RESEARCH PAPER

Demarcating day-light-length from temperature effects in PGMS rice using back tracking method

Njiruh Paul Nthakanio[1*], Xue Qingzhong[2]

[1]Embu University College, Department of Agricultural Resource Management. Po Box 6-06100, Embu, Kenya

[2]Zhejiang University[2], Huajia Chi Campus, Agronomy Department. 310029, Kaixuan Road, China

Abstract

Two near isogenic rice lines ZAU11S106 (Photoperiod sensitive genic male sterile) and ZAU11F121(a ZAU11S106 whose PGMS gene has undergone reverse mutation to become a non-PGMS) were used. These two lines were studied to define the effects of temperature and day light length on PGMS gene expression using *forward gene expression tracking* method. In this method, ZAU11S106 and ZAU11F121 were divided into 8blocks into which they were grown up to mordial stage. This was a growth stage before the pollen matured to become fertile or sterile, a point called critical sterility point (CSP). At this growth stage, the first block was exposed to short day light length (SDLL) treatment. After four (4) days first and second row were put under SDLL treatment. A row was included under the treatment after every four days till the first row headed after which the treatment was withdrawn and all rice allowed to grow up to maturity under long day light length (LDLL) and high temperature growth conditions. The PGMS grown under long day and high temperature growth conditions had higher spikelet sterility than those grown under influence of long daylight length and lower temperature growth conditions. Thus, high temperatures complement photoperiod in inducing sterility in PGMS rice.

* **Corresponding Author:** Njiruh Paul Nthakanio ✉ njiruhpaul@gmail.com

Introduction

Use of hybrids has become a major way of increasing rice yield due heterosis. To enable crossing breeding without self-pollination, the male gametes in female parents need to be emasculated (Mao, Deng, 1993). Among the common methods used to emasculate male gametes in female parents include; the use of cytoplasmic male sterility (CMS) and the Photoperiod/Thermo sensitive genic male sterility genes. Photoperiod-sensitive genic male sterile (PGMS) rice is sterile in long daylight length and fertile in short day light length growth conditions (Shi 1981; 1985; Shi and Deng 1986) while TGMS rice is sterile in high temperature and fertile in low temperature growth conditions (Ali *et al.,* 1995). Since the expression of PGMS and (Thermo-sensitive genic male sterile (TGMS) genes are environmentally controlled the varieties bearing the traits are referred to as environmental genic male sterile (EGMS) lines. One of the major challenges in use of EGMS lines is the inability to get complete sterility in the sterile phase (Shu *et al.,* 1996) that can lead to contamination of hybrid by inbreed seeds. So, there is need for timely rice exposure to the right day length and temperature to ensure complete sterility especially when it is done under natural (uncontrolled) conditions. The right time of exposure is possible if photosensitivity of PGMS rice is understood. PGMS has two photoreactions (Yuan *et al.,* 1993). The first-photoreaction (FPR), which is common in all rice varieties, is only responsible for vegetative growth and it is important in hastening growth and plants heading while the second photo-reaction (SPR) is the fertility determining reaction. Knowledge on individual effects of photoperiod and thermo or the combination of the two on SPR is important to enable timing of sowing of PGMS rice lines and hence cross breeding.

Quantitative methods have been employed in breeding to evaluate traits, especially those of quantitative nature. By use of conditional models it has been possible to demarcate intricate genetic effects such as epigenetic quantitative traits (Wu *et al.,* 2001). Within the principles of the model partitioning the photoperiod and temperature effects in PGMS ZAU11S106 was undertaken in this research with a view of determining individual effects. This will enable proper categorization of environmental genic male sterile (EGMS) lines into PGMS and TGMS. The importance of this is to distinguish between TGMS lines (that are largely controlled by temperature) and PGMS lines (that largely are under photoperiod control) (Ku *et al.,* 2001).TGMS lines have unstable male sterility and fertility reversibility due to temperature fluctuation (He *et al.,* 1999), leading to contamination of hybrid seeds by inbred seeds during hybrid seed production. Another challenge is that, in the tropical regions PGMS may not adequately replace the traditional cytoplasmic male sterile (cms) lines because photoperiod may not be long enough to induce complete sterility. Thanks to the development of TGMS that are adaptable to tropical rice growing regions thus, enabling development of two-line method of producing hybrid rice seeds (Virmani 1996, Lopez and Virmani, 2000, Latha and Thiyagarajan, 2010, Kanya *et al.,* 2013).

