°)	Stigma exertion (%)	Sterile lemma length (mm)	Pollen sterility (%)
Replication	2	16.11	4.37	18.77	34.02	30.38	4.59	0.007	0.0003	0.001	0.0005	2.15	0.0005	0.004	3.38
Treatment	118	206.91*	235.10*	50.68*	12.31*	96.32*	72.38*	0.185*	0.0474*	0.491*	0.0202*	107.84*	0.0802*	0.056	1196.76*
Error	236	4.22	3.92	6.44	1.04	6.81	2.29	0.004	0.0002	0.002	0.0005	2.03	0.0005	0.026	2.13

\*Significant at 5 % level

**Table 3: Descriptive statistics for morphological traits at fertility and sterility favouring environments of TGMS lines**

Traits	Days to 50% flowering		Plant height (cm)		Panicle length (cm)		Panicle exertion (%)		Flag leaf length (cm)		No. of productive tillers		No. of grains/panicle	Single plant yield (g)
	F	S	F	S	F	S	F	S	F	S	F	S	F	F
Environment	F	S	F	S	F	S	F	S	F	S	F	S	F	F
Mean	76.0	78.0	83.4	70.4	21.4	22.3	80.1 (64.4)	72.7 (59.1)	28.2	20.6	15.6	16.7	165	27.2
Min. mean value	57.0	57.0	69.8	47.3	17.7	17.3	65.2 (54.0)	48.8 (44.3)	23.7	12.6	9.8	6.2	34	7.7
TGMS lines	TNAU 115S, TNAU 139S	TNAU 115S, TNAU 139S	GDR 5S	GT 48/14	GT 48/14	GT 48/14	TNAU 115S	GDR 48S	GDR 74S	TNAU 59S	TNAU 115S	TNAU 115S	TNAU 112S	TNAU 101S
Max. mean value	91.0	94.0	97.5	94.2	26.3	27.3	100.0 (89.7)	97.6 (81.7)	34.5	36.4	23.0	26.9	326	67.7
TGMS lines	TNAU 126S	TNAU 129S	TNAU 145S	TNAU 1S	GDR 24S	GDR 39S	TNAU 104S	TNAU 104S	GDR 53S	TNAU 118S	TNAU 45S	GDR 3S	TNAU 114S	GDR 44S
No. of genotypes more/less than grand mean	53	57	40	62	46	64	59	52	59	38	65	74	64	55
SE (d)	1.8	1.9	2.3	1.0	0.8	0.3	4.6 (3.3)	1.3 (1.1)	1.8	1.6	0.2	1.6	2.0	2.3
CD	4.9	3.3	6.3	2.9	2.3	0.8	12.9 (9.3)	3.7 (3.0)	4.9	4.4	0.7	4.5	6.0	6.5
CV%	4.1	2.2	3.8	2.7	4.5	4.6	5.1	4.1	6.4	7.3	7.2	9.4	5.1	6.3

F: Fertility favouring environment

S: Sterility favouring environment

Value in parantheses are transformed value

**Table 4: Descriptive statistics for floral traits at sterility/fertility favoring environments in rice TGMS lines**

Traits	Anther length (mm)	Anther breadth (mm)	Stigma length (mm)	Stigma breadth (mm)	Pollen fertility (%)	Spikelet fertility (%)	Glume opening angle (°)	Stigma exertion (%)	Sterile lemma length (mm)	Pollen sterility (%)
Environment	S	S	S	S	F	F	S	S	S	S
Mean	1.94	0.3	1.71	0.37	84.1 (67.1)	62.8 (56.8)	20.2	42.0 (40.3)	1.98	91.1 (82.0)
Min. mean value	1.26	0.13	0.51	0.21	21.0 (5.7)	13.1 (27.3)	10.0	10.9 (19.3)	1.67	6.7 (13.8)
TGMS lines	TNAU 53S	TNAU 157S	GDR 16S	GDR 4S	TNAU 104S	TNAU 104S	TNAU 135S	TNAU 98S	GDR 39S	TNAU 129S
Max. mean value	2.59	1.22	2.48	0.66	98.6 (83.1)	97.0 (79.2)	33.3	100.0 (84.4)	2.5	100.0 (89.7)
TGMS lines	TNAU 111S	TNAU 124S	TNAU 118S	GDR 15S	TNAU 95S	GDR 10S	GDR 27S	GDR 41S	GDR 38S	TNAU 18S
No. of genotypes more/less than grand mean	64	73	79	38	86	69	57	59	65	85
SE (d)	0.03	0.008	0.02	0.006	2.7 (2.1)	1.1 (0.9)	0.3	0.6 (0.4)	0.09	1.4 (1.2)
CD	0.08	0.02	0.07	0.02	7.6 (5.7)	3.1 (2.4)	0.7	1.1 (0.24)	0.24	3.8 (3.4)
CV%	3.6	5.1	2.8	6.3	2.8	5.5	7.0	5.0	8.3	1.8

