

## **PRODUCTION OF HYBRID BASMATI RICE IN KENYA: PROGRESS AND CHALLENGES**

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### **Abstract**

Photoperiod sensitive genic male sterile (PGMS) and Thermosensitive genic male sterile (TGMS) rice varieties require a long day light length and high temperature respectively growth conditions for them to be completely male sterile. In hybrid rice seed technology complete male sterility is required in pollen recipient parent so as to avoid contamination of hybrid seeds by selfbred seeds. Natural weather conditions necessary to achieve this are difficult to obtain in the tropics thus limiting the use of the PGMS/TGMS lines. In this research PGMS and TGMS rice plants were grown under a tropical daylight length that was prolonged to 14hour using solar light illumination gadget while minimum daytime temperature was raised to  $\geq 36^{\circ}\text{C}$  using greenhouse. This was done under non-automated ordinary greenhouse conditions. The objective was to induce complete male sterility in PGMS or TGMS and to produce hybrid seeds. Under greenhouse growth conditions it was possible to induce complete male sterility in PGMS and TGMS. When grown outside the greenhouse the PGMS and TGMS were male fertile which is required for their own self propagation. The male sterile plants were cross pollinated with elite basmati rice to obtain  $F_1$  seeds. This paves the way for hybrid rice production in Kenya. One other major challenge is that the characteristic aroma found in basmati is not felt in hybrid plants because it is under a recessive gene control.

**Key Words:** Basmati, heterosis, hybrid, PGMS, TGMS

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### **INTRODUCTION**

In Kenya, rice (*Oryza spp*) is a major food crop after maize and wheat and the demand is increasing especially in the urban population. Unfortunately, rice yield per hectare is low compared to other rice producing countries like China, Japan, India and USA. Kenya produces about 100millions tones against a demand of over 300 million tones per year with an average of 3.6 to 4.0 tonnes per hectare (1). The 1960s and 70s green revolution that transformed rice and wheat plants from tall to short stature using semi-dwarf (*sd1*) gene significantly increased their yields (2). By 1980s and 90s, the high yielding varieties (HYV) produced in green revolution had reached a breeding plateau (3). Super hybrid rice seed technology and Biotechnology are among the breeding methods expected to push rice yield above the breeding plateau. However, the technologies seem to evade Africa, Kenya included, thus leaving the problem of food insecurity intact. In Kenya, Basmati rice is preferred by consumers because of its aroma and good cooking qualities. However, basmati yield is very low compared to other non-aroma rice lines (4). This has kept the average rice yield in Mwea, which is the main rice growing region in Kenya, low.

Hybrid technology, exploits heterosis (5), to raise yield above the breeding plateau currently experienced with pure inbred lines (6,7). The challenge has been lack of an effective method to emasculate male gametes of female parent so that they can be pollinated by a male parent. Both the Cytoplasmic male sterility (CMS) and Environment genic male sterile (EGMS) rice

lines are suitable candidates for use in hybrid rice technology (8). Sterility in CMS, which is due to sterile factors in cytoplasm (9), has a number of disadvantages among them is high expense to produce hybrid seeds because it utilizes three lines namely; the CMS (A line), the maintainer (B line) and the restorer (R line) (10), compared to EGMS that utilizes two lines. The alternative to CMS is the use of photo-period sensitive genic male sterile (PGMS) rice or thermosensitive genic male sterile (TGMS) rice collectively known as environment genic male sterile (EGMS) lines. The PGMS lines are completely male sterile in long day-light length (>13.75hour) and revert to fertility under short day-light length (<13.75) growth conditions (11,12,13). On the other hand TGMS are completely male sterile when grown under temperature greater than 30°C in their critical sterility determining phase (14). In their male sterile phases these lines can be cross pollinated with a fertile male to produce hybrid seeds which is less costly compared to use of CMS method that uses three lines. The challenges of producing hybrid seeds around the equator using EGMS are short day of 12hours and temperature below 30°C. This is below the required minimums needed to induce complete sterility (15) and thus a likelihood of contamination of hybrid seeds with self-bred ones. Secondly, aroma trait in Basmati rice is under recessive gene control, thus F<sub>1</sub> from a cross between Basmati and non-aroma line do not have aroma. Therefore, a deliberate effort is needed to develop a basmati female line parent with PGMS/TGMS gene for use in Basmati hybrid rice breeding programme (BHRBP). EGMS materials, though non-Basmati, have so far been tested for adaptability in Kenya paving way for their utilization in hybrid seed production technology. This paper reports on the progress and challenges faced in development of Basmati hybrid seeds in Kenya.