Although sterility in PGMS is largely under photoperiod control, temperature too has some effects. High temperature reduces the photoperiod required to induce complete sterility (Yuan *et al.,* 1993). Given interaction effects of these factors, more information on the precise time when second photoreaction (SPR) occurs is essential in ensuring proper timing of sowing dates under natural conditions. The most sensitive stage of fertility/sterility induction in PGMS rice is at the dyad stage of meiosis (Njiruh and Xue, 2013). Partitioned effects, indicates that photoperiod and temperature interactions tend to have a major influence on the sterility/fertility induction in PGMS compared to individual factor effects. The study was aimed at quantitatively determining photoperiod, temperature and their interaction effects in inducing sterility in PGMS rice. This will facilitate synchronization of sowing so that the PGMS rice critical sterility point coincides with high enough temperature and long enough photoperiod that ensures complete male

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sterility is realized for female parent at the time of cross pollination in hybrid rice seed production

Materials and methods

Plant materials

A PGMS lines ZAU11S106 and a control ZAU11F121 were sown on the 18th March, 4th May and on 14thMay at Zhejiang University experimental fields at Hangzhou in China, 30°15N. Sowing was programmed so that the heading took place in summer time during the long day light length and high temperature growth conditions. Here long day refers to at least 13hour day light including morning and evening twilight which reign Hangzhou region within the months of July and August while natural short day refers to below 11hour day light length including the morning and the evening twilight within the month of September. Natural high temperature refers to the summer temperatures between July 1st and August 30th (>33°C and >26°C day and night temperature respectively and low temperature refer to between 26°C and 33°C day time and 20°C to 26°C night time temperatures respectively.

Methods

Short day Treatment

To induce short day light length conditions while retaining the high summer temperatures, rice plants were covered with an opaque black cloth to completely block out the light. Line ZAU11S106 and the control ZAU11F121 were sown in rows each with six plants. Among the six plants three were ZAU11S106 and the other three were ZAU11F121. Inter-row spacing was 30cm and inter-plant spacing was 15cm. Plants sown on May 14thwere left to grow under natural conditions for 57 days after sowing and thereafter, short day treatment was started. Treatment started on July 11that the stage when the plants were 5 to 4 leaves before heading. Each day the plants were covered at 15.30hour (afternoon) and uncovered when darkness set in, thus giving them 11hours of daylight and 13 hours of darkness. At the beginning (zero up to four days) row one (R1) was covered, from 4th up to 8th day rows R1 and R2, were covered and from 8th to 12thdays rows R1, R2 and R3

were covered. After every four days next line was included in SDLL treatment until plants in the first row headed after which the treatment was withdrawn. This is what was called *forward tracking* method. Other treatments were;

i) Short day light length (SDLL) treatment of plants sown on March 18th:They were given SDLL treatment starting from July 20th. At this time, the flag leaf had just emerged and 15 days to heading time of the control (untreated line sown at the same time).

ii) SDLL treatment of plants sown on May 4th: They were given SDLL treatment starting from July 17th. This was the time the plants were 1 to 2 leaves before heading or 30 days to heading of the control. Short day treatment continued until the plants headed. Covering was done as described above. A set of plants were covered starting between 15.30 hour until darkness then uncovered while the other set was covered at night and uncovered at 8.30am in the morning. Staggered sowing was to enable realization of plants at four leave, two leave and flag leave nearly at the same time and during long day light length growth conditions. This also enabled calculation of natural day light length effects on heading and fertility of PGMS rice.

Fertility test

Pollen fertility was tested using 1 % I/KI. A day after heading a piece of spikelet with three glumes was excised from each plant and fixed using Canoys solution II. Anthers extracted from the sampled glumes were placed on a cover slide with 1% I/KI and macerated using forceps to release the pollen then observed under a light microscope. After maturity the ears/spikes were harvested to determine the seed set rate. Spike fertility was determined by counting the number of seeds per spike divided by the total number of glumes times 100.

SDLL treatment and induction or transduction of maturity/fertility

Induction was taken to be a situation where SDLL treatment needed to persistently be maintained until pollen matured to obtain complete sterility. On the other hand, transduction was taken to be a situation

where SDLL treatment was needed only at critical sterility point (CSP) to obtain complete pollen sterility. To determine if panicle/fertility initiation was by transduction or induction the SDLL treatment effects on plants sown on 18th March and 4th May were compared with those sown on May 14th (treatments described above) and in Table 1. Sowing on 18th March was to allow materials to grow under a natural short day (March to May) and mature in a natural long day light length growth conditions.

Calculations, formulas and Statistical analysis

All analysis were done using Excel computer package (office for windows 2008).

1. Promoter strength (r)= Ri%-(Ri+1)%/ SDLL treatmentin days. The interval between treatment was 4 days, where i=1, R=row; R1, R2 ....Rn.

2. Photoperiod sensitivity and fertility rate, Psf=reduction or increase in fertility by 1%at a given photoperiod and temperature.

3. Photoperiod sensitivity and maturity duration (PSm) is change in maturity at a given photoperiod and temperature. $=\Delta$maturity (days)/Δ day-light length (hours).

Model building

At 100% sterility of the PGMS the long day (LD) and high temperature (HT) fertility was zero (0) and this was called C. Under farm condition short day(SD) conditions could be induced by covering the plants to induce near darkness conditions. This was called short day (SD) and high temperature (HT) treatments.

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SD + HT + error = A =Δ= Z.
LD         +  HT    = o=C    Δ =Z    short day(SD) effect

SD    +    HT    =A
SD    +    LT    =B          Δ=Y     low temperature (LT) effect
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In field conditions seed set rate values due to short day and low temperature were referred to as SD+LT effects. The difference between (SD+LT) and (SD+HT) could be attributed to low temperature (plus interactions) hence;

(SD+LT)-(SD+HT)=(LD+LT)= Δ=Y

B-A =Y

Total Effect =Environmental effects+ Genotype effects = Te=Ee+Ge   (1)

Ee =(A-C) + (B-A) = Z + Y -Ge

Te = (A-C) + (B-A) + Ge = Z + Y + Ge   (2)

Within the changes due to photoperiod, there were changes due to genotype i.e. different genotypes will react differently and therefore, genotype effects was due to photo (p) and thermo (t) sensitivities.

