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Research Article

Genetic studies on TGMS lines for development of superior two line rice hybrids

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Abstract
Development of desirable floral and out crossing related characters in TGMS lines plays a key role in two line rice hybrid programme. A set of six TGMS lines were raised during summer 2018 and observed under high temperature regime for floral, morphological, agronomic traits to find out commercially exploitable TGMS lines for two line hybrid seed production. Variability studies were conducted for several characters *viz.,* anther length, anther breadth, panicle exertion rate, stigma exertion rate, glume angle, spikelet sterility, pollen sterility which shows high GCV, Heritability, Genetic Advance could be utilized for selection. The three TGMS lines *viz.,* TNAU 39S, TNAU4S, TNAU 95S showed the wider glume angle opening (>29º), higher panicle exertion rate(>87%), stigma exertion rate (>72%) and stable pollen sterility percentage (>99%). The floral and morphological characterisation studies shows that TGMS lines *viz.,* TNAU 39S, TNAU 45S, TNAU 95S had stable pollen sterility expression with good desirable floral traits *viz.,* higher pollen sterility, wider glume angle opening, high stigma and panicle exertion % for better extent of out-crossing rate and these lines can be utilized for two line hybrid rice breeding programme with high heterotic expression and superior yield.

Keywords
Rice, TGMS lines, two line hybrid, floral traits.

Introduction

Thermo-sensitive Genic Male Sterility is a novel panorama in hybrid rice breeding in recent years. Heterosis in rice was first reported by Jones 1926. In India, heterosis was reported by Ramaiah (1935) and Kadam *et al.,* (1937). Yuan Long Ping, father of hybrid rice (1960) found out the genetic basis of heterosis in rice. Hybrid rice is also showing enough heterosis of 15-25% over semi- dwarf varieties. Development of wild abortive (WA) type of cytoplasmic male sterility (CMS) led the beginning of hybrid rice technology and China become most successful in hybrid rice production.

Male sterility is pre- requisite for exploitation of hybrid vigour in self pollinated crops like rice. China first utilised three- line system using A,B and R lines. On the other hand, two- line system or environmental- sensitive genic male sterility (EGMS) which is due to interaction of nuclear genes with environmental factors such as photoperiod, temperature or both. TGMS line Annong 1S was isolated as spontaneous mutant in China. Later, Yang and Wang (1989) induced a mutant (5460S) in the *indica* variety IR 54 in which male sterility/fertility alteration is conditioned by different temperature regimes. The first TGMS mutant induced by gamma rays in Japanese rice variety Remei by Maruyama *et al* (1991). EGMS system is highly convenient than CMS system since it eliminates the maintainer line for the hybrid seed production. Magnitude of heterosis is also 5-10% higher than in CMS system as it does not have cytoplasmic consequence. Moreover, two-line breeding system is attractive and potential tool for exploiting heterosis, has a wide chance of parental line combinations and highly proficient. The present investigation was carried out to unravel the genetic studies on TGMS lines by characterization of floral traits, morphological and agronomic traits to identify the promising TGMS lines with desirable characters for two line hybrid seed production.

Materials and Methods

The experiment carried out for the study consisted of six TGMS lines *viz.,* TNAU 39S, TNAU 45S, TNAU 95S, TNAU 63S, TNAU 97S and TNAU 93S were raised during summer 2018 at Agricultural College and Research Institute, Killikulam. One set of sowing was taken in the date 12.01.18. The materials were collected from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. It is completely sterile during the temperature above 32ºC/24ºC for hybrid seed production and it shows fertility at temperature below 28ºC/18ºC and it can be used for seed multiplication. Pollen sterility was evaluated using 10-15 freshly emerged panicles of all the plants. Anthers were tweezed out and observed under microscope with a drop of 1% Iodine

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Potassium Iodide (IKI) solution. With the help of weather data collected from Department of Meteorology, AC&RI, Killikulam (Fig.1) Sterility % was tracked on every two days from the starting of flowering in each line between the month of May to July 2018 and the maximum temperature was above 32ºc.The pollen sterility was calculated as follows

Pollen sterility %=

$$\frac{\text{(Number of (unstained withered+ unstainedspherical +partly stained round) pollen)}}{\text{(Total number of pollen counted (including fertile)}} \times 100$$

Scale	Description	Pollen sterility (%)
1	Completely sterile	100.0
3	Highly sterile	99.0-99.9
5	Sterile	95.0-98.9
7	Partially sterile	70.0-94.9
9	Partially fertile to fertile	< 70

The primary panicles of male sterile line are bagged at booting to heading stage before anthesis. Total number of ill filled grains and total number of spikelets on main panicle of each plant was counted separately and spikelet sterility per cent worked out.

