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Influence of Weather Factors on Fertility Alteration in Thermosensitive Genic Male Sterile Lines of Rice (*Oryza Sativa* L.)

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ABSTRACT: *Four thermosensitive genic male sterile lines (TGMS)* viz., *TNAU 27S, TS09 12, TS09 15 and TS09 25 have been subjected to monthly sowing plan for the determination of Critical fertility temperature (CFT) and critical sterility temperature (CST). All four TGMS lines* viz., *TNAU 27 S, TS 09 12, TS 09 15 and TS 09 25 had observed with significant amount of positive correlation between pollen sterility with maximum and mean temperatures and all the eight panicle developmental stages from differentiation of first bract primordium (S_1) to pollen ripening stages (S_8) of the lines TNAU 27 S and TS 09 25 were sensitive to mean temperature. TS 09 15 had seven stages except S_3 of panicle development were sensitive to temperature whereas in TS 09 12 sensitive stages of panicle development for temperature were S_1 and S_4-S_8. TGMS line TNAU 27 S had CST and CFT as 25.95°C and 25.83°C respectively. The CST and CFT of TGMS line TS 09 12 was 26.45°C and 25.78°C. In TS 09 15 mean temperature 25.45°C were noticed as CFT and 25.80°C CST. The CST and CFT of TS 09 25 were 26.73°C and 26.58°C respectively. Among all lines TGMS line TNAU 27 S showed that the very longest period of sterile phase from March 22 to August 11(143 days) and more than fifty per cent self seed set during December 20 to February 23 (66 days). TS 09 25 registered longest period of fertile phase which can be effectively used for TGMS seed multiplication. Relative influence of weather factors on pollen sterility / fertility alteration behaviour will vary among different TGMS lines due to different source of male sterility genes and genetic backgrounds. All four lines exhibited sterile phase during March to April (around not less than 26 consecutive days) therefore hybrid seed production by using of these lines can be taken up in Coimbatore with flowering coinciding with the sterile phase. From this study, Coimbatore is ideal place for hybrid seed production during summer as well as TGMS seed multiplication during December to February.*

Key words: *TGMS lines, pollen sterility, correlation, CFT/CST.*

INTRODUCTION

Rice is life for more than half of humanity in the world. It is estimated that the world's rice production has to increase by about 65 per cent by 2030 to keep pace with the population growth. Hence meeting all these requirements hybridization is the important technique for breaking yield barriers [1]. Thermosensitive genic male sterility (TGMS) is a useful genetic tool for the development of two-line hybrids in rice [2]. At the thermo sensitive stage of panicle development, the TGMS gene(s) cause/s male sterility under high environmental temperatures and result/s in fertility under low temperatures [3]. The TGMS line, identified by Chinese and Japanese scientists, was completely sterile under high temperature (> 32°C) but was fertile under reduced or low temperature of < 24°C [4]. For successful exploitation of this novel male sterility system in heterosis breeding, TGMS lines need to be characterized for their sterility/fertility alteration behaviour in given environment. The foremost step to start two line breeding is characterization of TGMS lines and determination of fertility behaviour of particular line to find out critical sterility temperature (CST) and critical fertility temperature (CFT). Because TGMS lines are sterile in certain temperature regime there is need to determine CST and CFT. Characterization of TGMS lines with respect to their fertility/sterility alteration behaviour will provide clear cut idea of utilization of that particular line for predicting appropriate timings for hybridization

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programme (at sterile phase) as well as seed multiplication (at fertile phase).

MATERIALS AND METHODS

Field experiment

Four TGMS lines *viz.*, TNAU 27S, TS09 12, TS09 15 and TS09 25 were utilized for field experiment and TGMS lines were sown sequentially from the month of February 2010 to January 2011 at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. Pollen fertility was examined by using a 1 per cent iodine potassium iodide (I–KI) solution. All round and dark brown-stained pollen was scored as normal fertile and irregular-shaped, yellowish or light brown coloured pollen grains were scored as sterile. The panicles that emerged from the primary tiller were bagged before anthesis and the number of filled grains and chaffs in the panicle were counted at the time of maturity. The ratio of filled grains to the total number of spikelets was expressed as seed setting rate [5]. The fertility status was fixed as under following the IRRI Standard Evaluation System [6].

