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Tracking the Expression of Photosensitive Genic Male Sterility Gene in Rice

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Abstract

Photoperiod sensitive genic male sterile rice lines contain genes that induce complete male sterility in high temperature and long day light length period, but are male fertile under low temperature and short day light length growth conditions. These lines are good candidates for hybrid rice seed production. The main challenge limiting their use in production of hybrid rice seeds is that of determining the exact time of their growth period when sterility gene(s) is completely expressed. Knowledge of this will enable development of a breeding program that ensures that sterility inducing conditions prevail in critical sterility point. The objective of this study was, therefore, to determine the time in the rice growth period when the sterility gene(s) are expressed. Rice line ZAU11S106, a Photoperiod sensitive genic male sterile line, was used to test hypothesis that it is possible to estimate time within 48hours when PGMS gene is expressed. Sowing was done in 8rows in Hangzhou-China, so that plants matured in August when day light length was over 14hours and day temperatures were over 30°C. At 57days old plants in row one were given short day light length treatment while plants in row two were given the treatment when they were 61days old or 4days later. After every four days the next row was included in the treatment. This was done until the plants in row one flowered when the treatment was stopped. Plants given short day length treatment at 73 and 77 days old realized 6% and 0% seed set respectively. At 73 and 77 days old the plants were at dyad and tetrad stages of pollen development

respectively. The conclusion was that, the sterility gene was observed to be expressed within 48hours (four days) lying within 73 and 77 days or within the dyad and tetrad stages of pollen development.

Key words: Meiosis, Photoperiod, Pollen abortion, PGMS rice.

INTRODUCTION

Heterosis or hybrid vigour realized in F_1 has been used to increase yield in many out-crossing crops like maize (*Zea may*). In particular, hybrid seed technology has been practiced in maize since 1930s and it accounts for over 30% increase in yield (Duvick 1992, Duvick 1997). Two inbred genetically fixed varieties of a particular crop are crossed to obtain hybrids seeds. Plants from such seeds are special because they express *"heterosis"* or hybrid vigor. The basic principle is that, if two parents which are genetically distant from each other are crossed, the offspring tend to exhibit *"superiority",* particularly in terms of yield. Hybrid seed technology is practiced in many other crops including, wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.*)*, cotton (*Gossypium hirsutum* L.) and rice (*Oryza sativa L.*) (Igor and Hari, 1969; Burke and Arnold 2001; Immanuel et. al., 2010; Zou and Lou, 2010).

Search for high yielding rice (*Oryza sativa* L.) line has gone through a number of breeding metamorphic stages. The first major breakthrough was the incorporation of a semi-dwarf gene (*sd-1*) in Chinese variety Dee-geo-woo-gen (Khush, 1994, 1995) into the ordinary rice plant around 1955 providing a plant architecture that can accommodate use of more nitrogenous fertilizer hence higher yields (Hu, 1993). To succeed this method was the high yielding varieties (HYVs) such as the IR8 produced by International Rice Research Institute (IRRI) (Hossain *et al.,* 1999). This was nicknamed *"miracle rice"* because of its improved yield. IR8 varieties could produce 10 metric tones / hectare in research stations, and for a long time this remained the yield ceiling. To break this barrier two routes were adopted *"Super Rice"* or IRRI 15-tonner per hectare, which was achieved by a radical restructuring

of the rice plant architecture, and the other route is by hybridizing rice. Super hybrid rice (Asia Pulse, 1999; Yuan, 1997) has so far demonstrated the capacity to break the yield ceiling established by IR8 and other high yielding varieties (Heling, 1999; Yuan, 1997). Production of hybrid rice started in 1970s with the discovery of cytoplasmic male sterile, *"wild rice with abortive pollen",* or WA rice line (*Oryza sativa f. spontanea*) (Virmani, 1996). This is what is called the "three line system" because it involves a sterile, a maintainer and a restorer lines. Yield increase due to crossing breeding in rice, just like in maize, exploits hybrid vigour or *heterosis.* For rice, *heterosis* has been reported to increase rice yield by between 20 to 30 % above the current dwarf lines (Kush *et al*., 1994; Virmani *et al*., 1996; Virmani *et al*., 2003). The yield gains led China to start commercial production of hybrid rice in 1976 and lines that can yield up to 17tones per hectare have been developed (Yuan, 1997; Kuyek 2000). Most available estimates suggest that China's hybrid rice yields average 15-20 percent more than the high-yielding inbred varieties (Zou and Lu, 2010).

