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Evaluation of photoperiod and thermosensitive genic male sterile lines for hybrid rice seeds production in Kenya

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Abstract

Photo–thermo-sensitive genic male sterile (P(T)GMS) rice is a new hybrid rice technology that uses prolonged light length and high temperatures to induce sterility. This technology is being introduced in Kenya but such growth conditions are lacking in the tropics. To overcome this, we grew P(T)GMS under greenhouse conditions where day light length was prolonged to 14 hrs using solar illumination and day and night temperatures were maintained above 36C° and 24°C respectively. Sterility of P(T)GMS was determined by the level of abortive pollen and seed set rates. Hybrid seeds were produced by crossing three P(T)GMS lines (V1PGM, V2TGM and V3PGM) as female lines with Basmati 370 and Basmati 217 varieties as pollen donors. Under long and normal day lengths and high temperatures, pollen sterility ranged from 99-100% but no seeds were set in PGMS lines. However, TGMS recorded 3% and 2% seed set under similar conditions. Under natural conditions both PGMS and TGMS reverted to fertility. Agronomic evaluation showed outstanding ($P<0.01$) performance of hybrids over parents in flag leaf length (V2370; 35.8 cm), panicle exsertion (V2217; 8cm) and shorter flowering time (V3217; 85 days) under greenhouse conditions while flag leaf width (V1370; 1.48 cm) and tillering (100) performed better under natural conditions. Percentage seed set positively correlated with flag leaf related traits and flowering days but negatively correlated with panicle length, panicle exsertion and tillering. We conclude that the P(T)GMS lines are adaptable for hybrid rice seed production in Kenya but there is need to review the P(T)GMS lines' background so as to minimize incompatibility in hybrids.

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Introduction

The advent of green revolution led to drastic increase in global rice yield but despite this, it has not been possible to meet the food demands of the world's increasing population (Kikuchi and Ikehashi, 1984). This involves breeding for high yielding varities (HYV). The traditional high yielding pure-bred lines have reached yield plateau (Yuan, 1993) yet by 2050, the world is expected to increase rice production to 880 million tonnes from the present 580 million tonnes in order to keep pace with consumption (Nguyen, 2010). To increase rice yields above this plateau, researchers have adopted the technology known as "super hybrid" rice and "Green super rice" (IRRI, 2009). In rice growing countries like, China, India, Indonesia, Egypt and Vietnam among others, hybrid varieties have shown improved yield above the high yielding inbred varieties. Hybrid vigour has been reported to increase rice yield by between 20 to 30 % above the current yields of the dwarf lines (Kush, 1994; IRRI, 2009). Recent progress in plant-breeding research has indicated that a significant shift forward in the yield frontiers is achievable through hybrid rice technology.

In Kenya both irrigated and rainfed rice is produced in about 17,830 hectares with an average yield of 4.1 tonnes and 1 tonne per hectare respectively (Ministry of agriculture-Kenya, 2010). The total annual production is about 73,141 tonnes against consumption of over 300,000 tonnes. This yield is low when compared to 8.9-9.4 tonnes per hectare reported in other rice growing regions like China. To improve rice production in Kenya, a number of practices will need to be adopted to raise the yield to global average. "Super hybrid rice" and "Green super rice" (IRRI, 2009) need to be adopted so as to increase rice yield in Kenya.

To produce hybrid rice seeds, a completely male sterile female parent is required for pollination with a fertile male parent. Plants from such seeds are special in that they express hybrid vigour (Yang *et al.,* 1996; IRRI, 2009).

Over the years manual removal of male gametes from rice plants used as the female parents during cross breeding has been practiced. However, unlike cross pollination that occurs in plants such as maize whose male and female flower parts are separate, in rice (self-pollinating crop), the two parts are in one minute flower making manual male emasculation difficult (Akhilesh *et al*., 2012).

However, with the discovery of cytoplasmic male sterility (cms) breeding system, large scale production of hybrid rice became (Virmani, 1992; Virmani and Kumar, 2004). Cytoplasmic male sterility is under extranuclear genetic control (under control of the mitochondrial or plastid genomes). It shows non-Mendelian inheritance, where male sterility is inherited maternally (Weider *et al*., 2010). Rice line is described as male sterile when the male sterility controlling factor S in the cytoplasm and the recessive alleles (*rf*) of fertility restoring genes are present in the nucleus (Schnable *et al*., 1994; Virmani *et al*., 1997; Schnable and Wise, 1998). The maintainer line (B line) is iso-cytoplasmic to the cms line since it is similar to it for nuclear genes but differs in cytoplasmic factor (N), which makes it self-fertile, but it has the capacity to maintain the sterility of the 'A' line when crossed with it. A restorer or 'R' line possesses dominant fertility-restoring genes (*Rf*) and it is dissimilar to or diverse from the 'A' line (Schnable and Wise, 1998; Weider *et al*., 2010). Crossing a restorer line as a pollen donor parent with a cms 'A' line as a female parent restores the fertility in the derived F_1 hybrid plants. The restorer gene in the dominant homozygous (*RfRf*) or heterozygous (*Rfrf*) state can restore the fertility in the F_1 hybrid despite the presence of sterility factors in the cytoplasm derived from the 'A' line (Virmani *et al*., 1997; Virmani and Kumar, 2004; Santhosh *et al*., 2009).

Despite the tremendous impact of the cms system in rice breeding, with time, the use of this method has also proved to be costly,

since it uses three lines (sterile, maintainer and restorer), compared to photoperiod-sensitive genic male sterility (PGMS) that uses two-lines (Santhosh *et al.,* 2009). Besides, the use of cms in hybrid rice seeds production is limited because the commonly used female parent, Wild Abortive (WA) type (Dalmacio *et al.*, 1995), suffers from cytoplasmic incompatibility with certain rice male parents and also, for japonica and basmati varieties it has been difficult to get a good restorer line (Virmani and Kumar, 2004).

In spite of challenges faced in hybrid rice technology, several countries such as India, Indonesia, Bangladesh, Malaysia, Myanmar, Philippines, South Korea, Sri Lanka, Vietnam and Thailand have begun their own hybrid rice research and development programs (Virmani *et al.*, 1997). Results of these programs so far, have shown that the degree of success in the use of hybrid rice depends on the extent of heterosis and the efficiency of the seed production techniques (Virmani and Kumar, 2004). For heterosis exploitation in rice, the two-line hybrid rice system shows many advantages over the three-line system of hybrid rice, including greater ease in determining the pollinator line in the TGMS male sterility system and a 5 to 15 percent higher heterosis (Ku *et al.,* 2001). The use of PGMS in hybrid rice seed production started after the discovery of Nongken58S in 1971 (Shi, 1981). In long day-light length and high temperature growth conditions the PGMS is completely sterile and it is used as female parent in hybrid rice production (Shi, 1981, 1985; Ku *et al.,* 2001) but under short day-light length and low temperature growth conditions it reverts to fertility, thus it maintains itself (Lopez, 2000). Thermosensitive genic male sterile lines (TGMS) (Ali *et al.*, 1995), which are sterile in high temperature growth conditions and fertile in low temperature growth conditions (Reddy *et al.,* 2007) can also be used in two-line hybrid rice seed production system. PGMS and TGMS traits are under nucleus gene control (Zhang *et al.*, 1994; Wang *et al.*, 2003), unlike cms that is under cytoplamic influence (Oka, 1974; Lin *et al.,* 1992).