Spikelet sterility (%) =

$$\frac{\text{Number of ill filled grains per panicle}}{\text{Total number of spikelets per panicle}} \times 100$$

Scale	Description	Spikelet Sterility %
1	Completely sterile	100.0
3	Highly sterile	99.0-99.9
5	Sterile	95.0-98.9
7	Partially sterile	70.0-94.9
9	Partially fertile to fertile	< 70

Extent of out crossing on male sterile lines trait was monitored at the growth stage of dough and mature grain stage of a male sterile line grown in the field where pollen supply at its flowering time is abundant. Seed set on the out pollinated primary panicles was observed.

Five elite plants in the middle row of each lines from each replication were randomly selected and evaluated for floral traits *viz.*, anther length (mm), anther breadth (mm), panicle exertion rate (%), stigma exertion rate (%), glume angle (º), panicle length(cm), spikelet sterility %, pollen sterility %, extent of out crossing (%),morphological traits *viz.*, anther colour, stigma colour, basal leaf sheath colour, leaf blade colour, flag leaf angle, auricle colour, ligule length, ligule colour, ligule shape, collar colour, panicle type, flag leaf length, apiculous colour and agronomic traits *viz.*, plant height, number of tillers per plant, days to 50% flowering, chlorophyll content (SPAD reading). The mean data from each floral trait were individually subjected to statistical analysis using GENRES statistical package. The ANOVA was computed to separate the total variation in to different components and to test their significance. Genetic parameters like GCV, PCV, heritability and genetic advance were calculated according to the method suggested by Johnson *et al.*(1995). The correlation between the floral characters was computed based on genotypic and phenotypic correlation coefficients (Goulden,1952).

Results and Discussion

The prime objective in exploitation of two line rice hybrids is the identification of TGMS lines with stable performance in sterility behaviour. The advantages of TGMS system are simple and it does not requires maintainer line which reduces labour, expenditure and area requirement, allows a wider range of parental combinations and avoids the negative influence of male sterility- inducing cytoplasm. The observations recorded on the floral, morphological and agronomic traits of TGMS lines are listed in Table.1 and Table.2. Out of six TGMS lines, three lines showed stable performance and three lines showed complete sterility under high temperature regime at AC & RI Killikulam. These lines could be utilised for two line hybrid rice programme for development of superior hybrids.Table.2 indicated that there is no difference in leaf blade colour, panicle type and basal leaf sheath colour except TNAU 39S which is light purple. The anther colour was light yellow in TNAU 39S, TNAU 63S, TNAU 93S where as white in others. Stigma colour is purple in TNAU 39S, TNAU 45S, TNAU 95S and white in TNAU 63S, TNAU 97S, TNAU 93S (Fig.2). Flag leaf angle is erect in all genotypes except TNAU 93S which is horizontal. Ligule colour is light brown in TNAU 45S and TNAU 95S and white colour in others and ligule length is low in TNAU 39S (0.88cm) and high in TNAU 97S (2cm). Apiculus colour is red in all genotypes and white in TNAU 97S and TNAU 93S. Flag leaf length is higher in TNAU 95S (34cm) and low in TNAU 63S (20cm). Minimum plant height was recorded in TNAU 39S (71cm) and maximum in TNAU 95S (110cm).

Among these lines, the anther length varied from 1.66mm (TNAU 93S) to 2.48mm (TNAU 95S). The general mean for the trait anther length was 2.17mm and TNAU 95S was exceeded the general mean significantly. The anther breadth ranges from 0.72mm (TNAU 45S) to 0.48mm (TNAU 93S).

The general mean was 0.58mm and two lines *viz.*, TNAU 39S and TNAU45S were significant (Fig.3). Similar results were observed by Kanimozhi *et al.*,(2018) in which anther length ranges from 2.66mm to 1.89mm and anther breadth ranges from 0.56mm to 0.43mm. The glume angle opening was higher during the peak hours of anthesis and it ranges from 33.62º (TNAU 95S) to 21.84º (TNAU 93S). The range of panicle exertion percentage was 94.94 (TNAU 95S) and 82.41 (TNAU 63S). The general mean was 88.45 and two lines *viz.*, TNAU 39S and TNAU 45S were significant. Stigma exertion percentage was recorded high in TNAU 95S (85.51) and low in TNAU 63S (63.42). Three lines *viz.*, TNAU 45S, TNAU 95S and TNAU93S were significant over the general mean (74.47%).