F: Fertility favouring environment

S: Sterility favouring environment

Value in parentheses' are transformed value

**Table 5: Best performing rice TGMS lines identified for morphological and floral traits at both fertility and sterility favoring environments**

S.No	Characters	Best performing TGMS lines identified based on mean performance
1.	Days to 50% flowering	TNAU 115S, TNAU 139S, GDR 21S, GDR 3S, GDR 27S, TNAU 104S, TNAU 112S, TNAU 132S, TNAU 106S, TNAU 132S
2.	Plant height (cm)	GDR 5S, GDR 15S, GDR 74S, TNAU 14S, TNAU 31S, TNAU 45S, TNAU 95S, TNAU 139S, TNAU 147S, TNAU 46S,
3.	Sterile lemma length	GDR 22S, GDR 38S, GDR 48S, GDR 52S, TNAU 4S-1, TNAU 31S, TNAU 82S, TNAU 113S, TNAU 131S, TNAU 145S
4.	Panicle length (cm)	GDR 24S, GDR 23S, GDR 22S, GDR 18S, GDR 27S, GDR 39S, GDR 41S, GDR 47S, TNAU 107S, TNAU 101S
5.	Panicle exertion (%)	TNAU 16S, GT 48/14, TNAU 14S, GDR 53S, TNAU 98S, TNAU 104S, TNAU 112S, TNAU 103S, TNAU 45S, TNAU 147S
6.	Flag leaf length (cm)	GT4/14, GDR 53S, TNAU 1S, TS 29, F4 17S, TNAU 114S, TNAU 118S, TNAU 101S, TNAU 92S, TNAU 111S
7.	Anther length (mm)	GDR9S, GDR 21S, TNAU 4S, TNAU 14S, TNAU 100S, TNAU 103S, TNAU 112S, TNAU 147S, TNAU 45S, TNAU 111S
8.	Anther breadth (mm)	GDR 38S, GDR 55S, TNAU 53S, TNAU 71S, TNAU 100S, TNAU 113S, TNAU 114S, TNAU 116S, TNAU 124S, TNAU 145S
9.	Pollen fertility (%)	GDR 52S, TNAU 147S, GDR 55S, TNAU 14S, TNAU 16S, TNAU 19S, TNAU 95S, TNAU 103S, TNAU 100S, TNAU 45S
10.	Pollen sterility (mm)	TNAU 18S, TNAU 14S, TNAU 103 S, TNAU 101 S, GDR 29S-1, GDR 35S, TNAU 45S, TNAU 19 S, TNAU 100 S, TNAU 147 S,
11.	Stigma length (mm)	GDR 40S, GDR 70S, TNAU 4S, TNAU 45S, TNAU 100S, TNAU 104S, TNAU 112S, TNAU 113S, TNAU 119S, TNAU 132S,
12.	Stigma breadth (mm)	GDR 15S, TNAU 14S, TNAU 45S, TNAU 67S, TNAU 103S, TNAU 112S, TNAU 19S, TNAU 126S, TNAU 100S, TNAU 147S
13.	Stigma exertion (%)	GDR 10S, GDR 34S, GDR 38S, GDR 41S, TNAU 18S, TNAU 39S, TNAU 112S, TNAU 142S, TNAU 92S,
14.	Glume opening angle	GDR 27S, GDR 29S-1, GDR 35S, GDR 47S, TNAU 14S, TNAU 45S, TNAU 19S, TNAU 100S, TNAU 147S, TNAU 103S
15.	Spikelet fertility (%)	GDR 10S, GDR 16S, GDR 27S, GDR 43S, GDR 48S, TNAU 118S, TNAU 147S, TNAU 126S, TNAU 145S
16.	Number of productive tillers	GDR 3S,, GDR 28S, GDR 29S, GDR 58S, GDR 68S, TNAU 4S, TNAU 4S-1, TNAU 59S-1, TNAU 129S, TNAU 151S,
17.	Number of grains per panicle	GDR 44S, GDR 53S, TNAU 4S, TNAU 45S, TNAU 86S, TNAU 114S, TNAU 123S, TNAU 139S, TNAU 15S, TNAU 145S
18.	Single plant yield (g)	GT4/14, GDR 4S, GDR 28S, GDR 29S, GDR 29S-1, GDR 43S, GDR 44S, GDR 34S, TNAU 114S, TNAU 131S,