To produce hybrid seeds a sterile female parent and fertile male parent (pollen donor) are needed. This is achieved by male emasculation of the pollen recipient or the female parent which makes it labour and skill-intensive and thus increases cost of production especially if it is done manually (Virmani and Kumar, 1997). Despite this hybridization remains the major method of increasing the rice yield. According to Yuan Longping (2010), China has reached the yield plateau for hybrid rice (IRRI, 1998; Yuan, 2010) using the three line system, however another major boost is expected from adopted "super hybrid" rice program. The Green Revolution, led by IRRI's high-yielding varieties (HYVs), led to dominance of a few lines such that by the mid-1980s just two HYVs occupied 98 percent of the entire rice

growing area of the Philippines (Kuyek, 2000) leading to genetic erosion and reduced biodiversity. The problem has been increased by use the cytoplasmic male sterile (cms) lines in hybrid seed production that has lead to increase of cytoplasmic uniformity leaving the hybrids vulnerable to disease and other environmental catastrophe (Leving, 1990). PGMS rice is expected to reduce the problem of genetic degradation because it can be used with many restorer lines (both indica and japonica rice lines) to produce hybrid seeds, unlike in cms where they are limited due to maternal (female parent) and paternal (male parent) incompatibility, which lead to F_1 sterility (Oka, 1974; Lin *et al.,* 1992). Wide compatibility is realized because PGMS (female) lines with diversified germplasm background can be produced unlike in cms whereby wild abortive (WA) is the major maternal line used (Virmani, 1996). Since the cost of production of hybrid rice seed using PGMS is expected to be lower then, these lines are the most suitable candidates in Hybrid rice seed production technology.

Discovery of PGMS rice line in 1970s (Shi, 1981; 1985) ushered in the use of two-line system as a major method of producing hybrid seeds (Mao, 1993). PGMS rice lines are completely male sterile in long day light length (LDLL) and revert to fertility under short day light length (SDLL) growth conditions (Shi, 1985; Shi and Deng, 1986). They do not require a maintainer line like in the case of cytoplasmic male sterile plants since they maintain themselves. In LDLL (above 14 hours) and in temperature of above 26°C it is completely sterile and reverts to fertility in optimal day length and temperature (Yuan *et al.,* 1993; Ku *et al.,* 2001).

To effectively use PGMS rice lines in hybrid rice seed production, a good breeding program needs to be developed. This will include evaluation and monitoring of PGMS gene(s) to determine the time when it is switched on and off. Under sterility inducing conditions the fertility gene is off and the pollen is completely sterile and seed set rate is zero (Xu *et al.*, 1995, Ku *et al.,* 2001, Njiruh and Xue, 2011). When PGMS are grown under short and low temperature growth conditions they produce fertile male gametes, a time when they are used to propagate themselves for the next generation. The PGMS character is genetically controlled and can be inherited from one generation to another (Shi, 1985, Virmani, 1994). The trait is controlled by genes *pms1* and *pms2* in chromosomes 7 and 3 respectively (Zhang *et al.,* 1994) and *pms3* on chromosomes 12 (Mei *et al.,* 1999). This has enabled breeding for new lines with the PGMS trait and among them include W6154s, W7415s, NS5047S, 31111s, WD1S, 8801S, 6334s, N5047S (Virmani 1994; Xue *et al.,* 1999). The objective of this research was to track PGMS gene and determine when it is expressed in rice growth cycle. This will enable synchronized sowing so that optimum sterility inducing conditions prevail at the time of gene expression. In this report the PGMS gene expression has been found to be confined at the tetrad to completion of meiosis stages of pollen development.

MATERIALS AND METHODS.

PLANT MATERIALS.

Plant materials used were male sterile (PGMS) line ZAU11S106 and a male fertile line ZAU11F121 that was used as a positive control. The male sterile line (ZAU11S106) is a PGMS developed from japonica line N5047S protoplasts (Xue *et al.*, 1999). All the

materials were sown in concrete trough (Fig.1) at the Zhejiang University –Huajiachi Campus experimental fields at Hangzhou in China, 30°15N in a synchronized manner so that they flowered in the hot summer months of July to September. In this research LDLL refer to over 14hour of day light time including morning and evening twilight while SDLL refer to 11hour daylight including the morning and the evening twilight. High temperature refer to >33°C and >26°C during day and night respectively and low temperature refer to 26°C and 20°C during day and night respectively.