Ge = p+ t

For now it is difficult to separate the Ge from Ee, so, unless otherwise stated Te will be taken to be phenotypic effects (Pe). Photo factors were divided into two; photoperiod p(d) and photo-intensity p(i).Thus p=p(d)+p(i). Photoperiod was further divided into photoperiod within low temperature (p(d)(LT) and photoperiod within high temperature (p(d)(HT) while photo-intensity p(i) can be subdivided into photo-intensity within low temperature (p(i)(LT) and photo-intensity within high temperature (p(i)(HT). Therefore;

p=p(d)(LT)+p(d)(HT)+p(i)(LT)+p(i)(HT).   (3)

Thermo factors were also divided into two namely; Thermo-period t(d) and Thermo-intensity t(i). Therefore t=t(d)+t(i).Thermo-period was further divided into thermo-period within short day t(d)(SD) and thermo-period within long day t(d)(LD) while thermo-intensity p(i) can be subdivided into thermo-intensity within short day t(i)(SD) and thermo-intensity within long day t(i)(LD).Therefore;

t=t(d)(SD)+t(d)(LD)+t(i)(SD)+t(i)(LD)   (4)

Therefore PE due to photo and thermo reactions can be written as;

Phenotype effects (PE) = [p(d)(LT)+p(d)(HT)+p(i)(LT)+p(i)(HT)][t(d)(SD)+t(d)(LD)+(t(i)(SD)+t(i)(LD)]   (5)

Results

Effect of short day treatment on fertility

SDLL treatment and high temperatures (HT) growth conditions before critical sterility point (CSP) was

found to induce seed set in PGMS (Fig.1). This was illustrated in the R1 to R4treatments that yielded higher seed set rates than in later treatments. Plants treated for 12 days or more had over 16% seed set rate and over 42% pollen fertility. SDLL treatment for 12 days gave a seed set rate of 20.48%, which was the highest in this category of treatment. When plants were treated for 8 and 4 days, the seed set rate was 6.42% and 4.9% and pollen fertility rate was 0% and 4% respectively. The untreated ZAU11S106 control

had a seed set rate of 4.84%. In ZAU11F121 (fertile line) all plants treated as well as the control had over 38% seed set rate (Fig.2). ZAU11F121 plants treated for 24 days had the lowest (38.53 %) while those treated for 8 days had the highest (68.74%) seed set rates. The uncovered control of ZAU11F121 had over 50% seed set rate. ZAU11F121 subjected to 8 and 4 days of SDLL treatment had a seed set rate of 63.6% and 68.74% respectively.

Table 1. Comparison of SDT for March and May Sowing. T=treatment, cK control, Field = plants growing in the field. Covering days were days of short day treatment. Days to heading were time (in days) from sowing to the time the panicle emerged out of the flag leaf.

Sowing date	Variety	Heading date	Covering days	Seed set	Pollen fertility	Days to heading
18th March	S106 T	18th August	16	0	0	139
18th March	S106 Ck	18thAugust	0	0	0	138
14th May	S106 T	4th August	24	16.25	40.83	81
14th May	S106 cK	26th August	0	4.85	6.19	103
18th March	S121cK	16th July	0	67.48	93	120
18th March	S121T	27th July	0	69.06	88	121
14th May	S106 Field	27th August	0	9.26	5.91	104

Table 2. Comparison of SDT on ZAU11S106 and ZAU11F121. These were sown in May 4th sowing. Covering days were days of short day light length treatment. Days to heading were time (in days) from sowing to the time the panicle emerged out of the flag leaf. T=treatment, cK control, Field = plants growing in the field.

Sowing date	Variety	SDT days	Seed set rate	Pollen Fertility	Days to heading
14th May	ZAU11S106 T	4	4.9	0	100
14th May	ZAU11S106 ck	0	4.84	23.3	103
4th May	ZAU11S106 T	17	0.46	13	99
4th May	ZAU11S106 cK	0	1.19	4.74	108
4th May	ZAU11F121 T	17	62.23	87	92
4th May	ZAU11F121 T	0	77.6	94.33	88
14th May	ZAU11S106Field	0	9.26	5.91	104

Plants given SDLL treatment at flag and 2-leaf stage had 0% and 0.46% seed set respectively (Table.1&2) for ZAU11S106 and for the control (untreated ZAU11S106) had an average of 1.19% (Table 2). SDLL treatment led to pollen fertility of 13%and 4.74% for treated and for untreated (control) respectively. Plants treated at flag and two leave stages recorded lower seed set rate than the untreated. For this group of plants, SDLL treatment had a gene expression

power of -0.043% while four days treatment had 0.015% (Table3). Seed induction for treatments at two leave stage exceeded the control by only 0.06% (Table3). Most of the pollen in the treated plants were light yellow when stained with 1% I/KI, unlike innormal fertile parents which, stained blue/black.