Therefore, the PGMS/TGMS will reduce the problem of genetic degradation because it can be used with many restorer lines (both indica and japonica) to produce hybrid seeds, unlike in cms where they are limited due to maternal line (or cms) and paternal line (restorer line) incompatibility, which leads to F_1 sterility (Lin *et al.,* 1992).

PGMS (female parental) lines with diversified background can be produced unlike in cms whereby wild abortive (WA) is the major maternal line used (Virmani and Kumar, 2004). However, sometimes it is difficult to achieve complete sterility in the PGMS and the TGMS which brings about the problem of contamination of hybrid seeds by self-bred seeds. Methods of determining purity of seed or seedlings are therefore needed. Commonly used methods include conventional ones where F_1 plants agronomic traits are observed during growth or after maturity to determine selfs. Alternative methods include the use of DNA molecular and morphological markers. Use of DNA molecular markers has effectively been used to select for traits such as bacterial blight resistance, (Shanti *et al.*, 2001; Bagal *et al.*, 2012). However, it requires a specialized laboratory and skilled manpower for effective use in breeding and this tends to exclude the farmer. This leaves morphological markers as more realistic because they are visual and phenotypic. In this study deliberate effort was made to determine the suitability of PGMS/TGMS in hybrid rice seed breeding in Kenyan ecosystems by evaluation of their agronomic traits. Specifically, the study undertook to:-1). Determine the effectiveness of inducing complete sterility of PGMS/TGMS under greenhouse growth conditions in Kenya, 2). Produce F_1 hybrid rice using the PGMS/TGMS as female parents 3). Test for field performance of F_1 hybrids compared to parental lines.

Materials and methods

Plant material

Plant material used were two PGMS lines (V1-IR-73827-23-76-15-7S (TTB03) and V3-IR-75589-31-27-8-33S (TTB01)) and one TGMS (V2-IR-77271-42-5-4-36S (TTB02)) varieties, here code-named as V1PGM,

V3PGM and V2TGM in that order. These materials were sourced from International Rice Research Institute (IRRI) under the supervision of Kenya Plant Health Inspectorate Service (KEPHIS). The Basmati 370 and Basmati 217 used were the local elite lines obtained at the study site KARI-Mwea located in Central Kenya (0.7°S, and Longitude 37.37E) where daylight length and night length are nearly equal (12hours). In this study high temperature will refer to daytime and night time temperatures of ≥35°C and ≥22°C respectively, while normal temperature will refer to temperature of ≤30°C and ≤22°C.

Method

Three sets of experiments were conducted over time: the first experiment to determine sterility induction conditions in PGMS and TGMS (P(T)GMS) rice lines, second was to generate F_1 hybrids (Basmati 370 and 217 vs P(T)GMS lines) using environmental influence emasculation method and third was to compare performance of hybrids vis-à-vis their parents. The first experiment was conducted between August-November 2011, the second experiment from February-May 2012 and third experiment from June-September 2012. For each period, two sets of experiments (in greenhouse (GH) and natural conditions (NC) were conducted.

Sterility Induction in PGMS and TGMS Lines

The P(T)GMS introduced in Kenya agro-ecosystem were first assessed for their ability to induce sterility under greenhouse conditions and to revert to fertility outside greenhouse growth conditions here referred to as natural conditions. The seeds of the three varieties namely V1PGM, V2TGM and V3PGM were sown in two concrete troughs (6.4m x 1.25m) in the green house. Trough one was divided into three blocks. Rice lines V1PGM, V2TGM and V3PGM were sown in blocks 1, 2, and 3 of trough one in that order. Each block had ten rows each with 6 plants at a spacing of 15 x15 cm. Trough two was also divided into three blocks. Blocks 1, 2, and 3 were sown with varieties V1PGM, V2TGM and V3PGM in that order.

All the plants in troughs one and two were allowed to grow in greenhouse conditions until primordial stage. At this stage, half of the hills of each variety were carefully uprooted and transferred (ensuring minimal root disturbance) to the natural conditions in buckets where they were allowed to grow until maturity for evaluation under normal day-light length (NDL) and normal temperature (NT) a treatment referred to as NDL+NT. The remaining plants of each variety in trough two were allowed to grow in greenhouse conditions until maturity but under minimum night and day time temperatures range of 24°-36°C respectively . These treatments are here referred to as NDL + HT. Plants in trough one were all given long day-light length (LDL) treatment by illuminating them with solar light while covered with a black cloth (to trap and concentrate the light around the plants) from 1800 hour to 2100 hour so as to expose them to 14hours of day-light period and high temperatures (HT). This was referred to as LDL+HT. This treatment was done until all the three lines flowered when it was withdrawn. All other cultural rice growing practices were held constant.

Assessment of pollen and spikelets sterility

At anthesis, twenty plants were randomly selected from both greenhouse and natural conditions for pollen sterility analysis. A total of nine spikelets from each plant were collected: three each from top, middle and bottom of the panicle mainly between 8-11am during this period. The spikelets were fixed in 70% ethanol and taken to laboratory for analysis of pollen fertility.

Anthers were extracted from the glumes using forceps and placed on a microscope glass slide with a drop of 1% potassium iodide (I/KI) solution (Virmani and Kumar, 2004) after which they were macerated using the forceps to release the pollen cells. Anther-husks were removed from the glass slide leaving the microspores. A cover slip was placed on a glass slide and observed under x10 objective of light microscope. Pollen fertility was scored by counting yellow staining abortive pollen grains against the blue black staining

fertile ones and was expressed in percentage as shown below (Eq. 1):-

Equation.....1

$$\text{Pollen fertility}(\%) = \frac{\text{Total nuber of fertile pollen grains in 4 microscopic fields}}{\text{Total number of fertile pollen grains in 4 microscopic fields (fertile + sterile)}}$$

Spikelet fertility was also assessed at seed maturity stage from two panicles from each of the selected plants and expressed in percentage by taking the actual count of filled seeds to the total number of spikelets in a panicle (Eq. 2).

Equation....2

$$\text{Total fertility}(\%) = \frac{\text{Number of filled grains}}{\text{Total number of spikelets in a panicle}} \times 100$$

Production of F_1 hybrids

Seeds of P(T)GMS and Basmati 370 and Basmati 217 were pre-germinated after which they were sown in nursery and later the seedlings were transplanted in the greenhouse at 21 days old. The Basmati varieties were sown at an interval of 10 days before, during and after the commencement of the main experiment in buckets under natural growth conditions outside the greenhouse. This was to ensure that pollen to pollinate P(T)GMS was available throughout their flowering period. The P(T)GMS seedlings were divided into two sets; one set was transplanted in three concrete troughs (6.4m x 1.25m) in greenhouse growth conditions while the other set was planted in buckets under natural growth conditions.

The troughs were subdivided into sub-blocks of which the ones in trough one were designated as T1B1 and T1B2, T2B1 and T2B2 for blocks in trough 2 and T3B1 and T3B2 for trough 3. Each of the V1PGM, V2TGM and V3PGM lines was separately planted in rows in its own block. A distance of 60cm was maintained between blocks to prevent any possible inter-block contamination. Each block had ten rows each with 6 plants at a spacing of 15 x15 cm. Temperature in the greenhouse was maintained as described under materials throughout the growth period. The troughs were irrigated daily in the evenings. Weeding was carried out manually at the second week after transplanting and a second weeding during panicle initiation. A basal dose of N-P-K fertilizer (15:15:15) at the rate of 60 kg/ha was applied at 15 days after transplanting, immediately after removing the weeds. Subsequently, urea was top-dressed at the rate of 90 kg/ha at 30 days (at maximum tillering) after sowing.