METHODS

Determining the time of PGMS gene expression and the critical sterility point

The Rice ZAU11S106 and ZAU11F121 (positive control line) plants materials used to determine the time when the PGMS gene is expressed were sown on May 14th in eight rows each with six plants and allowed to grow up to 57th (Fig.1). Among the six plants, three were ZAU11S106 and the other three were ZAU11F121. At the 57th day plants in the first row were covered with an opaque black cloth at 4.00pm and uncovered at 9.00pm Hangzhou-China time so that they only experienced 11hours of normal daytime light. This is what was referred to as short day light length treatment (SDLLT) throughout this research. For purpose of counting number of days under SDLLT, 57th day was taken as baseline time and was designated a value of zero (0) and the 58th day was counted as day one. After four days the first and the second row of plants were put under SDLLT. After every four days a new row of plants was included among the plants under SDLLT. This was done as described in Table1 until plants in the first row flowered when SDLLT was stopped.

Linking PGMS gene expression to stage of pollen development Before plants in each row were subjected to SDLLT, samples of two whole panicles were picked from each plant in each row. Thus, samples were collected from row1,2,3,4,5,6,7 and 8 when the plants were 57, 61, 65, 69, 73 77, 81 and 81 (cK) days old respectively. Among the two samples collected, one panicle from each plant was fixed in Canoy's solution II for pollen analysis. The other sample was dissected to obtain a panicle that was directly imaged with a scanner to illustrate the stage of panicle growth. Anthers from each fixed sample were separately extracted from panicles using forceps and placed onto a drop of 1% potassium iodide (I/KI) solution on a microscope glass slide. They were macerated using forceps to release the pollen cells after which anther-husks were removed from the glass slide leaving the microspores only. A cover slide was placed on the microspores on a glass slide, observed, and photographs taken under x40 of light microscope. Stages of pollen development and shades of microspore staining with 1% I/KI were matched to the stages of panicle growth from various rice materials under SDLLT. Blue black staining was an indication of fertile pollen while yellow staining was indication of sterile pollen. The time of ZAU11S16 SDLLT that had all pollen staining yellow was taken to be the phase when sterility gene expressed or the critical sterility point (CSP).

Determination of spikelet fertility At post ripening stage three tillers with full grown panicles were picked from each hill in each row of the plants under SDLLT plus control for seed set evaluation. Glumes with filled up grains were counted and fertility was calculated as total number of grains per spike to total number of glumes per spike x 100.

Data analysis Data was analyzed using Microsoft excel version 2010.

RESULTS

DETERMINING THE TIME OF PGMS GENE EXPRESSION AND THE CRITICAL STERILITY POINT

ZAU11S106 plants in rows 1,2,3,4 and 5 that received SDLLT for a duration of 28,24,20,16 and 12 days headed after 81, 81, 90, 96 and 101 days old after sowing in that order (Table 1A). This is illustrated in Fig. 1B where plants in the rear of the trough have follower ahead of the one infront. The seed set of plants in rows 1,2,3,4 and 5 were16%, 18%, 8%, 20% and 6% respectively (Table1). ZAU11S106 rice plants in rows 6, 7 and 8 (negative control) that received SDLLT for 8, 4 and 0(Ck) days respectively, all recorded 0% seed set (Table1). The boundary between rice plants with seeds and seedless was between row5 and row6 or 12 and 8days of SDLLT. The seed set rate between row5 (p-value =0.2019) and row6 (p-value=0.0023) was significant at α=0.05.

Plants of ZAU11F121 (positive control line) from all rows subjected to SDLLT, recorded over 39% seed set except for plants in row2, which recorded 15% and had a significant difference from the rest at α=0.05 (Table1B). A sample of panicle representing ZAU11S106 plants under SDLLT in rows 1-5 and from rows 6 and 7 are shown in Fig.2A&B respectively. Glumes of panicle shown in Fig.2A recorded some seed set while that in 2B did not have any seed count.

A B

Figure 1. Sowing pattern of ZAU11S106 and ZAU11F121 for SDLT. PGMS rice ZAU11S106 was sown in concrete troughs in rows each with 6 plant and allowed to grow for 57days after which SDLT was started. Figure A shows plants at initiation of SDLT and Figure B shows plants at cessation of SDLT. In Figure A only four rows are appearing (others two were not captured) but Figure B shows the total size of trough and number of rows. SDLT started with plants at the rear of the photo and they also flowered earlier than the controls at the front of the picture (Figure B).