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Table 3. Estimation of gene power index. SSR =seed set rate, difference in see between SD treated and control; Index1 =SSR/days of treatment; DH (heading date)= difference in seed set between SD treated and control; Index2=DH/days of SDT.

Variety	Treatment time Days	SSR	Index1	DH	Index2
ZAUS106	4	0.06	0.015	-3	-0.75
ZAUS106	17	-0.73	-0.043	-9	-0.132
ZAUF121	17	-15.37	-0.904	4	0.235

Responsiveness of fertility to short day treatment

Responsiveness of fertility to SDLL treatment was analyzed and it was found that 24 day treatment had a responsiveness of zero, same as no treatment at all. The responsiveness of fertility to SDLL treatment was highest in R5 (8days of treatment) (Fig.3a). At this time change in total fertility was highest. Using the effect of SDLL treatment on fertility, attempt was made to estimate the PGMS gene(s) promoter strength. Promoter strength=increase or decrease in fertility due to a given increase or decrease in photoperiod and temperature. It was realized that promoter was most responsive at R5. Gene promoter strength (r)= Ri%-(Ri+1)%/SDLL treatment in days (Table 5). This was also used to determine photosensitivity and it was realized that the most photosensitive point was at R5 that had a gene induction effect (Ie)=PS=3.5 fertility/day (Table 5). Temperature change before the critical levels (see materials) in all SDLL treatment was assumed to be 0. From graphs Var, ITE and AV and the r values all had a peak at R5 and a smaller one at R3 (Fig. 4).

Table 4. Short day light length treatment of ZAU11S106.R- SDT treatment in days, CH- Days of SDT to heading date, Var-variation in days from one heading to the next, SR- seed ser %, CD- covering (treatment) days, PF- pollen fertility, DH- period from sowing to heading (in days).

Treatment (Days) R	Days from stop of SDT to heading CH	Variance(Days) Var	Seed set (%)SR	Covering Days CD	pollen fertility (%)PF	Days to heading DH
R1	0.00	0.00	16.25	24.00	42.50	81.00
R2	0.00	0.00	18.41	20.00	44.71	81.00
R3	7.00	7.00	18.47	16.00	58.75	88.00
R4	13.00	6.00	20.48	12.00	72.25	94.00
R5	18.00	5.00	6.42	8.00	0.00	99.00
R6	19.00	1.00	4.90	4.00	0.00	100.00
R7	22.00	1.00	4.84	0.00	6.19	103.00

Effects of short day treatment on heading date

Effects of various short day treatments are shown in Fig.1 and 2. In Fig.1 plants given a 24 and 20 short-day light length (SDLL) treatments took 81days to head for both ZAU11S106 and ZAU11F121 (Ck). This was 22 days earlier than the control in wire mesh and 23 days earlier than those in the field. After 8 days starting from 11th July, any delay in short day treatment resulted into a delay in heading. For instance plants given12days of SDLL treatment took 88 days while those given the treatment for 20days took 81days to heading (a difference of 7days).ZAU11F121 plants given 24 and 20 days SDLL treatment took 81days to head just like in ZAU11S106. Also less than 12 days SDLL treatment had no effect on heading time for ZAU11F121 (compared to untreated control). SDLL treatment at flag leaf stage hastened heading for ZAU11S106 by a day (Table1). When ZAU11S106 plants were SDLL treated at two-leaf and flag-leaf stages, heading was hasted by 9 days. SDLL treatment for ZAU11F121 at flag leaf stage delayed heading by 1day but at 2-leaf stage it hastened heading by 9 days (Table2). However, ZAU11F121treated and Ck headed earlier than the ZAU11S106.

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Responsiveness of maturity rate to SDLL treatment

Treatment R1 and R2 had no difference in responsiveness but, treatment R3 had the highest individual effect (Fig.3b). Plants given SDLL treatment for 24 and 20days at R1 and R2 respectively, headed at day 81 after sowing but the ones given treatment for 16days (in R3) took 88days.SDLL treatment of less than 16days reduced maturity time although at a reducing rate. Results described above were compared with plants sown on March 18th and May 4ththat were given SDLL treatment at flag-leaf and two-leaf stage respectively

before heading (Table1).Heading for plants sown on May 14thplus SDLL treatment took 22days earlier than the CK (Fig.1). ZAU11S106 sown on May 14thand those sown on March 18th headed same time despite 58 day age difference. The untreated ZAU11F121 sown on March 18th matured later than the treated(Table 1). Plants sown on May 14th plus 4days of SDLL treatment matured same time with those sown on 4th May under natural conditions. However, plants sown on May 14thplus 4days of SDLL treatment matured earlier than the untreated by 3 days but one day later than the ZAU11S106 sown on May 4th.

Table 5. Responsiveness of fertility to SDLL treatment Var=TEi - TE+1 IE= Individual effect = Var divide by 4; AV= Var divide by to days of treatment TE=total effects.