#### **MATERIALS AND METHODS**

Two PGMS rice lines, V1-IR-73827-23-76-15-7S, V3-IR-75589-31-27-8-33S, and one TGMS line, V2-IR-77271-42-5-4-36S were imported from International rice research institute through KEPHIS, here they are code-named as V1PGMS, V3PGMS and V2TGMS respectively. They were crossed with Basmati370 and Basmati217 (B370 and B217) to get F<sub>1</sub> lines. All the materials were sown at KARI-Mwea which is located in Kirinyaga district in Central province of Kenya on Latitude -0.7°S, and Longitude 37.37E. In this research work unless otherwise stated, long day refer to a 14hours that included 12hour normal day light plus 2hour of illumination using solar lighting system) and a short/normal day refer to 12hours of lighting. On the other hand, high temperature and low temperatures refer to ≥36°C day time, and between 19°C and 24°C night time respectively. The greenhouse used was non-automated and extreme rise in temperatures was prevented by opening the doors and the sides of the greenhouse.

##### **Test of EGMS adaptability**

EGMS and Basmati rice seeds were soaked in 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 72 hours with a change every 24hours to break the dormancy. Then about 200 seeds were sown in a 3cm deep 6cm width and 8cm length plate with fine decomposed soil that acted as a nursery bed after which the seedlings were transplanted into three different concrete paddy troughs when they were 15cm tall (21days old). At pre-mitosis stage the plants were separated into three complete blocks (complete block design). First block was treated with long day-light length (LDL) and high temperature (HT) by extending the 12hour light of a normal day to 14hours using solar illumination gadgets for 14days in greenhouse. The second block of plants were exposed to normal 12hour daylight length (NDL) and high temperature (NT). High temperature was realized by containing the plants in the greenhouse temperatures. Normal day light length and normal temperature (3<sup>rd</sup> block) was the natural temperatures outside the greenhouse. Each block had three varieties each receiving LDL+HT, NDL+HT and NDL+NT. There were a total of nine (9) different treatments. In this paper LDL+HT and

NDL+HT will be referred to as sterility inducing conditions (SIC) and NDL+NT will be referred to as fertility inducing conditions (FIC) respectively.

#### **Evaluation of spikelet fertility**

After heading, six plants were sampled from each variety from which three glumes were picked from each plant for pollen analysis. Immediately after sampling, glumes were fixed in Canoy's solution II after which they were preserved at 4°C until use. Glumes were carefully opened to extract the anthers which were placed on a drop of 1% potassium iodide (I/KI) solution and macerated with forceps to release the pollen. A cover slip was placed on the sample and observed under light microscope and fertile pollen, which was identified as black blue were counted against abortive pollen that stained yellow. Hypothesis that LDL+HT, NDL+HT and NDL+NT had similar effects was tested using General Linear Modelling (GLM).

#### **Production of hybrid seeds.**

When the panicle emerged out the flag leave, glumes were capped 1/3 from the tip to expose the

stigma using a pair of scissors. Pollen from basmati370 and 217 were dusted on the panicle of the female plant and bagged to prevent undesired cross pollination. To verify if there was effective cross pollination F<sub>1</sub> seeds were sown and observed for F<sub>2</sub> generation.

#### **Data Analysis**

Data was analysed using SAS computer software, the GLM Procedure analysis for pollen sterility (PS) and seed set rate (SSR) for plants from the three varieties (V1PGMS, V2TGMS and V3PGMS) that had under gone various photo and thermo treatments. The model below was used for analysis.

```
data EGMS;
```

```
input Rep TR PS SSR;
```

```
datalines;
```

```
PROC GLM;
```

```
class Rep TR;
```

```
MODEL PS SSR = Rep TR;
```

```
Means Rep/LSD;
```

```
run;
```

## **RESULTS AND DISCUSSION**

### **Test for adaptability of EGMS to Kenyan Conditions**

The three EGMS varieties (V1-IR-73827-23-76-15-7S, V3-IR-75589-31-27-8-33S, and V2-IR-77271-42-5-4-36S), here coded as V1PGMS, V3PGMS and V2TGMS respectively, under greenhouse and outside the greenhouse growth conditions are shown in Fig.1. Under long daylight length and high temperatures in greenhouse, the three lines were completely sterile (Fig.1) and when grown outside greenhouse under normal temperatures and natural 12hour day-light length the three varieties recorded between 33.367% and 38.3333% seed set compared to those grown under SIC which recorded an average seed set of less than 1% (Table.1).