Pollen fertility (%)	*Fertility status*
60-100.00	Full fertile
30-59.99	Partial fertile
1-29.99	Partial sterile
0-0.99	Sterile

Weather factors

The weather parameters (viz., maximum temperature, minimum temperature, mean temperature, rainfall, and relative humidity and sunshine hours) during panicle development stage were considered and mean of these weather factors during the panicle development stage was calculated by counting back the days from date of heading of panicle (Table 1, [7]). The association of the weather parameters of each day from one to 26 days before heading, average of each factor of different stages of panicle development (S_1-S_8) and overall mean of each factor throughout these panicle development stages with pollen sterility has assessed by working out simple correlation coefficient (*r*).

Table 1
Panicle development stages and their durations in rice (*Oryza sativa* L.)

Stage number	*Stages*	*Duration (day)*	*Days before heading*
S_1	Differentiation of first bract primordium	2	25–26
S_2	Differentiation of primary branch primordium	3	22-24
S_3	Differentiation of secondary branch primordium	3	19–21
S_4	Differentiation of stamen and pistil primordium	4	15–18
S_5	Pollen mother cell formation	3	12–14
S_6	Meiotic division of pollen mother cell	3	9–11
S_7	Pollen filling	6	3–8
S_8	Pollen ripening	2	1–2

Source: Rangaswamy (1993).

Determination of critical stages of fertility alteration

The critical sterility temperature (CST-the temperature at which the line become sterile from fertile condition) and critical fertility temperature (CFT-the temperature at which the line become fertile from sterile condition) of all four TGMS lines were worked out. The period of complete pollen sterility was taken into account for determining the CST of each line. For the panicles that emerged in complete pollen sterility phase, the daily mean temperature during the panicle developmental stages (S_1-S_8) (Table 1) were worked out and lowest among these mean temperature was taken as the CST of that particular line. Similarly for determining CFT, the period of high pollen fertility was taken into consideration. For the panicles that headed in high pollen fertility phase, the daily mean temperature during the panicle developmental stages (S_1-S_8) were worked out and highest among these mean temperature was taken as the CFT of that particular line.

RESULTS AND DISCUSSION

Relative influence of primary weather factors such as maximum, minimum and mean temperature and secondary weather factors such as relative humidity, rain fall and sun shine hours on pollen sterility / fertility alteration behaviour will vary among different lines due to different source of male sterility genes and genetic backgrounds [8,9].

The influence of weather parameters on pollen sterility and fertility alteration behaviour of TGMS lines over different stages of panicle development were assessed by simple correlation.

Maximum, Minimum and Mean temperature

TNAU 27 S had significant association of pollen sterility with maximum temperature in S_1-S_4 stages of panicle development. Other three TGMS lines *viz.*, TS 09 12, TS 09 15 and TS 09 25 were observed positive correlation to almost all stages of panicle development (Table 2). The results of the study indicated that all

Table 2
Correlation coefficient of pollen sterility with different weather parameters in different stages of panicle development

Panicle development stages	*Maximum temperature*				*Minimum temperature*				*Mean temperature*			
	TNAU 27S	*TS 09 12*	*TS 09 15*	*TS 09 25*	*TNAU 27S*	*TS 0912*	*TS 09 15*	*TS 09 25*	*TNAU 27S*	*TS 0912*	*TS 09 15*	*TS 09 25*
S_1	0.517*	0.693**	0.703**	0.673**	0.617*	0.253	0.419	0.430	0.655*	0.539*	0.693**	0.651*
S_2	0.604*	0.689**	0.758**	0.621*	0.581*	0.199	0.548*	0.368	0.780**	0.451	0.727**	0.559*
S_3	0.616*	0.585*	0.580*	0.672**	0.582*	0.049	0.277	0.555*	0.685**	0.360	0.494	0.696**
S_4	0.688**	0.735**	0.686**	0.648*	0.726**	0.298	0.475	0.410	0.787**	0.653*	0.722**	0.647*
S_5	0.369	0.726**	0.792**	0.565*	0.703**	0.786**	0.834**	0.581*	0.672**	0.828**	0.914**	0.682**
S_6	0.456	0.593*	0.746**	0.588*	0.881**	0.736**	0.833**	0.540*	0.811**	0.723**	0.876**	0.674**
S_7	0.436	0.592*	0.678**	0.641*	0.793**	0.500*	0.834**	0.571*	0.691**	0.619*	0.828**	0.657*
S_8	0.451	0.564*	0.651*	0.556*	0.626*	0.353	0.618*	0.698**	0.639*	0.551*	0.741**	0.687**
Mean for 26 days	0.573*	0.702**	0.756**	0.687**	0.797**	0.437	0.691**	0.572*	0.778**	0.651*	0.823**	0.705**

