RESEARCH PAPER

Segregation disortion of anthocyanin morphological marker in F$_2$ population of cross between basmati and environment genic male sterile rice lines

Njiruh Paul Nthakanio[1*], Kanya James Ireri[2], Kimani John Munji [3], Wanjogu Rapheal Kinyanjui[4] , Kariuki Simon Njau[5]

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

[2]*University of Nairobi, School of Biological sciences, P.O. Box 30197, Nairobi, Kenya*

[3]*Kenya Agricultural research, Institute (KARI), P. O. Box 298, Kerugoya, 10300; Tel:+254-202028217, or Box 402-10303 Wang'uru, Kenya*

[4]*Mwea Irrigation Agriculture Development (MIAD) Centre. Unyunyuzin House, Lenana Road Nairobi, Kenya*

[5]*Kariuki Simon Njau. Kenyatta University, Department of Biochemistry and Biotechnology. Po Box 43844– 00100, Nairobi, Kenya*

Abstract

Basmati rice (*Oryza sativa* L.) is preferred by many Kenyans yet its yield per hectare is low. This challenge led to initiation of Basmati hybrid rice project in Kenya with a goal of leaping yield benefits of heterosis. Since quality traits in Basmati are under recessive gene control, then the two parents in hybrid programme must possess the traits to avoid their masking at F$_1$ by any other dominant gene. In the process of breeding for suitable parents, Basmati and Environmental genic male sterile lines V1-IR-73827-23-76-15-7S (V1), V2-IR-77271-42-5-4-36S (V2) V3-IR-75589-31-27-8-33S (V3) were crossed to produce F$_1$. All the F$_1$s plants had strong presence of anthocyanin on the base of their stems but segregated at F$_2$ population. Thus, the objective of this study work was to evaluate and understand the mode of anthocyanin gene segregation at F$_2$ population as a potential morphological marker. The F$_2$ population was analyzed to test hypothesis that anthocyanin marker(s) is under simple Mendelian gene control. However, the results obtained indicated that F$_2$ did not obey the Mendelian laws of gene segregation. This led to the conclusion that anthocyanin marker experience segregation distortion in F$_2$ population.

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

Introduction

To produce more rice (*Oryza sativa* L.) to feed the growing population require methods other than the use of traditional high yielding pure-bred lines. The dwarf high yielding varieties have reached yield plateau (Kropff *et al.,* 1994; Yuan, 1994). To increase rice yields above this plateau researchers have adopted the technology duped "super hybrid" rice and "Green super rice" (IRRI, 2010). In rice growing countries like, China, India, Indonesia, Egypt and Vietnam among others, hybrids 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, 2010, Virmani, 1994). To produce hybrid rice, two genetically fixed varieties are cross pollinated to produce filial generation one (F_1) or hybrid seeds. Plants from such seeds are special in that they express hybrid vigor (Yang *et al.,* 1996; IRRI, 2010). For hybrid seeds, production a completely male sterile female parent is needed that is pollinated with male parent.

Over the years manual removal of male gametes from the plants used as the female parents during cross breeding has been practiced. Unlike cross pollinating plants like maize that's male and female flower parts are separate, in rice (self pollinating crop) the two parts are in one flower making male emasculation difficult. However, with the discovery of cytoplasmic male sterility (cms) large scale production of hybrid rice was possible (Virmani and Sharma, 1992). The use of cms proved to be costly too, since it uses three (sterile, maintainer and restorer) lines, compared to environment sensitive genic **male** sterile (PGMS) that uses two-lines (Mao and Deng, 1993). 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), suffer from cytoplasmic incompatibility with certain male parents and also, for japonica and basmati varieties it has been difficult to get a good restorer line (Virmani and Sharma, 1992). Discovery of photoperiod sensitive male sterile rice (Shi, 1981; Shi and Deng, 1986.) solved many of the problems associated with cms. In long day-light length and high temperature the PGMS is completely sterile and it is used as female parent in hybrid rice production (Shi, 1981, 1985, Ku *et al.,* 2001) but in short day-light length and low temperatures growth conditions it reverts to fertility, thus it maintains itself (Lopez and Virmani, 2000). Thermosensitive genic male sterile lines (TGMS) (Ali *et al.,* 1995, Virmani *et al.,* 2003), which is sterile in high temperature growth conditions and fertile in low temperature growth conditions (Reddy *et al.,* 2000) 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.,* 1995), unlike cms that is under cytoplamic influence (Oka, 1974; Lin *et al.,* 1992). Since the fertility controlling gene is influence by environment they are referred to as Environmental genic male sterile (EGMS) lines. EMGS 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 lead to F_1 sterility (Lin *et al.,* 1992). PGMS (maternal) lines with diversified background can be produced unlike in cms whereby wild abortive (WA) is the major maternal line used (Virmani, 1996).