SDLL Ttreatment	TE	Var	IE	AV
R1	16.25	-2.16	-0.54	-0.09
R2	18.41	-2.16	-0.54	-0.108
R3	18.47	-0.06	-0.015	-0.00375
R4	20.48	-2.01	-0.503	-0.1675
R5	6.42	14.06	3.5	1.7575
R6	4.9	1.52	0.38	0.38
R7	4.84	0.06	0.00	0.00

Relationship between FPR and SPR

First photoreaction(FPR)come earlier than the second photoreaction(SPR) in the rice growth cycle. SDLL induces faster maturity and higher fertility. SDLL treatment after critical fertility did not induce fertility but could hasten maturity (Table 1 and 2). ZAU11S106 and ZAU11F121 given SDLL treatment matured at the same day but in the untreated ZAU121F matured 9 days earlier than ZAU11S106.

SDLL effects transduction and induction

The ability to transmit SDLL effects (after withdrawal of treatment) to later growth stages is what was called "transduction power". Effort was made to determine effects of SDLL treatment transduced after the stopping the SDLL treatment. This was determined by calculating the shortening of the maturity period of treated compared to Ck starting from the inception of treatment. The power was highest between the treatments R3 and R5 (Fig. 3a). R2 is the point where the rice starts to be sensitive to SDLL treatment. Treatment earlier than this had no added advantage in hastening maturity. In Fig.4,AV shows the power

transduced across the growth period since the start of treatment, while Var shows the power of transduction after treatment was stopped. From R1 to R4 the power tends to increase, thus SDLL treatment reduced maturity period at an increasing rate at R4 after which it started to be less sensitive.

Plants given SDLL treatment at flag-leaf had pollen fertility and seed set rate of 0% while the treatment at 2-leaves before heading had a pollen fertility of 13% and a seed set rate of 0.46% (Table 2). At two leaves to heading the SPR had taken place and plants were irreversibly sterile. These plants took 99 days to head and 9days earlier than the untreated ZAU11S106 (control).

Fitting the Fertility model

SD+HT =A = 16.25% seed set

Long day + High temp. =C = 0% seed

SD +LT =B = 63.99% seed set

SD+LT-[(SD+HT) +error(temp)]= (LD+LT)=Y

63.99 -16.25 = 47.74 (temp effect)

SD+LT also include SDxLT (interaction). Using Xue

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(1995) to derive a factor to demarcate the two, it was realized that LD+LT = 11.19% therefore, SDxLT= (47.74-11.19)= 36.55%. The multiplication sign is used to mean interaction. Therefore, [(SD+HT)+(SDxHT)]+ [(LD + LT)] +[(SD+LT)+(SDxLT)]= [16.25%+ 0] +[11.19% +36.55%]= 63.99%.

$$p = p(d)(LT)+p(d)(HT)+p(i)(LT)+ p(i)(HT) \quad (3)$$
Phenotype effects =p+ t=16.25%+47.74% or 16.25%+11.19% +36.55%= 63.99%.

P = p(d)+ p(i)= 16.25. Note that it is difficult to determine photo-intensity so, for now it will be assumed that it is =0, p(i)(LT) = 0 (the fertility is =0)

$$p= p(d)(LT)+ p(d)(HT)+ p(i)(LT)+ p(i)(HT)(3)$$

Thus, p=16.25 + 0 + 0 + 0

$$t = t(d)(SD) + t(d)(LD)+ (t(i)(SD) + t(i)(LD) \quad (4)$$

Data reported by Xue (1995) was used to calculate temperature co-efficient T = 0.9053t(d) + 0.0947t(i) = 47.74.

T(d) = 43.219022 = t(d)(SD) + t(d)(LD) (this was taken to be zero because it was difficult to partition).

i)   T(i) = 4.520978 = t(d)(SD) + t(d)(LD).

ii)   Phenotype effects = [p(d)(LT) + p(d)(HT) + p(i)(LT) + p(i)(HT)] [t(d)(SD) + t(d)(LD) + (t(i)(SD) + t(i)(LD)].   (5)
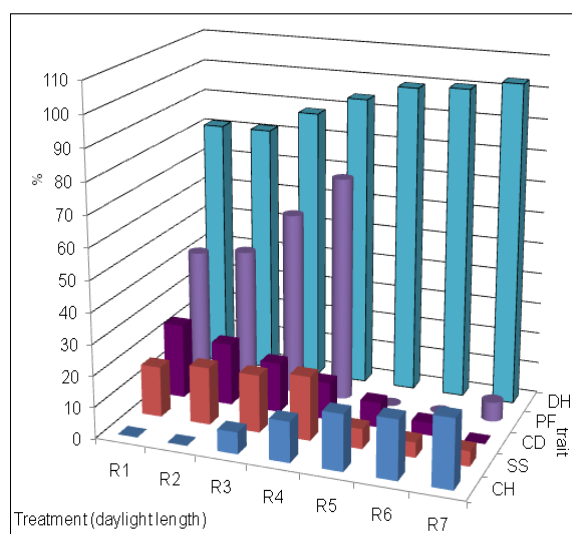
iii)   Phenotype effects = [14.711 + 0 + 1.539 + 0] + [43.219022 + 0 + 4.520978 + 0].