Panicle development stages	*Relative humidity*				*Rain fall*				*Sun shine hours*			
	TNAU 27S	*TS 09 12*	*TS 09 15*	*TS 09 25*	*TNAU 27S*	*TS 0912*	*TS 09 15*	*TS 09 25*	*TNAU 27S*	*TS 0912*	*TS 09 15*	*TS 09 25*
S_1	-0.277	-0.147	-0.391	-0.549*	0.103	0.166	0.320	-0.512*	0.090	-0.008	-0.020	0.410
S_2	-0.171	-0.388	-0.325	-0.677**	0.240	-0.162	-0.266	-0.797**	0.090	0.304	0.038	0.329
S_3	-0.363	-0.607*	-0.270	-0.675**	-0.469	0.290	0.258	-0.302	0.363	0.451	0.012	0.296
S_4	-0.376	-0.409	-0.350	-0.524*	-0.148	-0.133	-0.202	-0.412	0.237	0.359	0.147	0.084
S_5	-0.113	-0.303	-0.245	0.082	0.195	-0.201	-0.133	0.449	-0.056	-0.133	-0.277	-0.176
S_6	0.145	-0.270	-0.268	-0.324	0.134	0.189	-0.235	-0.432	-0.365	0.331	0.090	0.051
S_7	-0.029	-0.486	-0.231	-0.380	0.169	0.155	0.040	-0.338	-0.407	0.304	0.160	0.082
S_8	0.336	-0.437	-0.412	-0.320	0.329	0.068	-0.309	-0.297	-0.201	0.320	0.218	-0.098
Mean for 26 days	-0.150	-0.497*	-0.366	-0.533*	0.143	0.022	-0.171	-0.508*	-0.049	0.331	0.067	0.156

n =12; ** Significant at 1% level; * Significant at 5% level.

the eight panicle developmental stages from differentiation of first bract primordium (S_1) to pollen ripening stages (S_8) of the lines TNAU 27 S and TS 09 25 were sensitive to mean temperature. These results are in conformity with the findings of many scientists [10, 11 and 12]. TS 09 15 had seven stages except S_3 of panicle development were sensitive to temperature whereas in TS 09 12 sensitive stages of panicle development for temperature were S_1 and S_4-S_8.

TNAU 27 S, TS 09 12, TS 09 15 and TS 09 25 exhibited relative influence of maximum and mean temperature on pollen sterility with positively significant values. Except TS 09 12 other three lines had significant influence of minimum temperature on pollen sterility. This is in accordance with the findings of many research papers [13, 14, 15]. Pollen fertility was strongly and significantly correlated with the mean temperature, maximum temperature and minimum temperature during sensitive development stages of TGMS lines. Sensitive panicle development stages were from S_3 to S_6 and that continuous high temperature was necessary during these sensitive stages [16].

Relative humidity, Rain fall and Sunshine hours

Negatively significant correlation between relative humidity and pollen sterility was observed in TGMS line TS 09 12 during S_3 stage of panicle development (Table 2). TS 09 25 was noticed with significant correlation in negative direction during panicle development stages of S_1-S_4 for relative humidity and S_1 and S_2 stages for rainfall (Table 2).

Relative humidity and rain fall recorded the negative significant on pollen sterility in TS 09 15 and TS 09 25. These results are confirming the findings of various research papers [15, 16]. TNAU 27 S and TS 09 12 had no influence by secondary factors such as relative humidity, rain fall and sun shine hours) on fertility alteration. There no correlation has observed between fertility status and sunshine hours.