The temperature remained above 34ºc during the month of May (36/25ºc), June (36/25ºc) and July (35/24º). Spikelet sterility and pollen sterility was completely sterile in three lines *viz.*, TNAU 45S, TNAU 95S and TNAU 97S and were significant over the general mean 98.57% and 98.74% respectively (Fig.4).The period of sterility observed throughout the flowering days in a interval of every two days for all the lines (15-30 days after initiation of flowering). The extent of out crossing varied from 47.95% (TNAU 95S) to 32.57% (TNAU 63S).In the present study, the floral traits *viz.*, panicle length recorded higher GCV and PCV. Moderate GCV and PCV were recorded in anther length, anther breadth, stigma exertion rate, glume angle and extent of out crossing %. Low GCV and PCV were recorded for panicle exertion rate, spikelet sterility% and pollen sterility %. Low GCV and PCV indicates narrow genetic base for these floral traits. Improvement of these characters can be achieved through gene transfer methods or using mutagens to widen the genetic base followed by selection in advanced generation. The variability in anther length, anther breadth, glume angle, panicle exertion rate, pollen sterility% was presented in Table.3.

In the present investigation, high heritability and genetic advance was observed for the traits anther length, anther breadth, stigma exertion rate, glume angle, panicle length and extent of out crossing percentage. High heritability and genetic advance indicates heritability is due to additive gene action and thus selection is effective. Heritability and genetic advance are important parameters of selection. Panicle exertion rate, pollen sterility and spikelet sterility showed high heritability and low genetic advance which indicates non-additive gene action. In plant breeding, correlation studies measures the mutual relationship between various plant traits and determine the component characters on which selection can be relied upon for genetic improvement. Association analysis plays the key role to find the relationship among different characters which can be direct or indirect effects on selection of desirable characters. Nature of correlation can often be altered by selection and hybridization. Therefore correlation between extent of out crossing and its component traits are important for the future selection programme. In the present study, panicle exertion rate is highly significant with stigma exertion rate (Table.4). Glume angle is significant with anther length and panicle exertion rate. Panicle length is positively correlated with panicle exertion rate, stigma exertion rate and glume angle. Pollen sterility is highly significant with panicle length and spikelet sterility. Extent of out crossing is positively correlated with anther length, panicle exertion rate, stigma exertion rate, glume angle, panicle length, spikelet sterility and pollen sterility (Fig.4).

The results of floral and morphological characterisation studies shows that TGMS lines *viz.*, TNAU 39S, TNAU 45S and TNAU 95S had stable pollen sterility expression with good desirable floral traits *viz.*, higher pollen sterility, wider glume angle opening, high stigma and panicle exertion rate for better extent of out-crossing rate and these lines can be utilized for two line hybrid rice breeding programme with high heterotic expression and superior yield.

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Table 1. Mean performance of floral and out crossing related traits of different TGMS lines.

Genotypes	Anther Length (mm)	Anther breadth (mm)	Panicle exertion rate %	Stigma exertion rate %	Glume angle (⁰)	Panicle length (cm)	Spikelet sterility %	Pollen sterility %	Extent of out crossing %
TNAU 39S	2.26	0.67**	91.49**	72.18	29.31**	25.61	97.52	99.07	37.48
TNAU 45S	2.32	0.72**	87.81	79.22**	31.82**	28.80**	100**	100**	42.81
TNAU 95S	2.48**	0.58	94.94**	85.51**	33.62**	35.18**	100**	100**	47.95**
TNAU 63S	2.11	0.49	82.41	63.42	23.70	17.56	96.08	95.51	32.57
TNAU 97S	2.22	0.57	86.35	68.16	23.05	22.33	100**	100**	39.66
TNAU 93S	1.66	0.48	87.72	78.31**	21.84	21.84	97.84	97.91	35.81
Mean	**2.17**	**0.58**	**88.45**	**74.47**	**27.22**	**25.22**	**98.57**	**98.75**	**39.38**
SED	**0.109**	**0.009**	**1.018**	**0.874**	**0.376**	**0.493**	**0.568**	**0.274**	**2.126**
CD (5%)	**0.244**	**0.020**	**2.268**	**1.947**	**0.838**	**1.098**	**1.266**	**0.612**	**4.737**