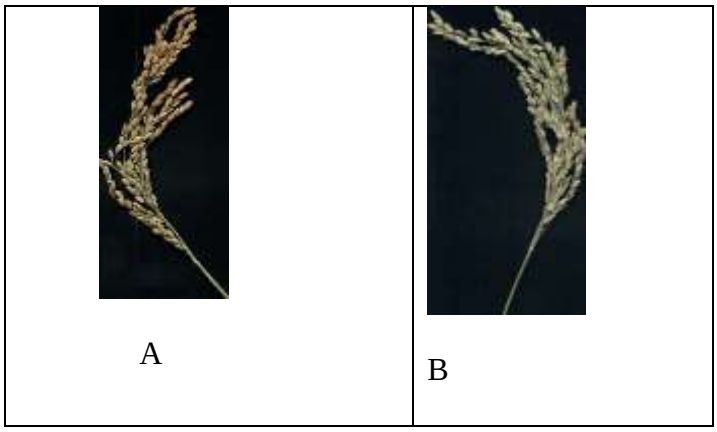
Table 1. Parameters used to track critical Point of PGMS gene expression. Table A shows data collected from ZAU11S106 while B shows data collected from ZAU11F121(control). In both table a &b column 1 shows number of hours of daylight the plants were exposed to SDLT while "plants row" in column 2 refer to order of sowing in the concrete trough. Column three indicate the dates on which SDLT was initiated and figures in brackets indicate the age of plant at the time of SDLT. Heading dates in column 4 refer to date when all plants in each row flowered. Column 5 and 6 shows total number of short day length treatment and days from sowing to maturity respectively. Plant fertility (%) or seed set (%) shown in row 7 indicate percentage seed of all plants in each row.

Table 1 A

Day length (hours)	Plant Row	Date of initiation of SDLT (Days after sowing)	Heading date	Total Days of SDLT	Days from sowing to Heading	Seed Set %	Z-values (P-value)
11	1	11-July (57)	4-Aug	28	81	16	0.9992
11	2	15-July(61)	4-Aug	24	81	18	0.9992
11	3	19-July(65)	11-Aug	20	90	8	0.4337
11	4	23-July(69)	17-Aug	16	96	20	0.9999
11	5	27-July(73)	22-Aug	12	101	6	0.2019
11	6	30-August(77)	23-Aug	8	102	0	0.0023
11	7	3- August(81)	26-Aug	4	105	0	0.0023
14	8	CK (81)	26-Aug	0	105	0	0.0023

Table 1B.

Day length (hours)	Plant Row	Date of initiation of SDLT (Days after sowing)	Heading date	Total Days of SDLT	Days to heading	Seed Set %	Z-values
11	1	11-July (57)	4-Aug	28	81	39	0.0615
11	2	15-July(61)	4-Aug	24	81	15	0.0000
11	3	19-July(65)	11-Aug	20	88	41	0.0865
11	4	23-July(69)	11-Aug	16	88	53	0.6214
11	5	27-July(73)	17-Aug	12	94	64	0.9663
11	6	30-August(77)	17-Aug	8	94	67	0.9920
11	7	3- August(81)	17-Aug	4	94	51	0.8648
14	8	CK (81)	11-Aug	0	91	54	0.9460



Figurer 2. Effects of SDLT and LDLT on PGMS rice ZAU11S106 panicle fertility. Panicle in Figure A was a sample of plants in row 1-5 shown in Table 1. These plants which recorded some seed set received SDLT for a period ranging from 12-28days or when plants were between 57 and 73 days old. Panicle shown in Figure B with seedless glumes was obtained in ZAU11S106 sown in row6 as recorded in Table 1. Plants in this row received SDLT for only 8 days when they were 77days old.

LINKAGE OF PGMS GENE EXPRESSION TO PANICLE AND POLLEN DEVELOPMENTAL STAGES

Samples of panicles picked from plants growing in rows 1 and 2 or at 57 and 61 days old are shown in Fig.3. These panicles had no grown glumes (Fig,.3A&B) and no pollen was observed after staining with 1% I/KI for both varieties ZAU11S106 (Fig.3 F&G) and variety ZAU11F121 (Fig.3 K&I). However, panicles sampled from plants in rows 3,4 and 5 that were picked at 65, 69 and 73days respectively (Fig.3 C&D) had glumes with distinct pollen at various stages of growth. For both varieties (ZAU11S106 and ZAU11F121) the following type of pollen were observed; pollen mother cells at 65th day (Fig.3H&M), dyads at 69th day (Fig.3.I&N), and tetrad at 73rd day (Fig.3. J &O) after sowing.