Sterile and fertile phase alteration

The present investigation, the TGMS line TNAU 27 S showed that the very longest period of sterile phase from March 22 to August 11 (143 days) (Table 3 and Plate1a & 1b) and more than fifty per cent self seed set during December 20 to February 23 (66 days)

Table 3
The critical sterility temperature (CST) and critical fertility temperature (CFT) of TGMS lines

TGMS lines	*Critical stages of thermo sentiveness*	*Sterile phase and duration*	*Pollen Sterility (%)*	*CST (°C)*	*Fertile phase and duration*	*Pollen fertility (%)*	*Spikelet fertility (%)*	*CFT (°C)*
TNAU 27 S	Differentiation of first bract primordium (S_1) to pollen ripening (S_8)	Mar 22 - Aug11 (143 days)	100.00	25.95	Dec 20 - Feb 23 (66 days)	56.26-66.78	42.53 - 58.69	25.83
TS 09 12	Differentiation of stamen and pistil primordium (S_4) to pollen ripening (S_8)	Apr 6 - Jun 1 (55 days)	100.00	26.45	Jan 4 - Feb 6 (34 days)	56.00-85.39	51.24- 76.69	25.78
TS 09 15	Differentiation of stamen and pistil primordium (S_4) to pollen ripening (S_8)	Apr 4- May 1 (26 days)	100.00	25.80	Jan 3 - Feb 6 (35 days)	61.22- 63.77	53.42- 57.21	25.45
TS 09 25	Differentiation of first bract primordium (S_1) to pollen ripening (S_8)	Mar 26 - Apr 27 (35 days)	100.00	26.73	Oct 25- Feb 27 (126 days)	53.39- 83.95	38.53- 62.35	26.58