To use this technology in Kenya it has been necessary to test for adaptability of EGMS for their utility in hybrid rice seed production. Under long daylight length (prolonged to 14hours in greenhouse) and high temperature growth conditions EGMS have been found to be completely sterile a time when they can be used for crossing, and when grown outside the green house growth condition they are fertile and can maintain themselves (Kanya *et al.,* 2013). The challenge is that, sometime it is difficult to achieve complete sterility in the EGMS. This is experienced where the day-light length is not long enough or temperature is not high enough to reach the critical point required for EGMS gene(s) expressions (Yuan *et al.* 1993). If the sterility genes are not completely expressed, complete male sterility of maternal parent

line is not achieved and there is a correlating problem of contamination of hybrid seeds by self-bred seeds. Therefore, productions of hybrid seeds require method(s) of determining hybrid pure from self-bred seeds. Commonly used methods include conventional ones where plants mature and observation of agronomic traits is done. This is too late because plant will have grown to maturity before separating hybrid from inbred seedlings. 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, (Bert *et al.*, 2008; Bagali *et al.*, 2010; Shanti *et al.*, 2010). However, it requires a specialized laboratory and skilled manpower for effective use in breeding. Use of morphological markers like anthocyanin maybe a better method because it is visual and phenotypic. In this research, it was realized that all F_1 seedlings had strong presence of anthocyanin at the base of the stem and thus it can be utilized as a selection marker.

Anthocyanin is a heritable trait (Holton' and Cornish, 1995, Roger, 2000) and has been studied since the days when Mendel studied peas colour. In plants like strawberries red colour is under MYB10 gene control and it is used as a selectable marker (Kortstee *et al.*, 2011). Deliberate breeding of EGMS rice with anthocyanin can done for use as a marker in hybrid seedling selection. Studies in F_2 population revealed that segregation was distorted and non-Mendelian. Elsewhere, cross between intraspecific crosses of rice has revealed a number of incompatibilities that is associated with low F_1 seed rate. Segregation distortion has been observed in F_2 and doubled haploid populations of temperate *japonica* rice and its common in Indica –japonica crosses (Yamagishi *et al.*, 2010). Many other plants have shown segregation distortion among them are *Pupulus trichokarpa* which is controlled by a lethal gene ith (Bradshaw and Stettler, 1994); maize (Edwards *et al.*, 1987), Lettuce (Landry *et al.*, 1987), rice (McCouch *et al.*, 1988) and barley (Ganner *et al.*, 1991). Specific chromosomal regions are responsible for F_2 segregation distortion due to presence of gametophyte (ga) or sterility gene

(S) (Nakagahra 1972; Sano 1990; Xu *et al.* 1997). Production of hybrid basmati seeds is complicated because quality trait like aroma is under control of recessive gene(s) (Ahn *et al.*, 1992), which means that it is masked by dominant trait. It is therefore, necessary to breed basmati rice line with EGMS gene that will be used as a female parent in crosses involving Basmati370 or 217 in hybrid rice seed production. Breeding and analysis of crosses between Basmati and EGMS is therefore, essential so as to select advanced lines with both aroma and EGMS traits. In this process evaluation of anthocyanin realized at F_1 was considered. The objective of this study work was to evaluate mode of anthocyanin gene segregation at F_2 plants as possible morphological marker that can be used in breeding of hybrid seeds. It was hypothesized that anthocyanin is under a normal one or two Mendelian gene segregation. We now report that anthocyanin segregation at F_2 in crosses between each of Basmati 217and 370 and V1PGMS, V2TGMS and V3PGMS did not tally either with 3:1 nor 9:3:3:1 Mendelian ratios, thus leading to deduction that there is a likelihood of gene segregation distortion.