At flower emergence stage the V1PGM, V2TGM and V3PGM lines were cross pollinated with Basmati 217 to obtain hybrid lines here designated as V1217, V2217 and V3217 while lines designated V1370, V2370 and V3370 were obtained from crosses between Basmati370 and V1PGM, V2TGM and V3PGM respectively. Pollination was done according to Kaushal and Raven (1998) whereby mature panicles were carefully cut off from mother plant (grown under natural conditions), covered with porous paper and kept separately under warm water (27-30^{o}C) for about two hours to facilitate opening of florets. Once the maternal (P(T)GMS) panicles fully opened (usually between 10.00-1400 hrs), the basmati panicles (pollen donor) were uncovered and pollen immediately sprinkled over the maternal flowers inside each block. Plants in blocks T1B1, T2B1 and T3B1 were crossed with Basmati 217 while T1B2, T2B2 and T3B2 were crossed with Basmati 370. The process was repeated twice to ensure higher seed set rate. At maturity, the F_1 seeds were harvested from each plant, counted per panicle and stored in separate porous paper bags. After three weeks, the seeds were incubated at 50^{o}C for 7 days to break dormancy. After incubation, the F_1 seeds were planted together with the five parental lines for characterization of plant vigour, seed production and agronomic traits.

Agronomic evaluation of F_1 hybrids and parents

The hybrid seeds were pre-germinated and seedlings divided into two equal sets; one set was transplanted in three concrete troughs in the greenhouse while the other set was planted in similar buckets under natural growth conditions. Two troughs in the greenhouse were each demarcated into eight plots. First trough was sown with hybrids V1217, V2217 and V3217 while second trough was sown with V1370, V2370 and V3370.

In each trough the five parental lines were sowed as control (cK). From each block twenty plants were randomly selected for scoring of agronomic traits which included plant height, flag leaf length, withd and angle, panicle exsertion and length, number of tillers, number of days from sowing to 50 % flowering and seeds per panicle. However, data for seeds assessment was only collected from the greenhouse conditions. In the greenhouse temperatures were maintained between 27-30°C throughout the growth period

Data analysis

Data were subjected to Analysis of Variance (ANOVA) procedure using R program software version 2.15.2 (2012-10-16) (R Development Core Team, 2012). Differences were declared statistically significant when $P < 0.01$ and where significant differences were detected, the Tukey's mean separation procedures were used at 1 % probability level.

Data normality was checked by Shapiro test while Inter-relationships among trait values were estimated using the Pearson correlation coefficient.

Results

Fertility assessment

Among the plants grown under LDL+HT growth conditions 100% of pollen grains from lines V1PGM and V3PGM stained yellow with 1% K/I and seed set was 0% (Table 1), while line V2TGM recorded 99% yellow pollen when stained with 1% K/I and 3 % seed set under the same conditions. The staining pattern of pollen from V1PGM andV3PGM grown under HT+NDL conditions had no observable difference from those under LDL+HT. However, V2TGM grown under HT+NDL and that grown under LDL+HT recorded 100% and 99% yellow-staining pollen with 1% K/I respectively. Seed set rate of V2TGM grown under LDL+HT and HT+NDL was 3% and 2% respectively (Table 1).

Table 1. Fertility evaluation of P(T)GMS lines in different conditions. LDL+HT (Long day length and high temperature), NDL +HT (Normal day length and high temperature) and NDL +NT (Normal day length and normal temperature). LDL+HT and NDL+HT were all carried under greenhouse growth conditions while NDL + NT treatment was carried outside the greenhouse growth conditions.

Cultivar	Treatment of spikelets	Percentage (%) Pollen fertility	Abortive pollen (%)	Percentage (%) seed set
V1PGM	LDL + HT	0	100	0
V2TGM	LDL + HT	1	99	3
V3PGM	LDL + HT	0	100	0
V1PGM	NDL + HT	0	100	0
V2TGM	NDL + HT	0	100	2
V3PGM	NDL + HT	0	100	0
V1PGM	NDL + NT	64	34	54
V2TGM	NDL + NT	60	100	30
V3PGM	NDL + NT	55	45	26

Panicles of rice lines V1PGM, V2TGM and V3PGM grown under LDL+HT did not have observable seeds in them (Table 1). This was the same as panicles sampled from the three lines grown under NDL+ HT growth conditions

Agronomic evaluation

Variations were observed in mean values of traits in this study. The mean plant height among the cultivars in greenhouse conditions was 112.9 ± 1.4 cm which ranged from 68–148 cm while in natural conditions mean height was 96.3 ± 1.6 cm and ranged from 52-125 cm. Basmati 217 and the F_1 hybrid cultivar V3370 recorded the highest heights of 152 and 148 cm under greenhouse and natural conditions respectively while V3PGM cultivars scored the lowest heights (68 and 52cm) under both greenhouse and natural conditions respectively (Fig. 1). Significant differences ($P<0.01$)

were observed between the parents and hybrids in both conditions with hybrids generally displaying intermediate heights while the maternal parents (PGMS and TGMS) were the shortest (Fig.1).

There were variations ($P<0.01$) in growth rates overtime between and within the growth conditions and between the parents and hybrids with local cultivars showing a superior growth compared to the exotic (P(T)GMS) counterparts and hybrids assuming an intermediary growth (Fig. 2). Generally, the growth rate in natural conditions was lower compared to that under greenhouse. Although the growth rates of hybrid cultivars were similar at the early stages (2-8 weeks) of development, real differences in height gains ($P<0.01$) set in from the 12^{th} week onwards. Of significant is that hybrid V2370 in natural conditions initially had a relatively lower growth rate but later greatly improved to score the highest growth rate (Fig. 2).

Table 2. Percentage seed set per panicle (Data for these results were collected from the greenhouse conditions only).

Cultivar	Mean number of spikelets	Mean number of seeds per panicle	Percentage (%) seed set
V1217	90	20	22.7
V1370	82	24	29.8
V2217	80	25	31
V2370	116	22	18.8
V3217	102	7	6.8
V3370	91	12	12.9
B217	100	66	65.7
B370	100	72	71.7
V1PGM	5	0	0
V2TGM	20	0	0
V3PGM	6	0	0

Flag leaf lengths varied ($P<0.01$) within and between the growth conditions. Mean flag leaf length in greenhouse was 26.6 ± 1.9 cm which ranged from 19.8–35.9 cm while in natural conditions it was 24 ± 1 cm and ranged from 19.5-27.2 cm (Fig. 3). The longest (35.8 cm) flag leaf was observed in hybrid V2370 under greenhouse conditions while maternal parent V3PGM had the shortest (19.8 cm) length in both greenhouse and natural conditions. Under natural conditions Basmati 217 had the longest leaf length. While hybrids recorded some of the longest leaf lengths in greenhouse, under natural condition they displayed intermediary lengths compared to their parents. Under both growth conditions, the maternal cultivars (P(T)GMS) had among the shortest leaves (Fig. 3).