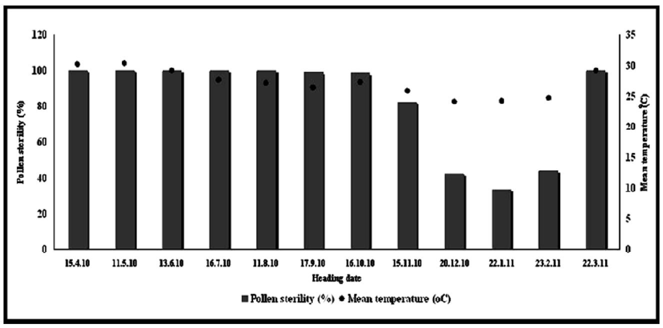


Figure 1: Fertility alteration behavior in TANU 27 S

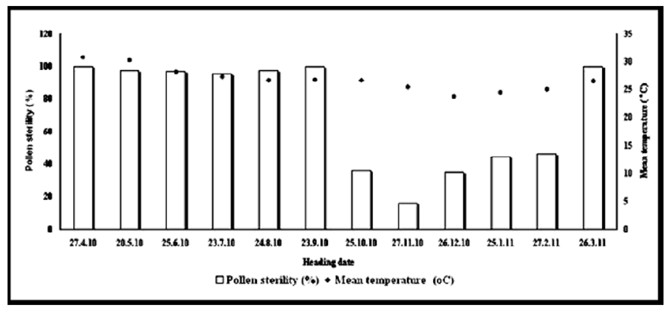


Figure 4: Fertility alteration behavior of TS 09 25

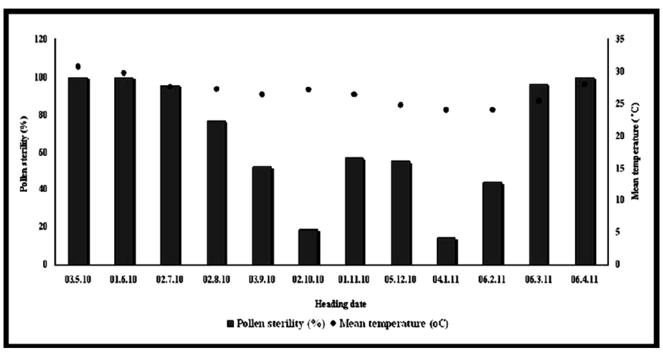


Figure 2: Fertility alteration behavior of TS 09 12

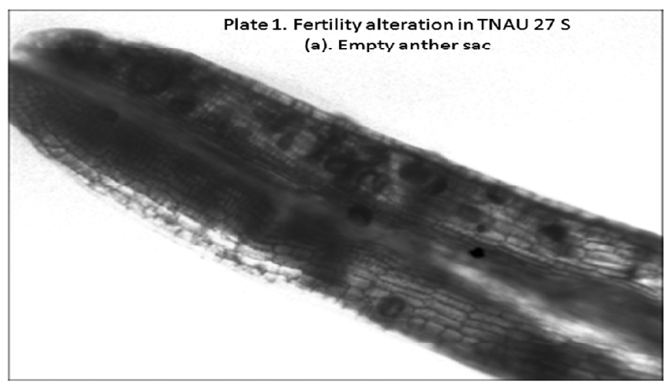


Plate 1. Fertility alteration in TNAU 27 S
(a). Empty anther sac

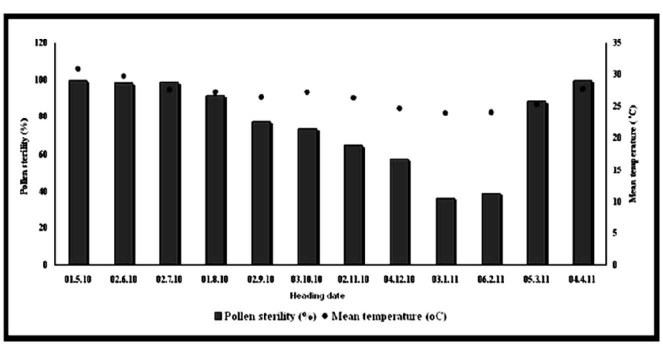


Figure 3: Fertility alteration behavior of TS 09 15

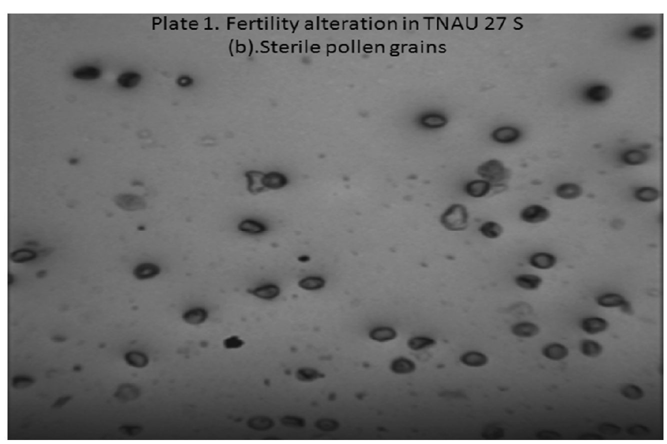
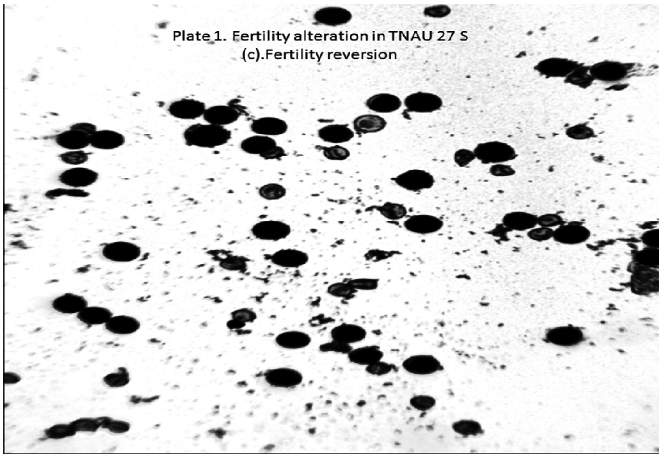


Plate 1. Fertility alteration in TNAU 27 S
(b).Sterile pollen grains



(Fertility reversion- Plate 1c) in Coimbatore condition (Fig 1, 2, 3, & 4). This line will facilitate to conduct hybridization programme for longer period in a year.

The temperature sensitive genic male sterility (TGMS) system is considered more useful than the photo period-sensitive genic male sterility (PGMS) system in breeding two-line hybrids under tropical conditions, where day length differences are marginal. TGMS lines required temperature of 28°C for expression of complete pollen sterility [17]. The TGMS lines which possess high pollen sterility under higher temperature condition and 30 per cent self seed set under low temperature conditions are considered as promising TGMS lines for commercial exploitation [17].