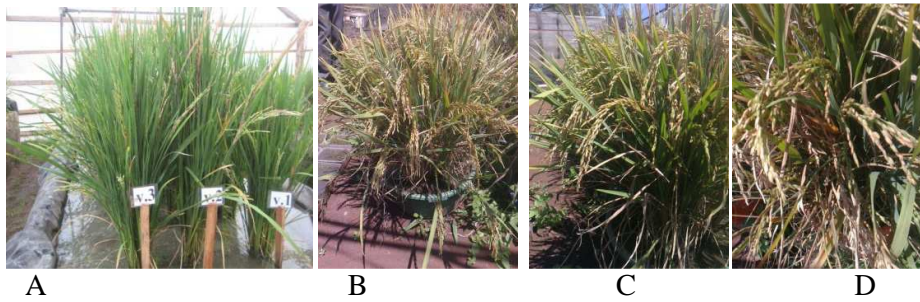


Fig.1 EGMS plants grown under SIC and FIC. Fig A are EGMS plants grown under SIC while Fig.B-D are EGMS plants grown under FIC.

**Evaluation of EGMS spikelet fertility**

Pollen from the three varieties grown under both sterility and fertility inducing conditions were

stained using 1% I/KI. All the plants grown outside the greenhouse recorded over 58% blue black staining pollen grains. However, blue black staining pollen grains from plants grown under LDL+HT and HT+NDL averaged 0.33% and 0% respectively (Table 1). After maturity, plants grown under SIC (inside greenhouse) had an average seed set rate of between 0 to 1% while the ones outside greenhouse had 33% (Table 1).

**Table. 1.** Effects of photoperiod and temperature on EGMS. LDL, HT, NDL and NT refer to long day-light length, High temperature, Normal day-light length and normal temperature respectively.

Significance test for pollen sterility				Significance test for seed set rate (%)			
Test for significance (t-grouping)	Mean pollen sterility (%)	Variety	Treatment	Test for significance (t-grouping)	Mean seed set rate (%)	Variety	Treatment
A	61.333	V3PGMS	NT+NDL	A	38.333	V3PGMS	NT+NDL
A	59.000	V1PGMS	NT +NDL	A	38.000	V2TGMS	NT+NDL
A	58.667	V2TGMS	NT+NDL	A	33.667	V1PGMS	NT +NDL
B	0.333	V2TGMS	LDL + HT	B	1.000	V2TGMS	LDL + HT
B	0.333	V3PGMS	LDL + HT	B	1.000	V1PGMS	LDL + HT
B	0.333	V1PGMS	HT +NDL	B	1.000	V3PGMS	HT +NDL
B	0.000	V2TGMS	HT +NDL	B	0.667	V2TGMS	HT +NDL
B	0.000	V3 PGMS	HT +NDL	B	0.667	V1PGMS	HT +NDL
B	0.000	V1PGMS	LDL + HT	B	0.000	V3PGMS	LDL + HT

GLM modelling analysis indicated that error within the treatments was not significant but there was significant difference between various treatments (Table 2&3).

Table 2. SAS System , The GLM Procedure for dependent Variable: pollen sterility

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	21292.88889	2129.28889	188.11	<.0001
Error	16	181.11111	11.31944		
Corrected Total	26	21474.00000			

Source of Error	DF	Type I SS	Mean Square	F Value	Pr > F
Within treatment	8	21254.66667	2656.83333	234.71	<.0001
Between treatment	2	38.22222	19.11111	1.69	0.2161

Table 3. SAS System , The GLM Procedure for dependent Variable: Seed set rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	7994.370370	799.437037	24.44	<.0001
Error	16	523.259259	32.703704		
Corrected Total	26	8517.629630			

Source of Error	DF	Type I SS	Mean Square	F Value	Pr > F
Within treatment	8	7794.962963	974.370370	29.79	<.0001
Between treatment	2	199.407407	99.703704	3.05	0.0755

All the three varieties grown under NT+NDL had significantly higher pollen fertility and higher seed set rate than when grown under LDL+HT and NDL+HT growth conditions (Table 1). Average pollen sterility of all the three varieties grown under LDL+HT was 0.00, and although the same varieties recorded average pollen sterility of 0.333 when grown under NDL+HT the difference was not significant. Also seed set rate between the three lines grown under LDL+HT NDL+HT had no significant difference (Table 1).