No variations ($P>0.01$) were observed in flag leaf widths among the rice cultivars grown under greenhouse conditions (Fig. 4) but variations ($P<0.01$) were observed under natural conditions with means of 1.2±0.06 cm ranging from 0.92-1.48 cm. Under these conditions, Basmati 217 had the narrowest (0.9 cm) while hybrid V1370 had the most broad (1.48 cm) leaves.

Similarly, mean flag leaf angle varied ($P<0.01$) among the plants with paternal cultivars displaying greater angles compared to hybrids and maternal (P(T)GMS) cultivars. Under these growth conditions, flag leaf angles ranged from 24.0-58.5° with a mean angle of 37± 3.3°. Hybrid V2217 had the least angle (24°) while Basmati 370 recorded the greatest angle (58°, Fig. 5). However, this was in contrast to the flag leaf angles of plants grown under natural conditions where Basmati 217 had the least (19°) while V3PGM had the greatest angle (35.5°).

Under these conditions angles ranged from 19.3-35.5° with a mean of 24.9±1.4°.

Panicle exsertion significantly varied (P<0.01) among the cultivars within and between the growing conditions as well. In GH conditions, exsertion ranged from 1–8 cm with a mean value of 4.7±0.3 cm while under natural conditions it ranged from 0.7-10.3 cm with a mean of 2.9±0.3 cm (Fig. 6). In GH conditions, V2217 had the greatest (8cm) while V1PGM had the least (1 cm) exsertion. Generally hybrids exhibited greater panicle exsertion compared to their parents under these conditions. However, the situation seemed to reverse under natural conditions where panicle exsertion for parental cultivars was generally higher than that of hybrids (Fig. 6). Under natural conditions V3PGM had the greatest (10.3 cm) while V3217 had the lowest (0.7 cm) panicle exsertions.

Table 3. Correlation Coefficients for Plant height (PH), Flag leaf length (FLL), Flag leaf width (FLW), Flag leaf angle (FLA), Panicle length (PL), Panicle exsertion (PE), Tillering (Ti), Heading days (HD) and percentage seed production (%SD).

	PH	FLL	FLW	FLA	PL	PE	Ti	HD	%Sst
PH	1	0.761*	0.021	0.350*	0.428*	0.689*	-0.673*	-0.392*	0.129
FLL		1	-0.043	0.111	0.533*	0.695*	-0.748*	-0.432*	0.368*
FLW			1	-0.087	-0.097	0.049	-0.039	0.178	0.351
FLA				1	0.02	-0.012	-0.154*	0.274	0.328*
PL					1	0.476*	-0.400*	-0.277*	-0.305*
PE						1	-0.556*	-0.598*	-0.299*
Ti							1	0.267*	-0.320*
HD								1	0.283*
%Sst									1

*Values in parentheses indicate there were correlations

For panicle length, differences (P<0.01) were also exhibited within and between growth conditions with GH conditions recording higher lengths ranging from 20.4-30.7 cm with a mean of 25.9±1.4 cm compared to their counterparts under natural conditions (18.8-27.3 cm with a mean of 22.4±0.6 cm). V2370 and V2TGM scored the highest (30.77 and 27.31) in GH and NC respectively while cultivars V1PGM and V3PGM recorded the lowest (20.4 and 18.8 cm) panicle lengths under GH and NC respectively (Fig. 7). Under greenhouse conditions hybrids generally had greater panicle lengths compared to their parents while under natural conditions they assumed intermediate lengths.

Comparison in tillering ability between the hybrids and parents revealed a poor response of the hybrids in GH but performed better under natural conditions (Fig. 8). The maternal parents (P(T)GMS displayed the best tillering (81) in GH but responded poorly under NC where hybrids exhibited better (100) tillering. Generally cultivars in NC exhibited better tillering (P<0.01) with a mean of 75±3 compared to those in GH conditions which recorded a mean of 52±3 (38, Fig. 8).

The number of days to 50 % flowering significantly varied among the cultivars with heading days ranging from 85-110. Hybrid cultivar V2217 had the shortest mean flowering time of 85 days followed by V2370 which attained 50% flowering in 88 days (Fig. 9). Much delay in flowering was observed in V1PGM followed byV3PGM which flowered at 110 and 109 days respectively. Generally the hybrids reported an early flowering compared to their parents (Fig. 9).

Seed production significantly varied (P<0.01) within cultivars with hybrids performing poorly. Hybrid cultivar V3B217 had the lowest (6.8%) while Basmati 370 had the highest (71.7%) percentage seed set followed by Basmati 217 (65.7%). The P(T)GMS cultivars did not produce any seeds under the prevailing conditions (Table 2). Percentage seed set

correlated with all the traits except for the plant height and flag leaf width (Table 3). Panicle length (r=-0.305, P<0.01), panicle exsertion (r=-0.299, P<0.01) and tillering (r=-0.320, P<0.01) negatively correlated with percentage seed set but flag leaf length (r=0.368, P<0.01) and flag leaf angle (r=0.328, P<0.01) had a positive correlation.