All four lines exhibited sterile phase during March to April (around not less than 26 consecutive days) therefore hybrid seed production by using of these lines can be taken up in Coimbatore with flowering coinciding with the sterile phase. Safest period of hybridization programme and TGMS seed multiplication for each line is given in Fig 5. From this study, Coimbatore is ideal place for hybrid seed production during summer as well as TGMS seed multiplication during December to February. In Tamil Nadu, Gudalur is the ideal place for TGMS seed multiplication due to prevailing of lower temperature. Care should be taken to prevent heading date of TGMS lines coincidence with monsoon period because rainy season usually occurs with low temperature and cloudiness (less sunshine hours) which may further induce the fertility in TGMS lines leading to impurity of hybrid seed.

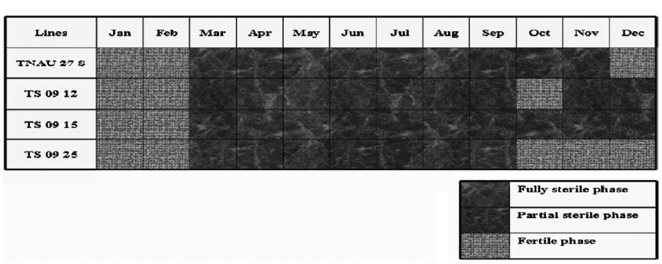


Figure 5: Safe periods of TGMS seed multiplication and hybrid seed production

Determination of CST and CFT of TGMS lines

In rice exploitation of heterosis by use of TGMS lines involves certain extent of risk will be expect if temperature fluctuations occur at critical stages of panicle development [18]. Therefore knowledge on sensitivity on critical panicle developmental stages to temperature will widen the way to predict suitable sowing time of TGMS lines for seed multiplication and hybridization operation. Sensitivity of weather factors on later stages of panicle development is very significant [19].

TGMS lines greatly differ in their sensitivity to temperature fluctuation. Only those lines with lower critical sterility point and those, which do not revert back to fertility with slight decrease in temperature are useful to develop two line hybrids [20]. This CST and CFT were found to vary in different TGMS lines as the TGMS gene of these lines are transferred into different genetic background. The critical temperature inducing sterility is relatively low (23°C) in temperate zone and 24°C in sub-tropics [21].

The present investigation revealed that the TGMS line TNAU 27 S had CST and CFT as 25.95°C and 25.83°C respectively. The critical sterility temperature (CST) and critical fertility temperature (CFT) of TGMS line TS 09 12 was 26.45°C and 25.78°C. In TS 09 15 mean temperature 25.45°C were noticed as critical fertility temperature and 25.80°C critical sterility temperatures. The CST and CFT of TS 09 25 were 26.73°C and 26.58°C respectively (Table 3).

All four TGMS lines had CST as 25.80°C (TS 09 15) and above. The range of difference between CST and CFT was 0.12°C (TNAU 27 S) to 0.7°C (TS 09 12). The difference between CST and CFT of TGMS lines were narrow. The narrow difference is undesirable, since there is a possibility of fertility reversion due uncommon prevalence of low temperature during hybridization programme. The narrow differences between CST and CFT of TGMS lines were also reported by many workers and they opined that low temperatures that would transform TGMS lines into fertile ones will not occur frequently during high temperature seasons. Even if it occurs, it would last for only a few days, thus the purity of hybrid seed will not be affected [21].

The TGMS lines possessed with high critical temperature ie. more than 27° C is not suitable for commercial exploitation, since a short fall of temperature during summer season may cause fertility reversion and result in selfed seed setting in TGMS lines [19, 22, 23]. Fortunately the TGMS lines undertaken for present study had critical temperature range less than 27° C. Therefore, the present investigation gave the conclusion of TGMS lines *viz*., TNAU 27 S, TS 09 12, TS 09 15 and TS 09 25 have clearly well defined sterile and fertile phases as well as these lines were exhibited a rhythmic pattern of fertility alteration in a year around. These lines were can utilize for seed multiplication during December to February months and hybridization during summer. Hence, these lines can be effectively utilized for commercial exploitation of hybrids in the future.

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