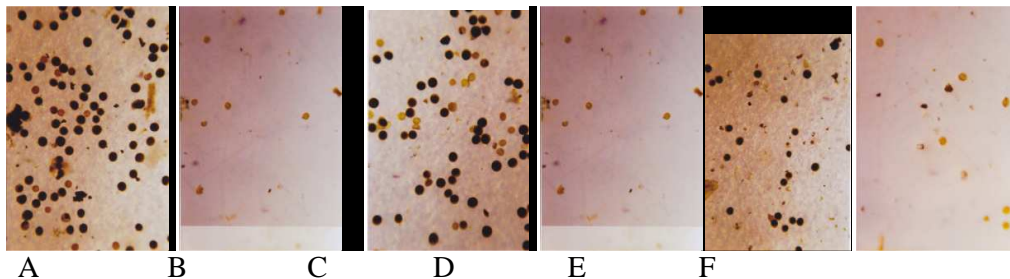


Fig. 3. Pollen of EGMS plants stained with 1% I/KI. Fig.A, C, and E shows pollen from plants grown under fertility conditions, and Fig.B, D and F shows pollen from plants grown under sterility inducing conditions.

### Production of hybrid seeds.

Hybrid seeds were obtained from all the six crosses. A total of six hybrid lines were produced. These were HB370V1, HB370V2, HB370V3 which, were obtained from cross pollination of V1PGMS, V2TGMS and V3PGMS to Basmati370 respectively. The other three, HB217V1, HB217V2 and HB217V3 were products of V1PGMS x B217, V2TGMS x B217 and V3PGMS x B217 in that order. All hybrids had conspicuous presence of anthocyanin at the base of the stem unlike parents that had no conspicuous anthocyanin other than TGMSV2 (Fig.4). Seeds from F<sub>1</sub> had no aroma but the hybrid plants were slightly shorter than the Basmati370 and 217 and taller than their female parents (Fig.4H-J), (data not included).