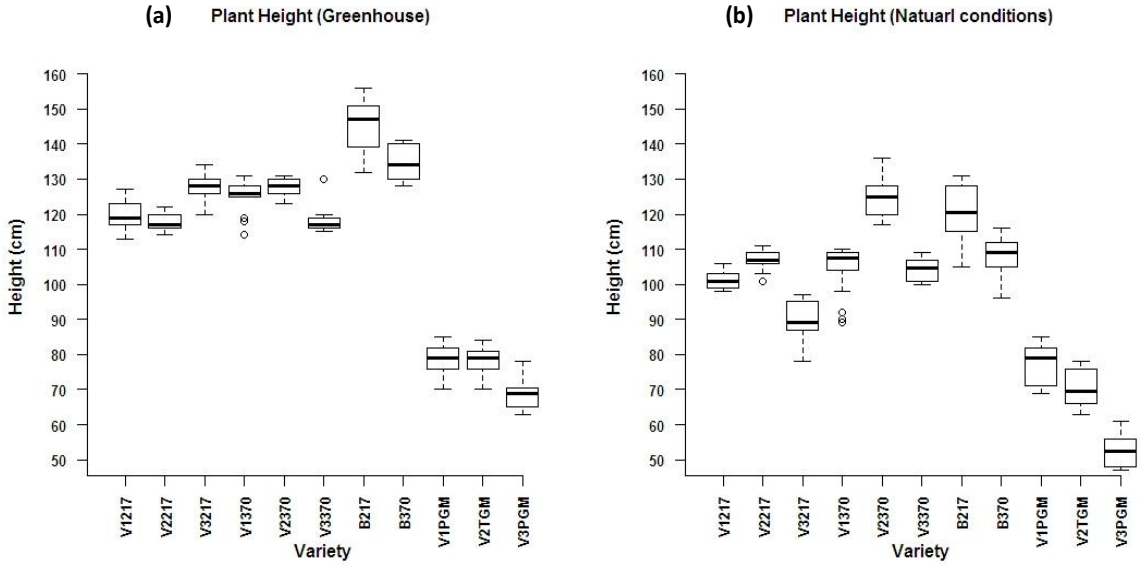

Fig. 1. Plant heights under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

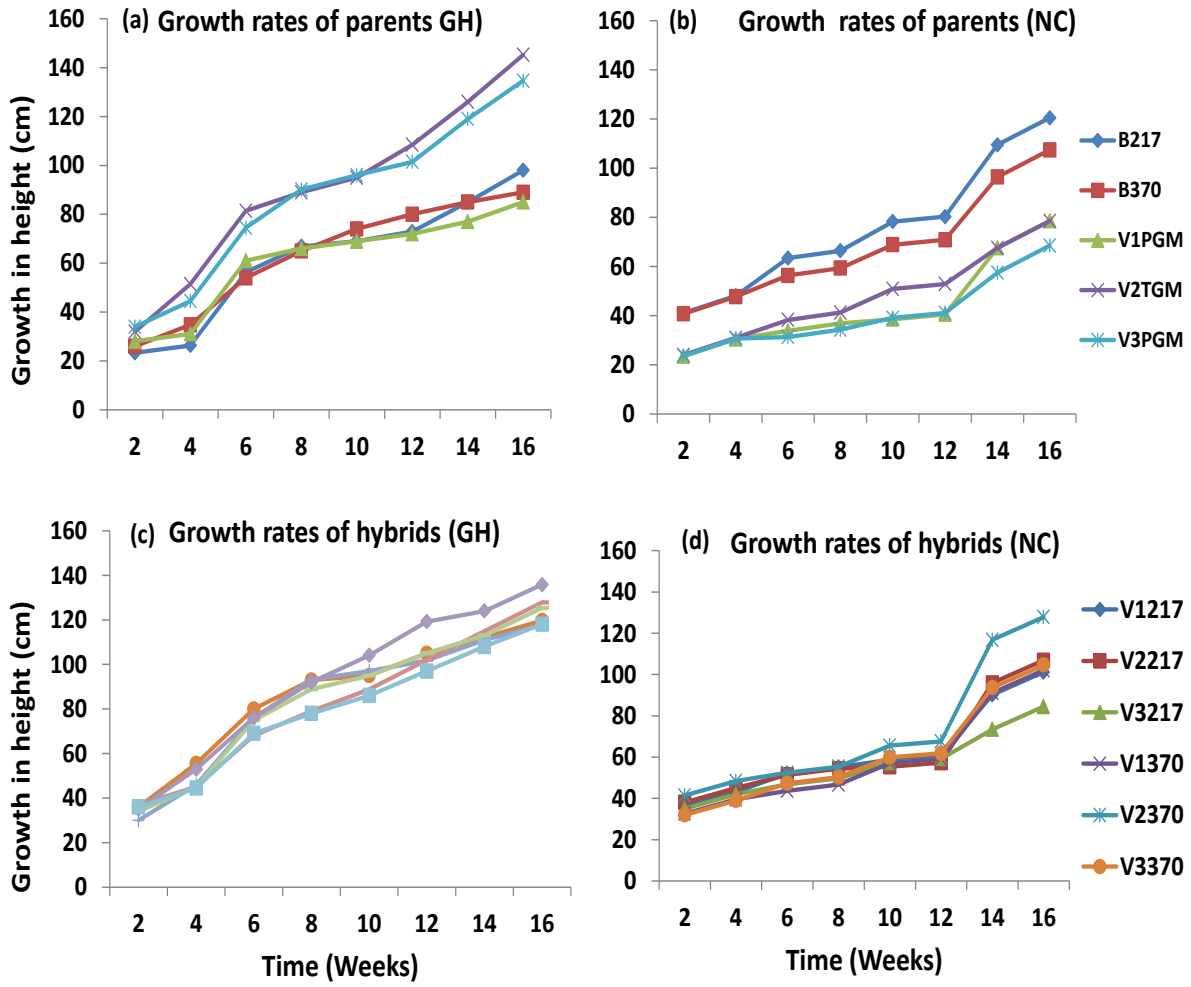

Fig. 2. Growth rates of parents (a and b) and hybrids (c and d) under greenhouse (GH) and natural conditions (NC) respectively. B217, B370, V1PGM, V2TGM and V3PGM represent the parents while V1217, V2217, V3217, V1370 and V3370 are the hybrids.

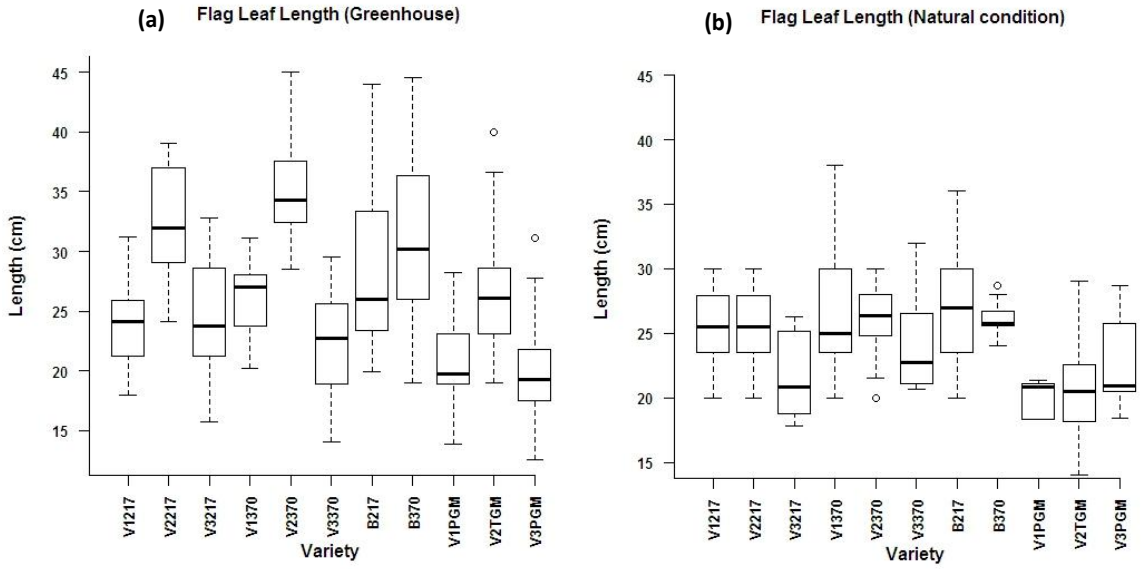

Fig. 3. Flag leaf lengths of cultivars under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

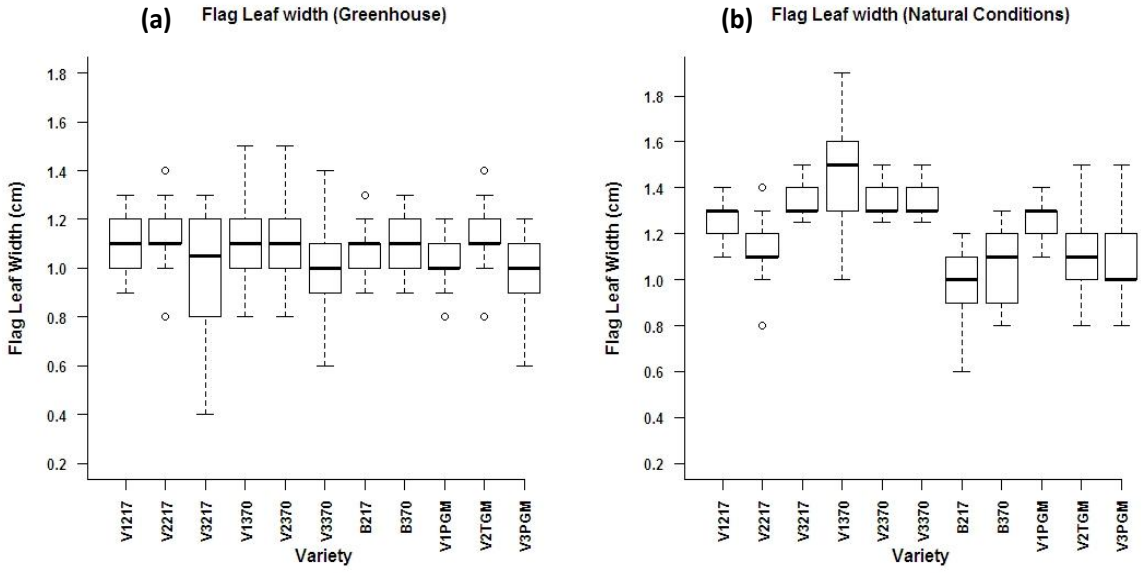

Fig. 4. Flag leaf width of cultivars under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