Table 2. Morphological and agronomic traits of promising TGMS lines

Traits	TNAU 39S	TNAU 45S	TNAU 95S	TNAU 63S	TNAU 97S	TNAU 93S
			Morphological traits			
Anther colour	Light yellow	White	White	Light yellow	White	Light yellow
Stigma colour	Purple	Purple	Purple	White	White	White
Basal leaf sheath colour	Light purple	Green	Green	Green	Green	Green
Leaf blade colour	Green	Green	Green	Green	Green	Green
Flag leaf angle	Erect	Erect	Erect	Erect	Erect	Horizontal
Auricle colour	White	Light brown	White	White	White	White
Ligule length (cm)	0.88	1.68	1.00	0.56	2.00	1.10
Ligulecolour	White	Brown	Light brown	White	White	White
Ligule shape	Acute	Acute	2-cleft	Acute	2-cleft	Acute
Collar colour	White	Brown	Light brown	White	White	White
Panicle type	Compact	Compact	Compact	Compact	Compact	Compact
Flag leaf (cm)	22.5	30.4	34.2	20.3	25.7	28.4
Apiculuscolour	Red	Red	Red	White	White	White
Agronomic traits						
Plant height(cm)	71	103	110	82	102	105
No. of tillers/plant	32	30	35	16	20	18
Days to 50% flowering	79	89	112	92	96	102
Chlorophyll content (SPAD reading)	47.4	35.7	36.3	29.9	33.9	28.5

Table 3. Estimates of parameters of variability for TGMS floral traits

Characters	GCV %	PCV%	ECV%	Heritability (h^2)	GA(%) of mean
Anther length	12.40	13.85	6.16	80.21	22.88
Anther breadth	16.39	16.50	1.92	98.65	33.53
Panicle exertion rate	4.81	5.02	1.41	92.11	9.52
Stigma exertion rate	10.81	10.91	1.44	98.26	22.08
Glume angle	18.35	18.43	1.69	99.16	37.65
Panicle length	24.44	24.56	2.39	99.05	50.18
Spikelet sterility	1.64	1.79	0.71	84.44	3.11
Pollen sterility	1.80	1.80	0.34	96.56	3.65
Out crossing %	13.27	13.27	6.61	80.12	24.47

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Table 4. Estimates of genotypic correlation among different TGMS lines for floral and outcrossing related traits

Floral traits	Anther length (mm)	Anther breadth (mm)	Panicle exertion rate	Stigma exertion rate	Glume angle(º)	Panicle length (cm)	Spikelet sterility %	Pollen sterility %	Extent of out crossing %
Anther length (mm)	1.000	0.652	0.511	0.210	0.836*	0.679	0.499	0.541	0.736*
Anther breadth (mm)		1.000	0.388	0.278	0.722	0.526	0.474	0.647	0.492
Panicle exertion rate (%)			1.000	0.810*	0.740*	0.904**	0.541	0.681	0.798*
Stigma exertion rate (%)				1.000	0.658	0.868*	0.621	0.607	0.825*
Glume angle (º)					1.000	0.898**	0.503	0.568	0.840*
Panicle length (cm)						1.000	0.725	0.747*	0.988*
Spikelet sterility %							1.000	0.941**	0.924**
Pollen sterility %								1.000	0.835*
Extent of Out crossing %									1.000

Significant at 5% level (*) and 1% level (**)