Materials and methods
Materials
The female parental plant materials used for cross breeding were courteously supplied by International rice research institute (IRRI)) under the supervision of Kenya Plant Health Inspectorate Service (KEPHIS). These included two PGMS (V1-IR-73827-23-76-15-7S and V3-IR-75589-31-27-8-33S, and one TGMS (V2-IR-77271-42-5-4-36S) varieties that are here code-named as V1PGMS, V3PGMS and V2TGMS respectively. The materials were sown and tested for adaptability at Kenya Agricultural Research Institute (KARI) Mwea-Kimbibi station, and at Mwea Irrigation Agriculture Development (MIAD) which are 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 12hours day which was extended to 14hours by illuminating using solar radiation system, and short/normal day refer to normal 12hours of daylight.

On the other hand, high temperature and low temperatures were the green house temperatures, which were minimum of 35°C during the day and 19°C at night respectively. Paternal parents Basmati370 (B370) and Basmati217 (B217) were elite lines grown at KARI Mwea.

Production of hybrid seeds

All the three female parents (V1PGMS, V2TGMS and V3PGMS) were each crossed with Basmati370 and Basmati217. The female parent were first sown on 19th June 2011 in concrete troughs in green house at KARI with a spacing of a 5X15 cm under long day-light length and high temperature growth conditions so as to induce male sterility. All the plants sown in troughs were allowed to grow in greenhouse conditions until panicle mordial stage when they were exposed to solar lamp illumination at 6.00pm up to 8.00pm to ensure long day light-length growth conditions and to induce male sterility. At panicle emergence (heading) the plants were carefully transferred outside the greenhouse in a bucket where each of the three lines were crossed with Basmati370 and Basmati217 and left to grow under natural conditions till maturity. After ripening the F1 seeds were harvested separately and each stored in a separate bag.

Evaluation of anthocyanin as selective marker for hybrid against selfbred rice lines

Evaluation of F1 plants for anthocyanin: In January 2012 sowing was done for the F1 and parental lines. The F1 and parental seeds were sown at KARI Mwea in January 2012 at spacing of 15cmx15cm. At seedling stage it was discovered that all hybrids had strong presence of Anthocyanin which was missing from all parents apart from V2TGMS. The growing plants were individually monitored for presence of anthocyanin compared to the pure parental lines at three levels. All evaluation was done in green house at KARI Mwea- Kenya in pot (buckets) and in paddy field at MIAD- Mwea.

Evaluation of F2 and anthocyanin segregation: In this phase the F2 were sown and compared against controls that included parents and F1 plants. All seeds

were pre-germinated and allowed to grow in nursery for 21 days before transplanting in the rice fields at MIAD-Mwea. Transplanting was done in 17 blocks. Block1-5 was sown with parental lines B370, B217, V1PGMS, V2TGMS and V3TGMS. The F1s (HV1B370, HV2B370, HV3B370, HV1B217, HV2B217, HV3B217) were sown in blocks 6-11 while the F2s segregating lines from each cross were sown in blocks 12-17. In this sowing phase number of seedlings per block for F1 varied between 20plants to 50plants. However, 410plants for F2 segregating seedlings were sown in 41 rows each with 10 plants. A spacing of 20x20cm inter-plant and inter-rows was maintained. Anthocyanin was evaluated by observation for presence of purplish/violet colouration at the base of the plant. Scoring was done by counting the number of segregants with purple stem base against others.

Data analysis

Emergence of anthocyanin must be due to gene interaction because non of the parents displayed strong noticeable presence of anthocyanin apart from V2TGMS. Since non of parents hand conspicuous anthocyanin other than V2TGMS, it was predicted that the anthocyanin was under interaction of at least two non-allelic gene, at least one from each parent. In this work interaction of two genes will be assumed. Data was recorded in Microsoft excel 2007 version. Segregation variance among the hybrid lines was analyzed using Z-test. The F2 segregation tendencies were compared among the lines and with conventional Mendelian ratios of 9:3:3:1 for two non allelic and non-linked interacting genes. Since it was difficult to demarcate the four possible phenotypic appearance characteristic of Mendelian gene segregation in a 9:3:3:1 model, it was assumed that segregation model was espistatic with 9 anthocyanin against 7 others or $9/7 = 1.285714$.