Discussion

Rice lines V1PGM and V3PGM when grown under LDL+HT conditions and NDL+HT conditions, all recorded seed set rate of 0% with no observable seed set difference among the two growth conditions. According to Shi and Deng (1986) and Xue *et al.*, (1999), PGMS rice plants require long day-light to be completely sterile, while TGMS rice plants require high temperature to attain complete sterility. However, under high temperature growth conditions, the critical photoperiod required to induce sterility is lower (Yuan, 1993; Lopez, 2000). This is because high temperature compensates for the reduced photoperiod required to induce complete pollen sterility especially for PGMS which are breeds for temperate regions where natural day-light length is long (in summers). In this research work under high temperature conditions (in greenhouse) and under normal tropical 12 hour day light we obtained complete sterility of PGMS lines (V1PGM and V3PGM). This observation is in tandem with that of Yuan (1993) who reported that under high temperature growth conditions, the photoperiod required to induce complete sterility in PGMS is reduced. Although V2TGM under LDL+HT and those under NDL+HT gave seed set of 2% and 3 % respectively, the difference between them and that observed in PGMS (V1PGM and V3PGM) lines was insignificant.

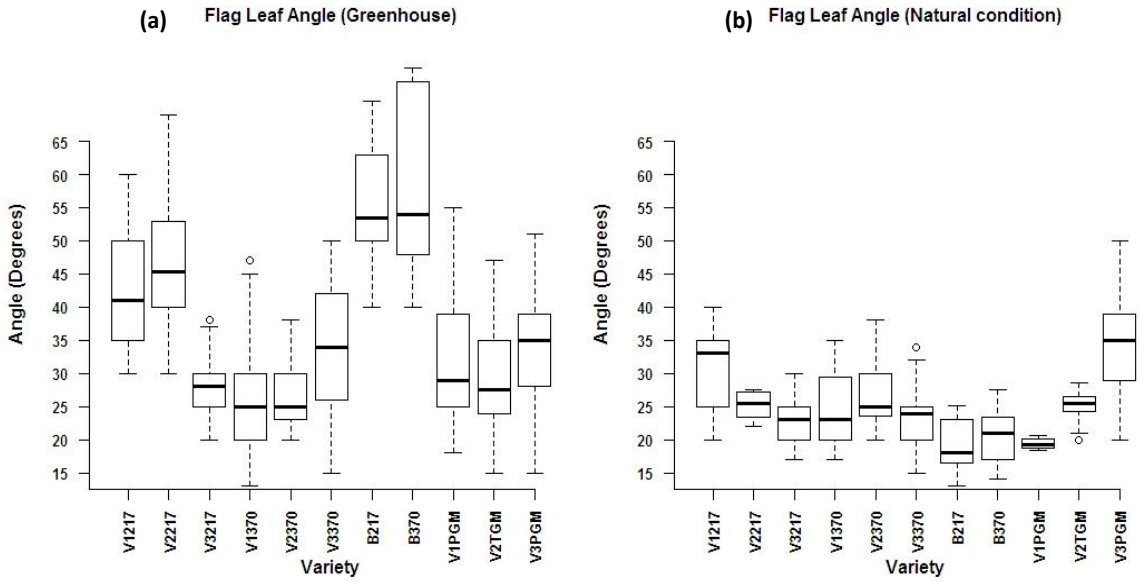

Fig. 5. Flag leaf angle of cultivars under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

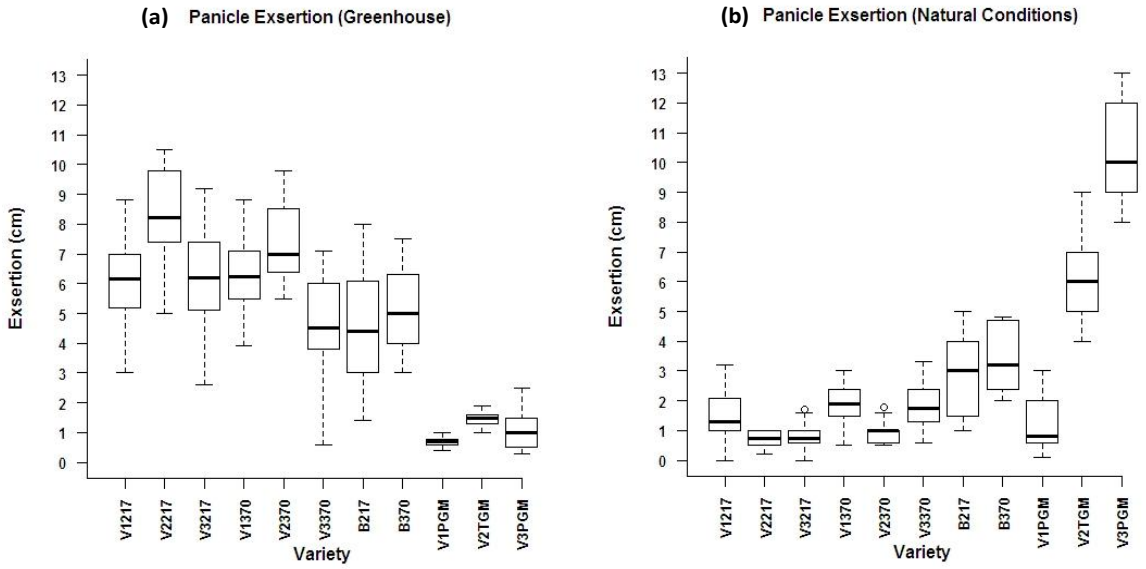

Fig. 6. Panicle exsertion of cultivars under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

Pollen sterility in V1PGM and V3PGM was 100% with a seed set rate of 0% (Table 1) while V2TGM had pollen sterility of 97% with a seed set rate of 3%. This shows that percentage pollen sterility can be used to correctly predict actual spikelet sterility in a hybrid rice breeding programme. TGMS and PGMS lines grown under long day-light length and high temperature conditions had their pollen fertility significantly reduced (Ku *et al.*, 2001); a condition which can also be used to predict spikelet fertility. Results from this research work indicate that fertility of the three lines (V1PGM, V2PGM and V3PGM) under LDL+HT growth condition was the same as those under NDL+HT growth conditions (high temperature). This is an indication that high temperature can effectively be used to mitigate the long day-light length requirement needed to realize 100% sterility in PGMS, a condition that is necessary to prevent adulteration of hybrid seeds with self-bred during crossbreeding (Njiruh *et al.*, 2011). The results

also indicate that sterility in the PGMS and TGMS rice lines under study could be induced by using high temperature alone without increasing day light length. This was particularly important in that by eliminating use of solar illumination not only reduces cost of resource use but also error margins (that could be introduced during switching off and on of the light source).