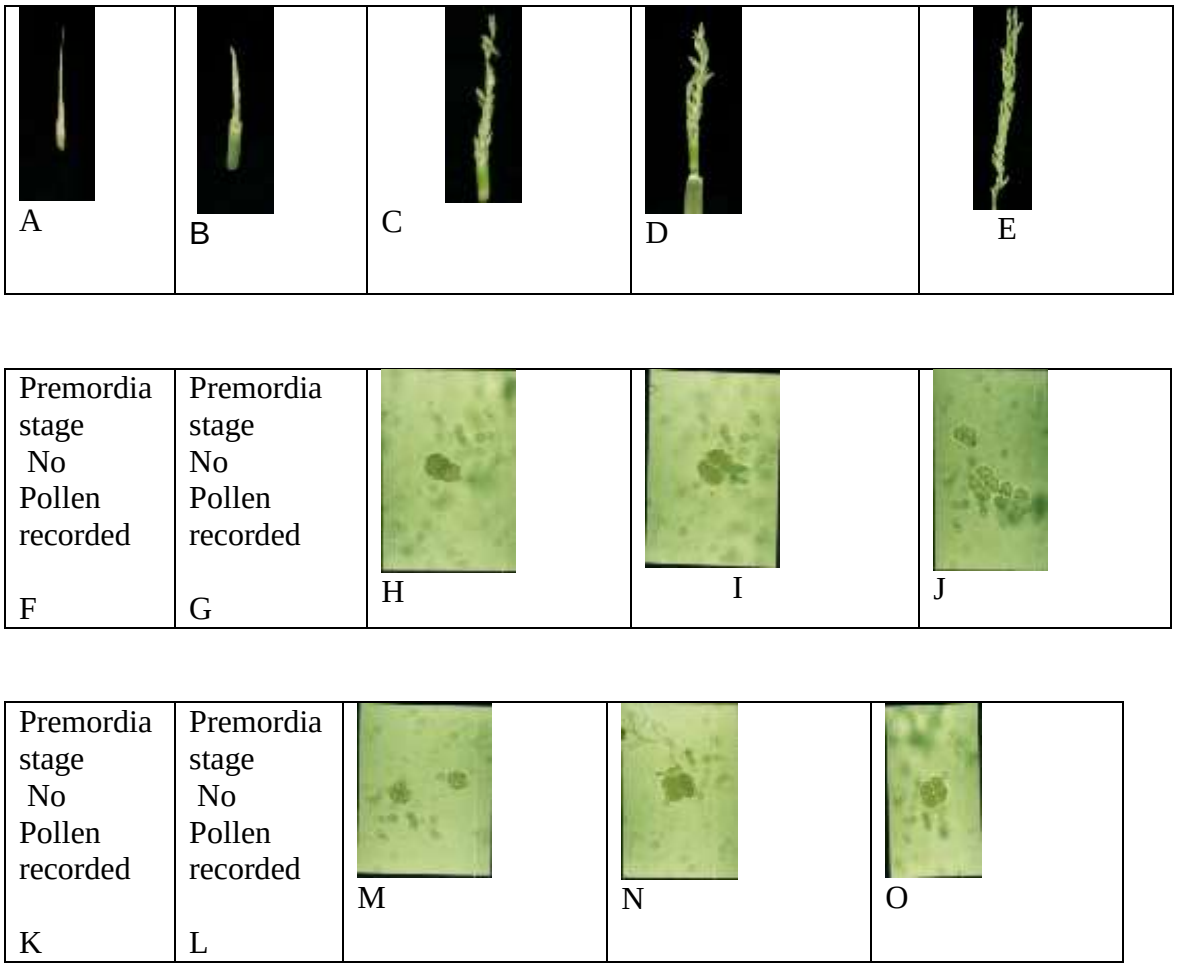


Figure 3. ZAU11S106/ZAU11F121 rice panicles, and pollen cells at various stages of development. Figure A-E shows samples of panicles obtained from ZAU11S106 in rows 1-5 taken before SDLT initiation. At this stage panicles from ZAU11S106 and ZAU11S121 did not display any observable difference among them (therefore the samples represent both lines). Meiotic pollen from ZAU11F106 are presented in Figure F-J while those from ZAU11F121are presented in Figure K-O. Samples were taken up to completion (tetrad stage) of meiosis.