Discussion

Effect of Short day treatment on fertility

When PGMS plants ZAU11S106 were given SDLL treatment earlier than in R5 there was below 5%fertility (Fig.1). This implies that to induce fertility SDLL treatment for four days before R5 was enough. Therefore, treatment earlier than R4 may not be necessary and after R5, SDLL treatment has limited effect since SPR has already taken place. Therefore the period between 4 and 8 days is the fertility determination point (FDP) or what is called critical fertility point (Yuan *et al.*, 1993, Njiruh *et al.*, 2013). At this time, SDLL treated plants were 18 to19 days to heading or 3- to 2-leaves before heading (including

the treatment days) (Fig.1). This is the time pollen mother cells differentiate and are undergoing the first meiosis (Njiruh *et al.,*, 2013). At this point a decision as to whether pollen are to be permanently sterile or fertile is taken. This is the point of SPR (Yuan *et al.,* 1993). Short day treatment at this point results to fertile pollen while long day results to sterile pollen. For instance 4 to 8 days of treatment resulted to 6.42% seed set rate while 1 to 4 days treatment resulted to near 0% implying that by the time of SDLL treatment SPR had already occurred and pollen were destined for abortion. This means that pollen fertility is so synchronized that about a single day of change in photoperiod or temperature can lead to changes in fertility status. In later treatments, after R4, seed set declined drastically and tended to below 5% in R6 (Fig.1) implying that SPR had taken place. If sterility was to be artificially induced, then only 4 days of SDLL treatment (R4) are sufficient. After the peak on R4 (Fig.3) later treatment had little or no noticeable effects on fertility. However, before this stage the effects of SDLL treatments remained in plant and plants remained sterile even if the treatment was withdrawn. SDLL treatment at critical sterility point (CSP) results to disintegration of pollen exine and intine that leads to irreversible abortion (Njiruh and Xue 2011).

Fig. 1. Respose of PGMS rice ZAU11106 to short day ligh length treatment. CH=days from stop of covering to heading; SS=seed set rate (%), CD=length of time of short day treatment PF=pollen fertility (%); DH=days to heading.
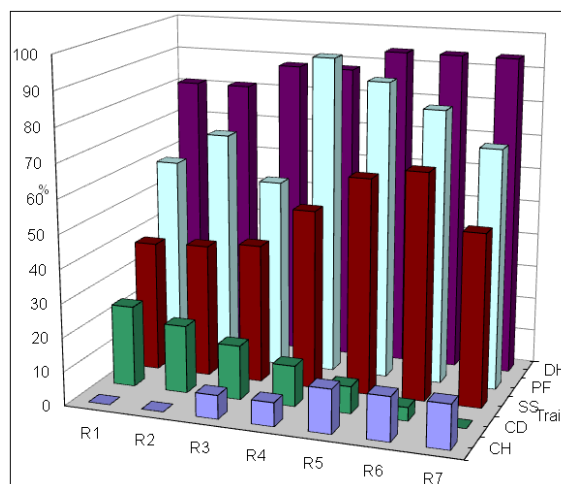
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Responsiveness of fertility and maturity to short day treatment

Photoperiod and temperature are major factors responsible for fertility/sterility in the PGMS rice. PGMS line ZAU11S106 is mainly responsive to photoperiod (Xue *et al.*, 1999). Partitioning of day length effects from and temperature effects was done and it was realized that ZAU11S106 is photosensitive but the contribution of interaction between photoperiod and temperature was bigger than the effect of each individual factor. For instance it was found that when PGMS were exposed to SDLL and HT treatments the seed set was 16.25% but when the same line was exposed to SDLL and LT treatments the fertility rose to 63.99%. The difference is due to temperature change however it is also essential to consider the interaction to avoid over exaggeration of temperature effects. Using Figures obtained by (Xue, 1995) (data not published) to calculate a co-efficient to enable obtaining long day and low temperature effects it was estimated that long day (LD) and low temperature (LT) could have contributed 11.18% seed set rate. Therefore SDLL and LT (interaction) contributed 36.56% thus forming an important entity in influencing fertility. Many reports indicate that PGMS activity is under genetic control (Zhang *et al.,* 1994, Mei *et al.,* 1999), there are enzymes that control its expression (gene products). Thus, two major options were considered; the sterility inducing enzymes are produced in LD and HT but not in SDLL and LT. Given that in long day and high temperature the cell tapetum layer is systematically destroyed hence cell abortion (Njiruh and Xue, 2011), then some gene products in LD and HT lead to cell pollen abortion and SDLL+LT reverse the process. Apparently, the growth point at R5 is the CFP. Thus, this is the time caution must be taken when producing hybrid seeds.