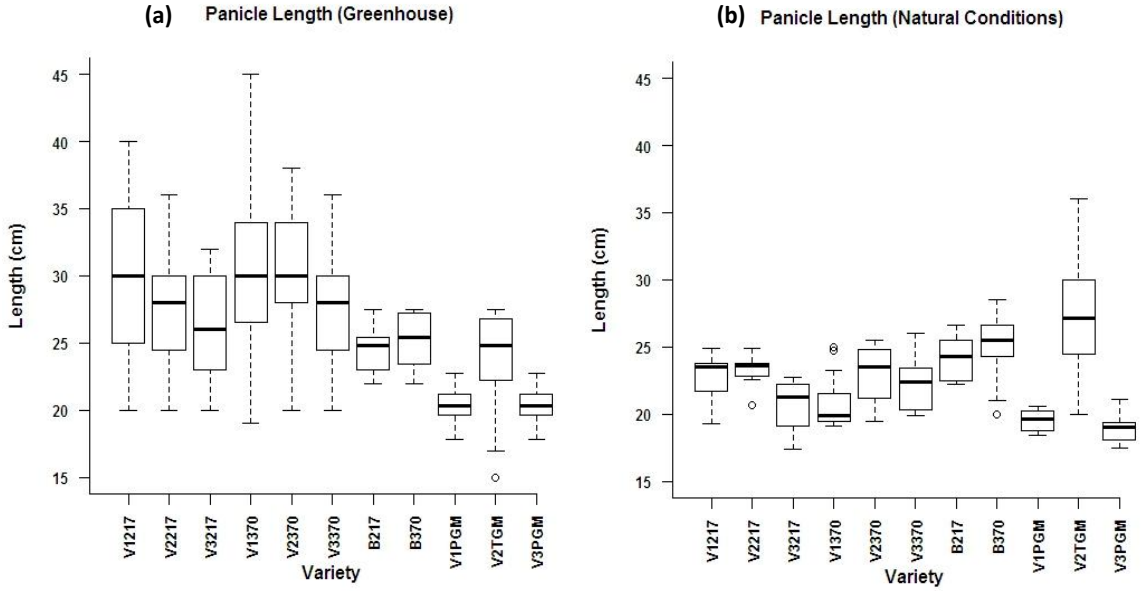

Fig. 7. Panicle length of cultivars under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents.

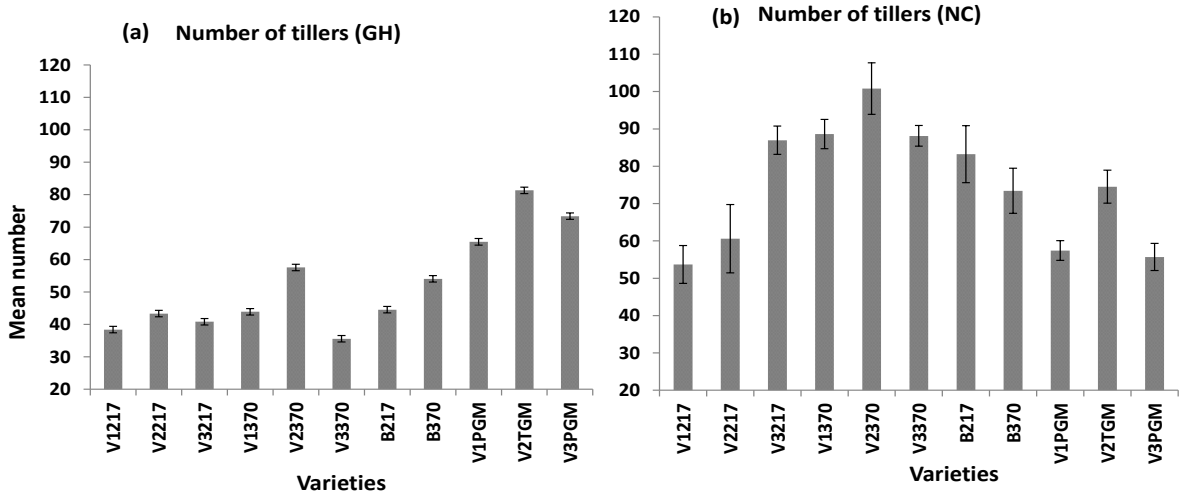

Fig. 8. Number of tillers per hill under greenhouse (a) and natural conditions (b). V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents (Y-axis is adjusted).

Significant variations were observed in agronomic traits among the five parents and six hybrids. Variations in plant height indicated that the local cultivars (Basmati 370 and 217) were taller than all the other cultivars while the maternal parents (P(T)GMS) were the shortest with hybrids displaying intermediate heights. These results are in line with the findings of Tua *et al* (2007) and Kanya *et al*., (2012) who found that hybrid rice had intermediary heights compared to their parents. However, this depends on other factors such as cultivar type, agro-ecosystems involved and the cultural agronomic practices applied (Zafar *et al.* 2004). Various explanations on the importance of plant height in plants have been postulated. According to Zafar *et al*., (2004) and Kende *et al*., (1998) plant height improves

access to light, hence increases seed production due to improved photosynthesis. But according to Evans (1998), dwarf plants have a superior advantage over tall plants in that they have increased grain yield through an improved 'harvest index' (the proportion of plant weight in the grain). This means that a greater proportion of the products of photosynthesis accumulate in the grains rather than in the leaves. Short stems also reduce high investment costs incurred in construction and maintenance of the stem and improve translocation of resources from stems and leaves to seeds (Jackson, 1985; WARDA, 1999; Falster *et al.* 2011). Thus production of hybrids with intermediate heights in the current study is significant in that it can be utilized to achieve balanced advantages of height. The growth rates indicate that local cultivars have an outstanding growth compared to the exotic cultivars. Although the growth rates were similar at the early stages (2-8 weeks) of development, real differences in height gains set in from the 12th week onwards especially for the hybrid cultivars. This comes late in the developmental stage and thus there may be no possibility of a competitive advantage, in terms of height for hybrids as compared to the local cultivars.

The present study showed variations in flag leaf lengths within and between the two growth conditions with hybrids developing longer leaves than their maternal parents. Long and broad flag leaves in hybrids are particularly important in that they provide more surface area for trapping solar radiation hence more photosynthesis (Hasegawa and Horie, 1996; Blake *et al.*, 2006). Flag leaves, compared to penultimate leaves contribute most photosynthetic products that are important in the grain filling (Blake *et al.*, 2006). Hence the higher and intermediate lengths observed in the hybrids in the current study is an indication of hybrid vigour and a potential advantage of producing more and quality filled grains compared to their parents. Flag leaves in cereals contribute about 41-43% of photosynthetic assimilates and that the distribution of these assimilates from leaves varied depending on the position of the leaf (Dere and Yildirim, 2006). It is also documented (Hasegawa and Horie, 1996) that about 80% of assimilates from flag leaves are translocated to the panicle and only ~5% of it comes from the fifth leaf from the flag leaf.