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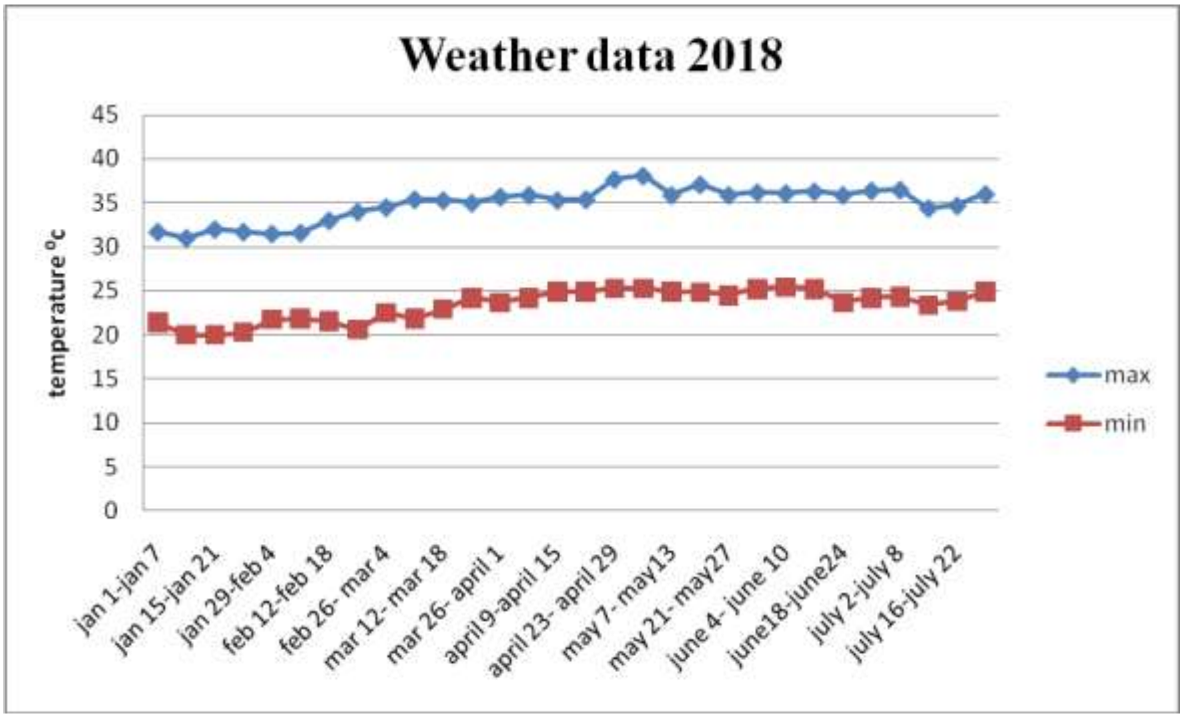

Fig.1. Maximum and minimum temperature observed at Killikulam.