Forward Tracking method (Ali *et al.*, 1995) has been used to determine the time of SPR in TGMS. This method involves daily recording of pollen fertility and later using weather chart to determine the date of critical temperature. This makes the method looks like an indirect backward tracking. In current research a *direct forward tracking* was used whereby plant growth was followed with SDLL treatment to determine when SPR took place (see methods above). This was called direct forward tracking method. This method can be used to predict the expected contamination of hybrid seeds with self bred seeds due temperature fluctuations.
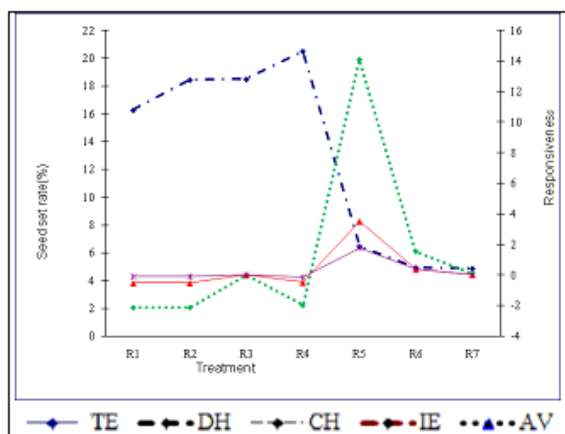

Fig. 2. Response of 11F121 control (cK) to short day light length treatment. CH-days from stop of covering to heading; CD=length of time of SDLL treatment in days; SS=seed set rate (%); PF=pollen fertility (%); DH=days to heading.

Effects of short day treatment on heading date

SDLL treatment of over 20 days long had no major observable difference on maturity rate (Fig.1 and 2). For instance plants treated for 20 and 24 days all matured in 81 days for both treated and the CK. However SDLL treatment between R2 and R3 was enough to speed up maturity by 22 days (21.36%) (Fig.2). After R5 SDLL treatment had limited effect. Therefore, between stage R3 and R5 is the time plant maturity is most responsive to photo reaction (Fig.3b) and at R5 is the time when fertility is most responsive to photoperiod. This indicates that the time of waning of FPR marks the peak of SPR. Yuan *et al.*, (1993) reports that at very early stages PGMS rice lines have unnoticeable response to photoperiod and this firmly supports our observation. Maturation in ZAU11S106 is largely controlled by photoperiod but temperature had only some small effects. When plants were given a SDLL and HT treatment at 24days, heading was realized at 81 days (Table1). This was 22 days earlier

than the untreated ZAU11S106. Also, plants that received SDLL and LT treatment for 28days matured in 81 days (22 days earlier than the untreated ZAU11S106). Effects of treatment reached a plateau between 24-28days. The upper limit of 28days was taken to be the time of growth when effects of SDLL stabilized. At this point the LT and HT seem to have had same effect on maturity. The difference in maturity between SDLL+ HT and SDLL+LT (28-22=6days) was taken to be due to interaction between photoperiod and temperature. This shows that maturity of ZAU11S106 is largely influenced by photoperiod and temperature is only a modifying factor.
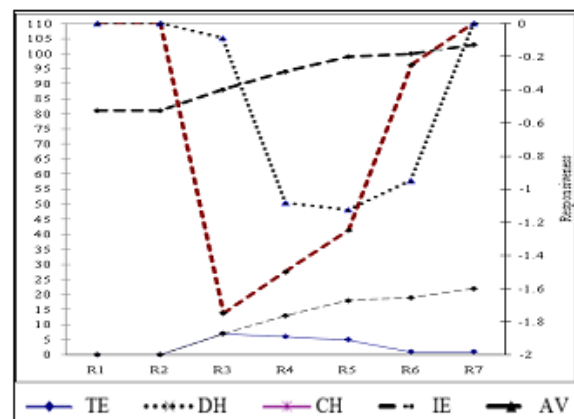

Fig. 3a. Responsiveness of fertility to SDLL treatment. Values of TE are read on left Y axis and all others are read on right Y axis; TE=Total effect; Var.= Inter-treatment variance in % seed set rate (L1-L2); EI= (L1-L2)/4; AV=Var divide by days of treatment, DH=days to heading and CH-days from stop of SDLL treatment to heading

Relationship between FPR and SPR
At R5, SDLL treatment had the greatest influence in fertility of ZAU11S106. This is evidently the most sensitive time of SPR. After this point, SPR had already taken place and any treatment did not have prominent effects on fertility. As the most sensitive part of FPR came to an end the most sensitive part of SPR was beginning (Fig. 1 &2). Plants in R5receivedSDLL treated for only 8 days plants and headed 18 days after cessation of the treatment. On the other hand, untreated ZAU11S106 to took 30 days to heading (starting from the SDLL treatment of stopped for R5). Therefore, the fertility determining

stage of PGMS (whether treated or untreated) was 18 to 22 (30-8) days. Within this span of time a short day treatment leads to fertility and a long day treatment leads to sterility. Once the SPR take place SDLL treatment does not affect sterility and long day treatment does not induce sterility. Fertility in PGMS is determined at primary premordia differentiation stage (Yuan *et al.*, 1993). This seems to be the time that corresponds with R5 or three to two leaves stage before heading. This is 69 days after sowing of plants in R5.It is an indication that fertility/sterility is determined within very narrow time span. Given that PGMS that received SDLL treatment matured at the same time with the control ZAU11F121 (Fig.1 and 2), there seem to exist a relationship between maturity and fertility genes.