## Results

Anthocyanin in F1 seedlings

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 the hybrid plants had strong presence of anthocyanin on their stem compared to parents that had no conspicuous anthocyanin, apart from V2TGMS (Fig.1). Strong presence of anthocyanin manifestation was observed in all mature F_1 plants such as presented in Fig.1A-F and in 21day old seedlings (Fig. 2A-F). However, there was no conspicuous presence of anthocyanin in the V1PGMS, V3PGMS, Basmasti370 and Basmati217 for both mature parents (Fig 1.G-I) and seedlings (Fig. not included) Although the V2TGMS parents had some anthocyanin observed (Fig 1. J) it was lower than that of the hybrid plants. Besides in the V2TGMS parental materials anthocyanin was non-segregating compared to segregation observed at F_2. In all the F_1 plants the level of anthocyanin present in the stem and the leave sheaths were conspicuously observed compared to the control parental plants.

Fig. 1. Anthocyanin in F_1 and parent plants. Fig *A-C represent cross between V1PGMS, V2TGMS and V3PGMS and Basmati370 while Fig. D-F represent cross between V1PGMS, V3PGMS and V2TGMS and Basmati217. Fig G represents Basmati370 and 217 while H-J represents V1PGMS, V2TGMS and V3PGMS in that order.*
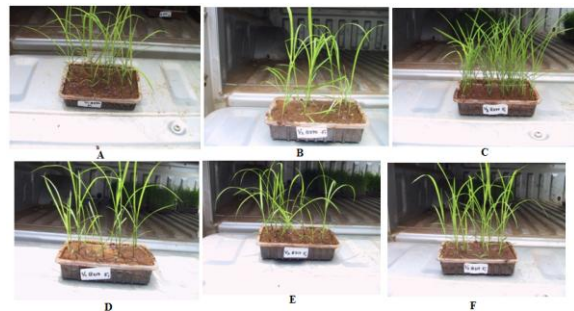
Fig. 2. Anthocyanin in F_1 hybrid seedlings. Fig A, B,C,D,E, and F represent cross between V1PGMS x370V1, V2TGMS xHB370V2, V3PGMSx HB370V3, HB217V1, HB217V2, V1PGMS x B217, V2TGMS x B217 and V3PGMS x B217 respectively.

F2 segregation: comparison of segregation ratios among hybrids

Out of the 410 plants originally sown, each for the six hybrids, some did not survive and the tally was HV1B217 =362plants, HV2B217= 366plants, HV3B217=357 plants, HV1B370=349 plants, HV2B370=341 plants and HV3B370=313plants (Table 1). Variances among the hybrid was done by determining segregation ratios of plants with anthocyanin to non-anthocyanin plants. Among the six hybrids the ratios were highest for HV2B370 and HV2B217 plants with a z-value of 0.212489173 and 0.491663097, and a p-value of 0.1664 and 0.3256 respectively (Table 1). HV1B217 and HV1B217 with z-values of 0.053277025 and 0.040099789 that corresponds to p-values of 0.0398 and 0.032 respectively were the lowest. The anthocyanin to non-anthocyanin plants segregation ratios of HV3B370 were not significantly different from others when

tested at α=0.05, however HV1B217 and HV2B370 and highest z-values.

F2 segregation: comparison of segregation ratios to expected 9:7 dihybrid ratio

Under z-distribution, the area occupied by each of the hybrids lines were below average or z-value of zero(0)

apart from that of HV1217 and HV2370 which had 0.298678 and 0.818121 respectively. These had p-value of 0.2282 and 0.5878 respectively which was not significant when tested against the Mendelian rations at α= 0.05 (Table 2).

Table 1. Segregation for anthocyanin among the F_2 plants. Comparison of anthocyanin segregation with an assumed 9:3:3:1 ratio. Average observation ratio =of all observed segregation ratios divide by n (n=7).

Line	Observed segregation ratios	Mean of observed ratios	STD δ	z-values	P value
HV1B217	0.732057	0.962876	-0.23082	0.053277025	0.0398
HV2B217	1.423841	0.962876	0.460965	0.212489173	0.1664
HV3B217	0.645161	0.962876	-0.31771	0.10094237	0.0876
HV1B370	0.762626	0.962876	-0.20025	0.040099789	0.032
HV2B370	1.664063	0.962876	0.701187	0.491663097	0.3256
HV3B370	0.549505	0.962876	-0.41337	0.170875277	0.135

Table 2. Test of F_2 segregating population against a Mendelian Dihybrid ratios of 9:3:3:1. Expected ratios are the assumed espistatic (9:7) ratios.