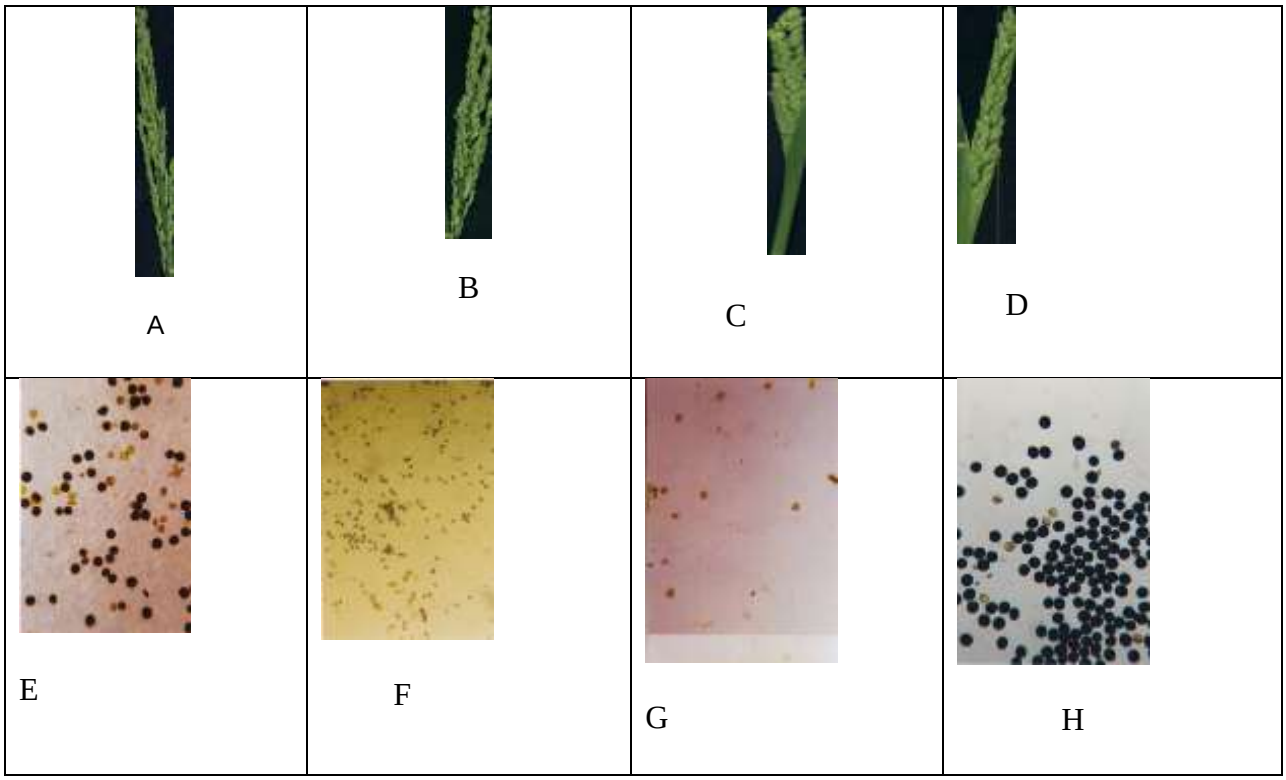
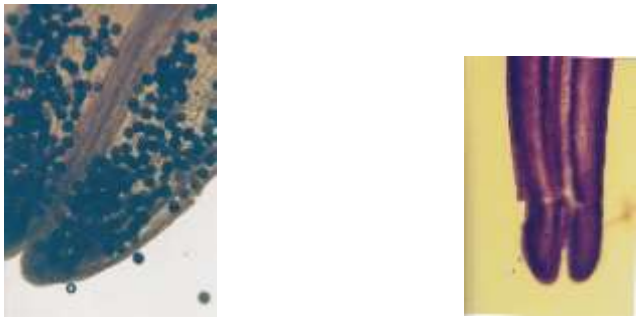


Figure 4. Manifestation of ZAU11S106 and ZAU11F121panilce and pollen cells after critical fertility/sterility point. Figure A,B, C and D shows mature PGMS panicle collected from rows 5,6, 8 (sterile control) and Fertile control (ZAU11F121). Pollen extracted from glumes were stained in 1% K/I. Figure E shows pollen collected from mature panicle from row5 which shows effect of SDLLT at critical fertility point where some pollen stained blue-black, resembling the pollen from ZAU11F121 rice panicles (Figure H), which stain blue black with 1% K/I. Figure F and G indicate pollen from row 6 and row 8 (control) respectively. Mature pollen from rows 1-5 had a number of their pollen staining blue and that from row 7 resembled that in row6 (photos not included).