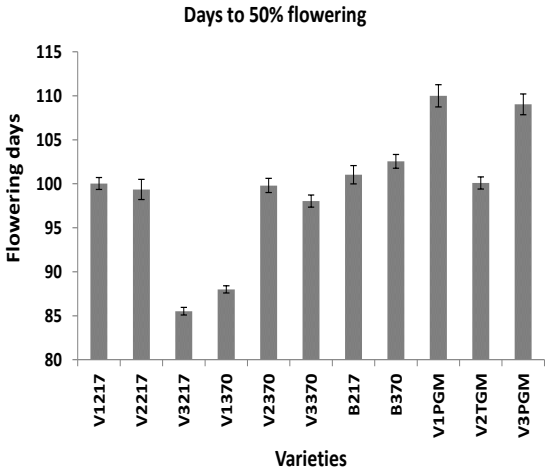

Fig. 9. Number of days to 50% flowering under greenhouse conditions. V1217, V2217, V3217, V1370 and V3370 represent hybrids while B217, B370, V1PGM, V2TGM and V3PGM are the parents (Y-axis is adjusted).

Further in the current study flag leaf angle of hybrids, which is also associated with increased grain filling, had mixed results; low, intermediary and high. In rice breeding, grain yield is a function of photosynthesis and an optimum distribution and arrangement of leaves may increase the plants' efficiency (Tari *et al.*, 2009). A greater penetration of light into the crop canopy combined with morph-physiological traits is appropriate for the productivity of the crop. Hence, modifications of flag leaf angles have been emphasized as a means of obtaining better light utilization, with more upright leaves permitting better capture of energy to the lower levels of the aerial structure of plants (Donald, 1968; Tari *et al.*, 2009). In the current study, hybrids with high and intermediary flag leaf angles are proposed candidates for selection in future rice hybrid production. It is however notable that the hybrids have greater flag leaf angles in natural conditions compared to their parents an indication that they would yield better under field conditions. Study by Sasahara *et al.*, (1992) showed that when half of the leaf's angle from the horizontal plane falls from 35^{o} to 52^{o}, the

be higher. According to them, this could be attributed to reduced leaf blade's inclination and that lower leaves received more irradiation. In the current study, leaf angles of hybrids V3370 (35°), V1217 (42°) and V1370 (49°) in greenhouse conditions were within the proposed range for optimal rice yield but only one hybrid (V1217) cultivar fell within this range under natural conditions. Except for V3PGM (35°), all other parents' leaf angles fell out of this range suggesting that the hybrid cultivars have an ecological advantage over parents on this trait.

Panicle length and exsertion also exhibited variations under the two growth conditions with hybrids performing better than parental cultivars in greenhouse condition but which was reversed under natural conditions. Panicle length and exsertion in rice is driven by uppermost internode elongation which is linked to internode elongation gene (*eui1*). This gene is responsible for complete panicle exsertion and is influenced by temperature variations (Hittalmani *et al*., 2002; Yang *et al*., 2003; Ma *et al*., 2009). Different temperatures were found to induce full expression of male sterile gene in P(T)GMS lines; the lower the temperature, the higher the expression level of *eui* gene and the better panicle exsertion (Bardhan *et al*., 1982; Xiao and Wang, 2008; Yang *et al*., 2003). In the current study, low temperatures in natural conditions compared to greenhouse conditions may have contributed to the strong variation realized in panicle exsertion. It is notable that the response of the hybrids in relation to the two traits more or less resembled that of their paternal parents under the two growth conditions suggesting that the two traits could be paternally influenced.

Our preliminary results show that hybrids have poor performance in seed set (<7%). The low yield in hybrids compared to parents however may have been anticipated given that this was a cross between indica and japonica sub-species. Crosses between indica and japonica result in hybrid sterility despite their high heterosis (Zhang *et al*., 1997). However, certain indica and japonica hybrids show normal spikelet fertility in which case one or both parents of these crosses must possess a dominant wide-compatibility gene (*S5n*) (Ikehashi and Araki, 1986) and such lines are designated as wide-compatibility varieties (WCVs). Sterility and non-sterility is thought to be controlled by three alleles *S-5i* (in indica), *S-5j* (in japonica) and *S-5n* from WC rice (Ikehashi and Araki, 1986; Zhang *et al*., 1997). Genotypes *S-5n/S-5i* and *S-5n/S-5j* results in fertile female gametes but the *S-5i/S-5j* genotype produces semi-sterile panicles because of the partial abortion of female gametes and this is what is postulated to have worked in our study as evidenced by the low percentage seed set.

In the current study, correlation coefficients between agronomic traits and % seed set revealed varied results. Some traits (flag leaf length, width and angle and days to 50% flowering) correlated positively while others (panicle length and exsertion and tillering) correlated negatively. However, while it is documented (Kende *et al*., 1998; Zafar *et al*., 2004) that plant height characters are important in improving rice production, in the current study there was no correlation between the two traits. Whereas this information is true in relation to some cultivars in the current study, to others it is conflicting, for instance; paternal parents had among the highest plant heights but recorded the highest (71.6%) % seed set while hybrids which had intermediary heights had the lowest % seeds set (6.8%). Nonetheless, such contradictory observations have been noted before by and Muhammad *et al*., (2012) who observed that height alone may not be used as the only measure for grain yield but rather a combination of more traits and exogenous factors (Islam *et al*., (2009).

Lack of relationship between flag leaf width and other leaf related traits in the current study was surprising given that many authors (Blake *et al*., 2006; Ahmadikhah *et al*., 2008; Dwivedi, 2012) have indicated strong relationships between flag leaf width, angle and length. This could possibly be explained by the negative heterosis realized after combining the local cultivars with the P(T)GMS cultivars. Similar non-correlation reports were given by Tari *et al*. (2009). However, correlation between early flowering and % seed set was a good outcome for hybrids given

that late flowering is not a good breeding selection goal because any gain in yield may be offset by losses in other traits of economic importance (Naokuni and Takeshi, 2011). In the current study, hybrids displayed an impressive early flowering compared to their parents an indication of a strong potential for yield improvement. The relationships between tillering, flowering days and % seed set in this study was in line with the findings of (Wang *et al.*, 2007) who found that grain number per panicle showed the greatest variation over tillers among all yield components, indicating that more tillers bring obvious negative impact on grain number per panicle, leading to reduced panicle weight. This suggests that moderate tillering would give better yields.

In conclusion, our results show that the use of P(T)GMS hybrid rice technology for seed production in Kenya is promising and that the technology can be applied using controlled temperature as the only environmental condition. We also confirmed that hybrid production has potential gains and losses in regard to agronomic traits; some traits in hybrids perform better than in either of the parents. Based on our results we hypothesize that plant height, flag leaf length (in both GH and NC) and panicle exsertion, days to 50% flowering and tillering (all in GH) were linked to male influence whereas flag leaf angle (GH) had female influence. However, performance of flag leaf width and angle, panicle exsertion and tillering (all in NC) was due to genotype and environmental interaction. Generally the hybrids had either an intermediary or higher performance except in percentage seed set where they performed poorly. We elucidate that greenhouse conditions could be used as initiation breeding conditions for the F_1 hybrids followed by adaptability evaluation later in natural conditions.

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