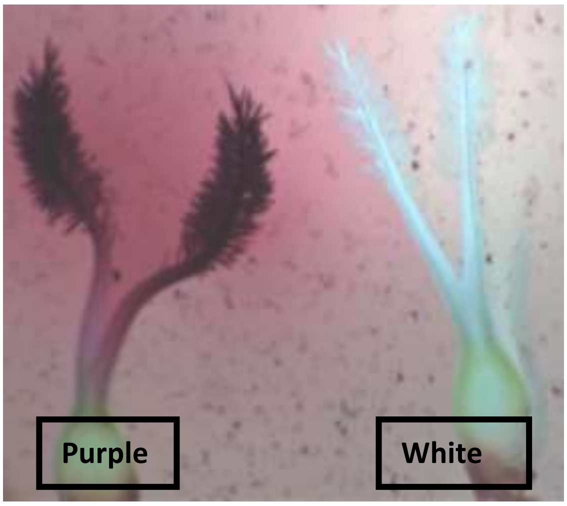

Fig. 2. Variations in stigma colour

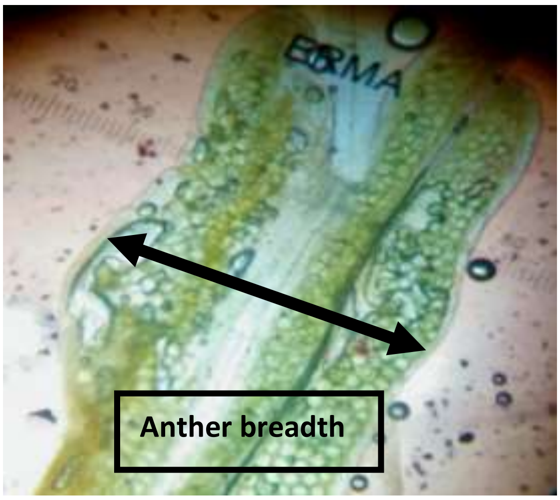

Fig. 3.Anther length and breadth measured using ocular micrometer

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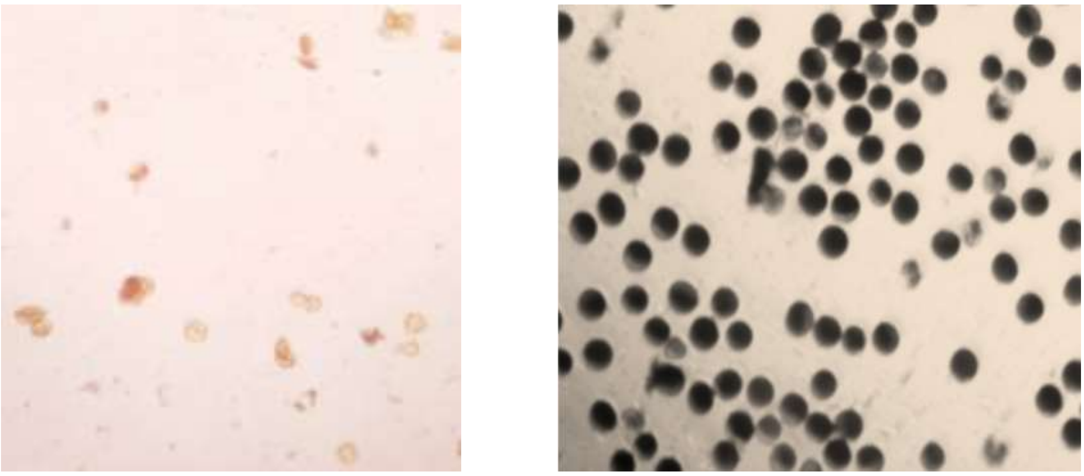
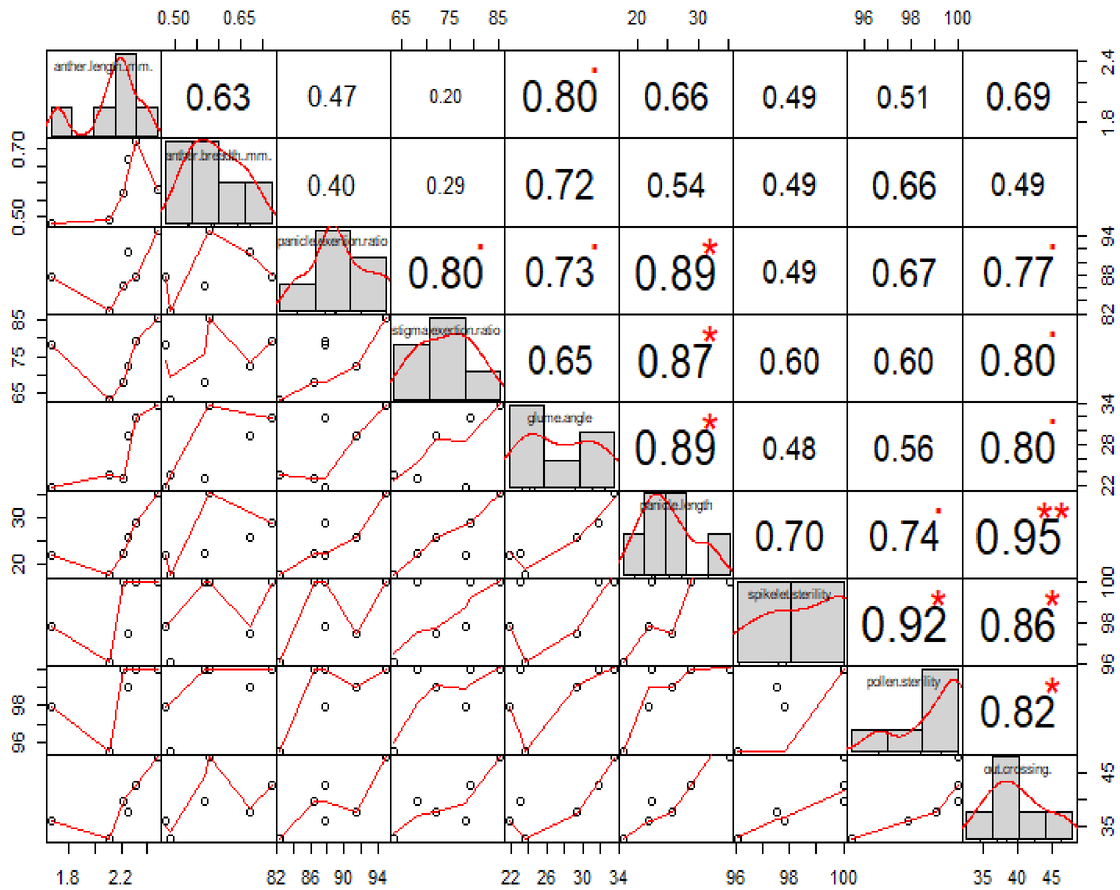


Fig.4. Pollen sterility/fertility expression in TGMS lines - unstained irregular sterile pollens and stained round fertile pollen grain.



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