At 65days old, the flag leaf and the next lower precedent leave emerged at the same node level, and the apex of panicle were at the level of third leaf (Fig.6A) while at 69 days old the flag and the precedent lower leave were separated by about a length of about 2cm but panicle apex was below second last leaf from the top (Fig. 6B). The flag leave of plants at 73days old appeared completely spread out and the distance between it and the precedent lower leaf was nearly at its maximum (Fig. 6C). At that time the apex of the panicle was approximately at

the middle of the flag and the next precedent lower leaf (Fig. 6D). This corresponds to the panicle growth illustrated in Fig.3E. Panicles from the plants at 77days and 81days old (Fig. 6 E&F) had mature pollen (Fig.4). The samples in Fig.4 were collected from rows 5, 6 for ZAU11S106, and from 8 for ZAU11F121. Pollen from plants in row6&7 (that were given SDLLT at 77 and 81 days old) all stained yellow same as those in row8 (ZAU11S106 control line) (Fig.4F) and no viable pollen was observed in their anther locules (Fig.5B). A conspicuous number of pollen from row1-4 stained blue black with 1% 1/KI (Fig.4G and 5A), however ZAU11S106 plant in row5 had a few of its pollen staining blue-black, while majority stained yellow, with 1% 1/KI (Fig. 4E).



A B

Figure 5. Locules of anther from ZAU11S106. Figure A shows locule of ZAU11S106 grown under SDLLT while B was obtained from ZAU11S106 grown under LDLL treatment.

A B C

D E F

Figure 6. Panicle development and stages of flag leave development in PGMS. Areas marked with a toothpick piercing the shoot shows the upper most tip of the developing panicle. Figure A shows position of flag leave and panicle at pollen mother cell stage. Figure B,C&D shows position of panicle and flag leave when shoots are at dyad , tetrad and completion of meiosis respectively. Figure E&F shows position the panicle when it is about to emerge from the flag leaf (at this pollen is mature).

DISCUSSION

EFFECTS OF SHORT LENGTH TREATMENT ON ZAU11S106 FERTILITY

ZAU11S106 plants in row1-5 recorded a seed set rate of between 6% and 20%, this was significantly higher than the male sterile control (Table1). For the plants in rows 6 and 7, a seed set rate of 0% was recorded same as that from ZAU11S106 (control line) in row8. At α=0.05 this was significantly different from the ones from row1-5. Among the plants under SDLLT, the boundary between plants with seeds and those without seeds was between row5 and row6. Plants in row5 which, were subjected to SDLLT for 12days, recorded 6% seed set while those in row6 received 8days of SDLLT recorded seed set of 0%. The z-test of the two indicated significant difference at α=0.05. Therefore the critical fertility point (CFP) lies within the four days separating the two treatments. CFP corresponds to stage of completion

of meiotic division (Fig.3E) or when the panicle apex had just grown above the leaf preceding flag leave (Fig. 4C). This is the stage when the female parents must be subjected to male sterility inducing condition so as to ensure complete sterility of male gametes (Yuan et al., 1993; Ku et. al 2001). ZAU11S106 plants grown under LDLL conditions between 73 and 77 days old became male sterile and even when subjected SDLLT for 8days this could not be reversed. Likewise ZAU11S106 subjected to SDLLT at 73days for 12days had some seeds. After 12 days of SDLLT, exposure of the plants to 17days of LDLL till flowering could not induce complete sterility (there was a 6% seed set realized). This means that fertility gene had expressed and LDLL treatment could not reverse it. Once the sterility inducing gene expresses the pollen are irreversibly destined for abortion (Yuan et al., 1993). This is a situation similar to programmed cell death (Njiruh and Xue 2011). At 73days of age the sterility inducing gene had not completely expressed and SDLLT could induce a 6% seed set in ZAU11S106. If such plants are cross pollinated subsequent seeds are not 100% pure hybrid. The 4days duration between 73 and 77 days is the phase of transformation from normal to sterile pollen for ZAU11S106 exposed to LDLL treatment; or from potential sterile to fertile pollen for ZAU11S106 under SDLL growth conditions. This is the time of partial fertility (Latha and Thiyagarajan, 2012).