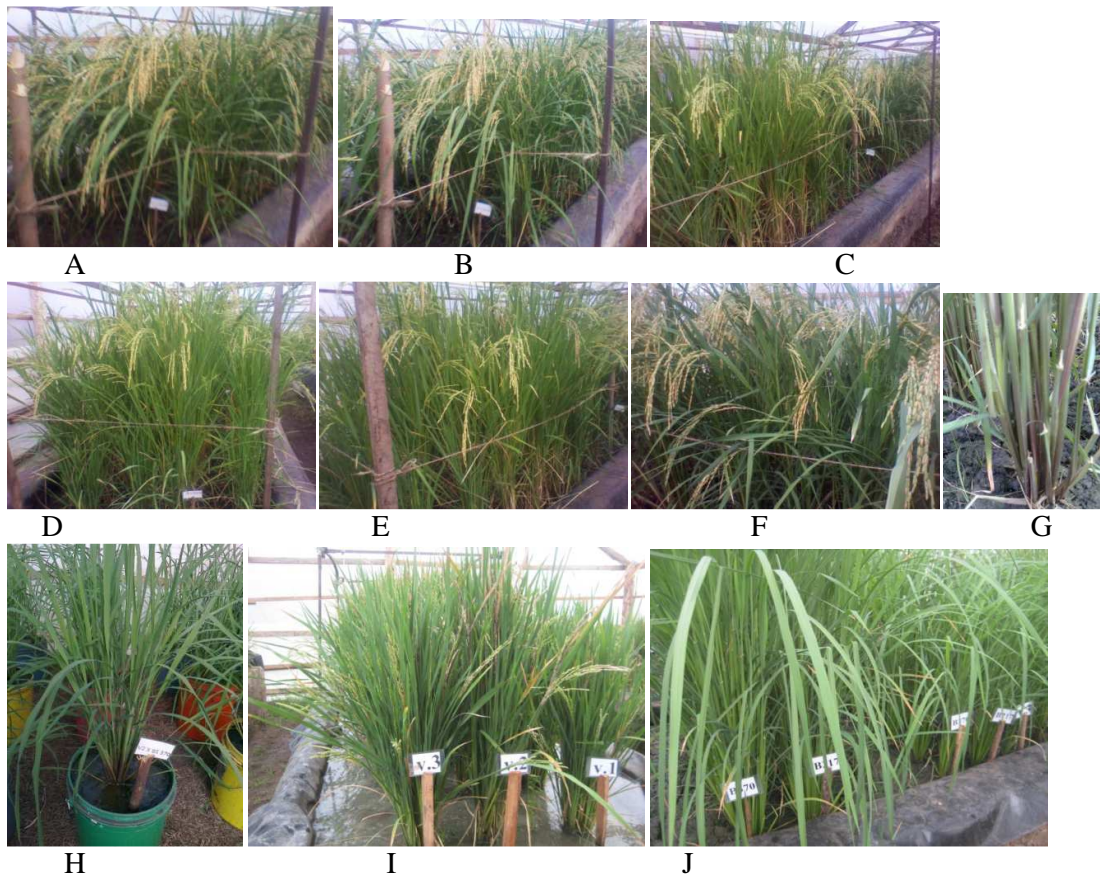
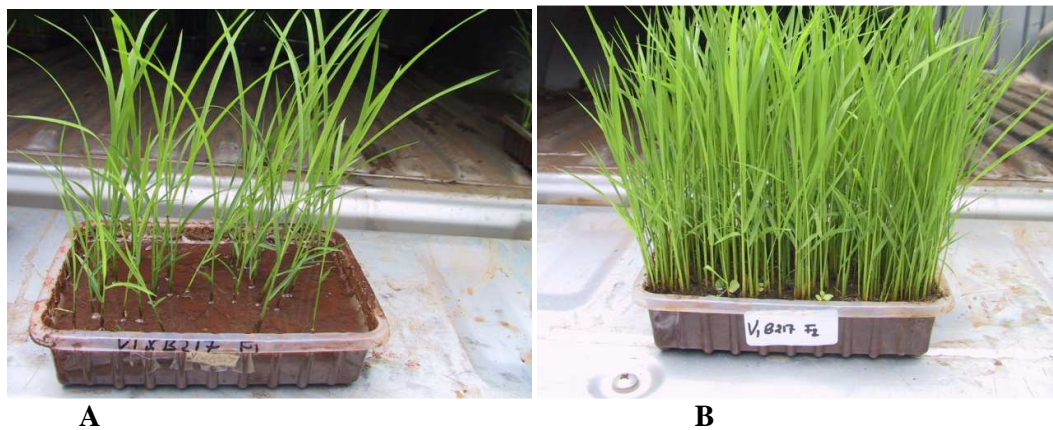


Fig. 4. Hybrid rice growing in the green house . Fig A-F are hybrid plants from crosses V1PGMS XB370, V2PGMSxB370, V3TGMSxB370, V1PGMSxB217, V2PGMSXB217 and V3TGMS X217 respectively. Fig. G&H show anthocyanin colour on hybrids while Fig.I&J shows parents.





C

D

Fig. 5. Anthocyanin levels in parents and offspring. Fig. A,B,C,&D represents seedling from V1PGMS x B217 (F<sub>1</sub>) , V1PGMS x B217 (F<sub>2</sub>) , B217 (parent) and V1PGMS (parent).

Seedling in Fig.5 were obtained from cross between Basamti217 and V1PGMS at different generations. They are used as a sample to show that anthocyanin can be used in hybrid plant selection. The seedlings were evaluated for presence of anthocyanin at the base of seedling as a morphological marker for selecting hybrids against non-hybrids. All the F<sub>1</sub> seedlings (Fig.5A) that were from a cross between Basamti217 and V1PGMS had deep presence of anthocyanin, while those from the pure parents' seeds (B217 and V1PGMS) did not have noticeable anthocyanin (Fig.5C&D). However, seedlings from segregating F<sub>2</sub> population had different shades of anthocyanin (Fig.5B).