Fig. 3b. Responsiveness of Maturity to SDLL treatment. Values of AV and IE are read on right Y axis and all others are read on left Y axis. Change in total effect (TE). DH (days to heading); CH (stop of SDLL treatment to heading); IE =(TE/ SDLL treatment days); AV= TE / CH
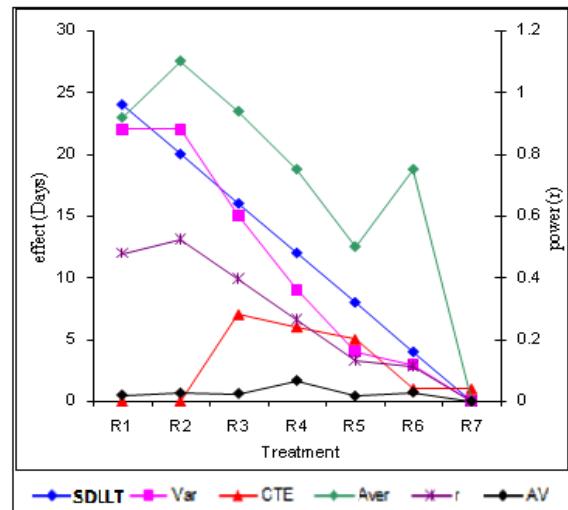
Signal transduction and induction
Table 4 shows the effects of SDLL treatment on fertility and maturity. It can be seen that long after SDLL treatment was stopped, in R2, R3 and R4, plants were fertile. This shows that after fertility induction point (FIP) plants remained fertile if they were given SD treatment otherwise they remained sterile. The ability to retain sterility inducing factors (SIFs)/fertility inducing factors (FIFs) is what was called transduction power. This requires SDLL treatment only for a short time span surrounding the CFP. It has been reported that PGMS pollen under

influence of long day light length conditions undergo abortion in a manner similar to programmed cell death (Njiruh and Xue, 2011). Thus, once programmed under SDLL growth conditions at CFP pollen grew to be fertile even if the treatment was withdrawn. If at CSP the pollen cells grew under HT they were programmed to abort or die. This could be the reason why 4 days of SDLL treatment, done 19days before heading could still transduce sterility (Table 4). From Fig.4, it can be seen that hastening of maturity was mainly due to induction since transmission power declined soon the treatment was withdrawn. This is unlike fertility where transduction and induction powers had no noticeable difference. Therefore, it can be concluded that once SPR take place sterility transducing factors (SIFs) persist within the plant leading to pollen abortion. On the contrary maturity-hastening factors seem to stay within the system but at a declining efficacy. Plants treated at flag-leaf and 2-leaves stages did not have any substantial seed set. This emphasizes point that SPR occurs between 4 and 2 leaf stage before heading. Plants in R4 that were exposed to SDLL treatment for only 8 days(at 69days of growth) had fertility of 20.48% while those allowed to grow for 74 days then SDLL treatment till heading had 0.46%. The positive control (uncovered ZAU11S106), had 1.19% while negative control (ZAU11F121) had 62.23% and untreated ZAU11F121 had 77.6% seed set (Table 2). This is an indication that sterility observed in ZAU11S106 was due to PGMS gene.

Fertility model
Estimated contributions of photoperiod and thermo effects suggest that the interaction between photo and thermo substantially contributes to SPR (Formula 5). In this research it can be seen that SPR took place within a short time and pollen were fertility days later after the SDLL treatment was stopped. Fertility was therefore due to transduction. On the other hand SDLL treatment even in the last days to heading hastened maturity. Hence, effect of SDLL treatment to maturity is largely by induction. In both fertility and maturity, the interaction of photoperiod and temperature was an important component.

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Fig. 4. Induction/transduction power. SDLLT= short day light length treatment; Aver =Average; Var =Variation from CK   Aver=SDLLT/Var, AV=r/SDT; r=Var/(SDLLT+Var); CTE=Change in total effect.

One of the major challenges in hybrid rice production is cost of labour since the whole process is labour intensive which pushes the cost of hybrid seeds beyond the reach of small scale farmer unless a lot of public subsidizing is done (Kuyek, 2000, Virmani, 2000).Use of EGMS varieties is expected to reduce this cost since it uses two lines instead of three lines used in the cms system. If proper synchronization and monitoring of temperature and day light length is done predictions of losses due to contamination of hybrid seeds with selfbred ones can be minimized. Realization that temperature complement photoperiod a great deal will enable utilization of the hybrid rice seed technology in the tropics where day light length is about 12hours but temperatures can be more than $33°C$.

Conclusion: Combined effects of photoperiod and high temperature induce a stronger second photoreaction in EGMS. This is an indication that the PGMS line under study can be used in areas with slightly shorter than its recommended critical day light length growth conditions but under influence of higher than its recommended critical temperatures.

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List of Abbreviations

1. CFP- Critical fertility point
2. Ck – Control
3. CMS – cytoplasmic male sterility
4. CSP – Critical sterility point
5. EGMS- environmental genic male sterility
6. FPR - first-photoreaction
7. High temperature
8. I/KI- Potassium iodide
1. LD - long day
10. LT- low temperature
11. R-row (e.g. R1 mean row one 1)
12. PGMS- Photoperiod-sensitive genic male sterility
13. SD- Short day
14. SDLL- Short day light length
15. SDT- Short day treatment
16. SPR- Second photoreaction
17. TGMS- Thermo genic sensitive male sterility.

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