LINKAGE OF PGMS GENE EXPRESSION TO POLLEN DEVELOPMENTAL STAGES

At 73days old, pollen development was at the tetrad stage of meiosis (Fig.3) and at 77days old meiosis was complete (Fig.4) and the panicle had completed the stamen and pistillate stages. ZAU11S106 plants that were exposed to SDLLT at 77days old for a period of 8days recorded 0% seed set rate. This was despite a follow up with 14days of LDLL treatment until

flowering time. Pollen of ZAU11S106 at 77days old were fully mature although the panicles were still enclosed in the flag leave. If plants were grown under LDLL conditions at this time they became male sterile but exposure to SDLLT at 73days or earlier resulted into fertile pollen. Before 77days panicle and pollen development for both ZAU11S106 (Fig. 3F-J) and ZAU11S121 (Fig. 3K-O) had no noticeable difference. However, after 77days ZAU11S106 grown under LDLL and high temperature conditions had no noticeable pollen in their locules (Fig.5) either because they could not stain or they had disintegrated. Under such conditions pollen tend to be sterile and abortive (Xue et al., 1999).

Transformation of pollen from sterile to fertile and vise versa was found to occur between days 73 and 77 old after sowing or at completion of meiosis. Once sterility or fertility reaction took place during the critical fertility/sterility period, transformation was irreversible in spite of the conditions that prevailed later. According to Yuan *et al*., (1993), at critical sterility/fertility period SDLLT make PGMS/TGMS rice irreversibly fertile while LDLL treatment make them irreversibly sterile. Microscopic observation of pollen development from pollen mother cell to tetrad stage from both ZAU11S106 and ZAU11F121 (control) plants with or without SDLLT were observed to have same meiotic features. ZAU11S106 plants given SDLLT at tetrads stage of pollen cell development recoded 6% seed set but those given SDLLT after tetrad recorded 0% seed set (Table 1). Apparently, the decision whether glumes was to be fertile or sterile took place between days 73 and 77. Therefore, ZAU11S106 needed for production of self-breed seed for its own maintenance need to be given SDLLT before 77days old and those that need to be completely sterile for hybrid seed production need be given LDLL growth conditions between 73 and 77 days after sowing.

In photoperiodsensitive genic male sterility (PGMS) rice, LDLL and high temperature induce up to 100% pollen sterility while in short day-light length growth conditions pollen differentiate to become fertile (Xue *et al*., 1999). Sterility is controlled by three major genes; *pms1, pms2* and *pms3* that have been mapped on chromosomes 7, 3 and 12 respectively (Zhang *et al.,* 1994; Mei *et al.,* 1999). According to Yuan *et al*., (1993) the critical time determining sterility or fertility in PGMS rice is the time from primary premordia through secondary premordia differentiation to differentiation of stamen and pistillate. Our observation indicate that all pollen (100%) from ZAU11S106 rice plants under SDLL growth conditions at 77days stained yellow with 1% I/KI and 0% seed set was realized (Fig.4 F&G). At 73days old, a time when pollen development was at tetrad stage of meiosis, SDLLT of ZAU11S106 for four days gave 6% seed set. This is an indication that PGMS gene(s) were expressed between days surrounding tetrad and completion of meiosis stages of pollen development, or between 73 to 77 days old after sowing.

When breeding for hybrid rice seeds female parent need to be 100% sterile to prevent contamination of hybrid seeds with self-breed seeds. Precise determination of the most critical stage of sterility expressing genes will enable synchronization of sowing, so that plants enter sporogenesis stage under influence of LDLL growth conditions. PGMS require sterility inducing conditions only at the critical sterility point, once this is realized pollen become irreversibly sterile (Njiruh and Xue 2011). Expression of PGMS gene leads to deformed tapetum and exine (Kaul, 1988; Xu *et al.,* 1995, Njiruh and Xue 2011). Tapetum is the innermost wall of microsporogium that provide enzyme, hormones and food to the

growing pollen mother cells (PMC). Our observation indicates that locules of anthers from ZAU11S106 grown under LDLL and high temperature growth had no noticeable functional pollen. Under sterility inducing conditions anther locules of PGMS plants are occupied by deformed pollen (Ku *et al.,* 2001). This is brought about by deformed tapetum (Njiruh and Xue, 2011), and such plants can easily be pollinated by fertile pollen from another plant to produce hybrid seeds free of contamination by selfbred seeds.

CONCLUSION

ZAU11S106 was completely sterile when given LDLL growth conditions at dyad and tetrad stage of pollen development. This corresponds to period 73 to 77 days old. Therefore the critical fertility point (CFP) or critical sterility point (CSP) falls within the 4days or 48hours between 73 to 77 days. This is equivalent to the immediate time when the flag leave of plants appeared completely spread out or when the apex of the panicle is mid-way between flag leave and the subsequent lower leave. .

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