## DISCUSSION

### Adaptability of EGMS rice in the tropics

Plants grown under long day-light length in greenhouse growth conditions were completely sterile, but when grown outside the green house they recorded significant amount of fertility (Fig.1). To induce complete sterility, minimum temperature when plants are at critical fertility phase needs to be above 30°C (10). Greenhouse growth conditions were able to maintain temperature above 30°C that ensured complete sterility of EGMS at Mwea-Kenya. This is an indication that use of EGMS in production of hybrid seeds is very viable. Complete male sterility is a requirement in female parents to avoid self-pollination hence contamination of hybrid seeds with selfbred seeds. The diurnal temperatures ranges along the equator / tropical regions is high, and go below the critical level needed to induce complete sterility in TGMS/PGMS. To obtain the critical minimum temperature required, a greenhouse is needed but this will add to the cost of hybrid rice seed production. However, if greenhouses can be

made from simple locally available materials, the ultimate yield benefits from hybrid seed technology may outweigh the cost of production.

#### **Evaluation of spikelet fertility**

Over 99% of pollen from plants grown under LDL+HT and NDL+HT conditions stained yellow and at maturity the plants scored a seed set rate of  $\leq 1\%$  (Table 1). This is an indication that the pollen were of abortive type and the panicle were sterile. GML analysis indicated that both the pollen sterility and seed set rate from plants grown under LDL+HT and NDL+HT had no significant difference (Table 1-3). Observations indicated that high temperature tended to compensate for shorter day-light length. Use of PGMS is suitable in temperate regions where daylight is more than 13 hours (14). Under elevated greenhouse temperatures complete sterility was realized in PGMS without prolonging the daylight length. This is a good score for production of hybrid seeds in the tropical regions. On the other hand, over 58% of pollen grains from plants grown under NDL+NT was blue black when stained with 1% I/KI (Table.1 and Fig.3). This is an indication that the pollen were fertile, which was confirmed by an average seed set rate of over 33% recorded after plant matured. Given this correlation, the pollen staining can be used to predict the level of male fertility in female parents before undertaking cross pollination and production of hybrid seeds. LDL and HT induce PGMS and TGMS genes to express (15, 16, 17). Expression of gene can be tested by staining pollen using 1% I/KI where fertile pollen stain blue black while abortive pollen stain yellow (18, 19). Temperatures between 26 -29°C have been reported to induce TGMS to sterility levels necessary for application in hybrid seed production (20, 21). This is a relatively good critical temperature, because it is not too high neither it is too low. Critical temperatures higher than this make it difficult to induce complete sterility in TGMS hence high risk of contamination of hybrid with self-bred seed. Very lower critical temperature is not good either because it is difficult to induce fertility so as to produce seeds for TGMS self-maintenance

The major challenge for using pollen staining to predict fertility is that plants will be required to grow up to heading time so as to avail pollen for staining. This is a long time lapse before a decision to produce hybrid seeds or not is made. At this time, if a high level of fertile pollen is realized, then supplementary methods like use of chemical emasculation can be used. However, this is unfavourable because it adds to environmental pollution. The way forward in the tropics is to breed female parents with lower critical sterility temperature requirement, so that temperature of less 30°C can induce complete sterility.

#### **Production of hybrid seeds.**

Hybridization is effective if plants to be used as female parents are completely male sterile. This condition was ensured by treating PGMS and TGMS with LDL+HT and NDL +HT under greenhouse growth conditions. By crossing the three EGMS varieties each with Basmati370 and 217 six hybrid types namely; HB370V1, HB370V2, HB370V3, HB217V1, HB217V2 and HB217V3 were obtained (Fig. 4). These lines were stable in that they displayed uniform plant canopy. However, the hybrids were not expected to have aroma since in Basmati it is under a recessive gene, *fgr*, control (22). This is a breeding challenge that needs to be overcome, by producing a basmati with PGMS/TGMS gene. If this is achieved, then both male and female parents in hybrid programme will have aroma and hence the F1s. Another challenge is that sterility in PGMS may not be stable in the tropics because daylight-length go below the critical minimum needed to induce complete sterility. This can be solved by breeding PGMS with shorter day-light length and lower temperature requirements needed to induce complete sterility. Given that PGMS/TGMS trait is under genetic control (23) then the trait can easily be transferred to other varieties to produce novel PGMS/TGMS varieties. As this research continues another expected challenge is that of lower grain filling in hybrid



rice due to incompatibility (24,25). This will necessitate introgression of wide compatibility gene S-5 (26, 27) in hybrid- producing parents.

## CONCLUSION

Production of hybrid rice is viable in Kenya, but a breeding programme will be needed before its benefits are realized.

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