## REFERENCES

- Abeysekera, S.W., Abeysiwardana, D.S., & Dehideniyz, E. (2003). Characteristics associated with out crossing rate of cytoplasmic male sterile *cms* lines in rice under local conditions. *J*, 1-6.
- Arasakesary, S. J., Manonmani, S., Pushpam, R., & Robin, S. (2015). New temperature sensitive genic male sterile lines with better outcrossing ability for production of twoline hybrid rice. *Rice Science*, 22(1), 49–52.
- Borkakati, R. P., & Virmani, S. S. (1996). Genetics of thermosensitive genic male sterility in rice. *Euphytica*, 88(1), 1–7.
- Chakraborty, S., & Hajarika, M.H. (1972). Estimation of various genetic parameters of yield and yield components of rice. *Oryza*, 31, 226-227.
- Ikehashi, H., Zou, J. S., Huhn, P. M., & Maruyama, K. (1995). Wide compatibility gene(s) and *indica x japonica* heterosis in rice for temperate countries. In: *Hybrid Rice Technology, New developments and future prospects*. IRRI, Philippines.
- Kumar, P., & Nautiyal, M.K. (2016). Two line hybrid production system and their application in rice. *Intl J. Agric. Sci.* 8(61), 3502-3504.

- Mahalingam, A., Saraswathi, R., Ramalingam, J., & Jayaraj, T. (2013). Genetics of floral traits in cytoplasmic male sterile (*cms*) and restorer lines of hybrid rice (*Oryza sativa* L.). *Pak. J. Bot.*, 45(6), 1897-1904.
- Matsui, T., Namuco, O.S., Ziska, L.H., & Horie, T. (1997b). Effects of high temperature and CO<sub>2</sub> concentration on spikelet sterility in *indica* rice. *Field Crops Res.*, 51, 213-219.
- Pandey, M. P., Rongbai, L., Singh, J. P., Mani, S. C., Singh, H., & Singh, S. (1998). The identification and nature of a new thermosensitive genic male sterility source, UPRI 95 - 140 TGMS in rice. *Cereal Res. Communications*, 26(3), 8–10.
- Suzuki, S. (1981). Cold tolerance in rice plants with special reference to the floral characters I. Varietal differences in anther and stigma length and effects of planting densities on these characters. *Japanese J. Crop Sci.* 31, 57-64.
- Tanno, H., Xiong, J., Dai, L., & Ye, C. (1999). Some characteristics of cool weather tolerant rice varieties in Yunnan province, China. *Japanese J. Crop Sci.* 68, 508.
- Singh, T. (2016). Study of morphological and floral traits of different lines in rice (*Oryza sativa* L.). *Int. J. Curr.Res.* 8, 27-33.
- Virmani S.S., & Ilyas Ahmed, M. (2001). Environment sensitive genic male sterility in crops. *Advances in Agron.* 72, 139-195.
- Virmani, S.S., Heterosis, & hybrid rice breeding. Springer Verlag, Berlin. (1994).
- Virmani, S.S., Aquino, R.C., & Kushi, G.S. (1982). Heterosis breeding in rice (*Oryza sativa* L.). *Theoret.Applied Genet.* 63, 373-380.
- Wa, C.M., & Yang, X.C. (2008). Fixation of hybrid vigor in rice: Opportunities and challenges. *Euphytica*, 160, 287-293.
- Wan, A.L., & Shong, Y.M. (1981). Studies on the relationship between flag leaf area and yield and yield components of rice. *Oryza* 31, 226-227.