Line	Anthocyanin to non-anthocyanin segregation ratios	Expected 9:7 Mendelian segregation	Mean	Z values	P-value
V1xB217	0.732057	1.285714	0.46246	-1.1972	0.383
V2xB217	1.423841	1.285714	0.46246	0.298678	0.2282
V3XB217	0.645161	1.285714	0.46246	-1.3851	0.4162
V1xB370	0.762626	1.285714	0.46246	-1.1311	0.3708
V2xB370	1.664063	1.285714	0.46246	0.818121	0.5878
V3XB370	0.549505	1.285714	0.46246	-1.59194	0.4441

Discussion

Breeding for hybrid seeds require a method of selecting hybrid seeds from pure breed seeds. Marker assisted selection (MAS), involving DNA molecular and morphological markers, are some of the available methods. DNA Marker assisted selection require identification of DNA sequence that associate with target phenotype in the desired line (McCouch, *et al.*, 1988) which is laborious. Since morphological markers are phenotypic, they can simplify the MAS. In this study, anthocyanin pigmentation was found to dominate the hybrid seedlings (Fig.1 A-F) more than

the in pure parents (Fig.1-J). Among the parents, anthocyanin was only noticeable among the V2TGMS plants and not segregating. At F1 anthocyanin was present in all plants to compared segregating F2 population in which some plants had no noticeable anthocyanin on base of stem. Therefore, anthocyanin can be used as morphological marker to select F1 seedlings (Fig 1. A-F) especially for crosses involving V1PGMS and V3PGMS. Elsewhere anthocyanin has be reported to be a promising marker in selection of recombinant from non-recombinant plants (De Jong, 2004; Kortstee, 2011). For instance, Strawberry

plants transformed with the MYB10 gene show anthocyanin accumulation in leaves and roots as opposed to non-transformants (Kortstee, 2011). Thus, markers aided selection is becoming important in the field traditionally dominated by conventional phenotypic breeding. Presence of anthocyanin in all the F_1 plants indicated gene dominance. Since, indications were that the two parents contributed to the anthocyanin, a two gene interaction was predicted. However, segregation in F_2 generation behaviour did not tally with the hypothesized 9:3:3:1 nor the 9:7 epistatic ratios. This is an indication that F_2 segregation could have suffered a distortion. Gametic selection has been reported to cause segregation distortion in F_2 progenies and even in anther culture derivatives (i.e., nonmorphogenetic microspore derived calli (NMC) (Guiderdoni,1989). This segregation distortion is associated with semi-fertility in F_1 hydrids (Ikehashi and Araki, 1984). Cases like this brings about a breeding alert because it may reduce the level of yields expected from hybrids compared to purebred varieties. Evaluation of F_1 obtained from crosses between Basmati and EGMS revealed that F_1 had low seed set rate than parents (Kanya *et al.*, 2013). Low seed set rate observed in F_1 could be a point of non-Mendelian F_2 anthocyanin segregation. However, at F_1 anthocyanin is stable and uniformly expressed. Since anthocyanin is under genetic control (Holton and Cornish, 1995) such as flavonoid biosynthetic gene CHS gene in parsley (Kreuzaler *et al.*, 1983), if the gene can passed to other varieties it can be used as a good breeding tool to supplement conventional breeding which is slow to select for a target trait (Xu and Crouch, 2008). Effects of low seed set rate at F_1 (incompatibility or distortion) can be reduced by introgressing wide compatibility gene $S5^n$ (Ikehashi and Araki, 1984) into cultivars of interests to reduce F_1 spikelet sterility. In situation where a target trait con-segregate with a DNA or a molecular marker then breeding time for such a trait can be drastically reduced. However segregation distortion like the one experienced in this research may slow down the speed of releasing new stable lines for use in breeding. Whichever the case MAS speedup breeding like in the case of pyramiding four Bacterial Blight resistance genes *Xa4, xa5, xa13* and *Xa21* into rice lines selection (Shanti *et al.*, 2010). Where else DNA primers/probe markers are expensive and require expertise, morphological markers are simpler to use because they are phenotype (Xu and Crouch 2008). In this research, anthocyanin markers were found to be associated with F_1s hybrid rice seedlings (Fig. 1). It is only in V2TGMS where anthocynin was present in both the parental and in F_1 plants. However, anthocyanin in parental plants was lighter compared to F1 hybrids and did not segregate at F_2. Since anthocyanin is observable at seedling stage, then it can enable sorting out hybrid from parental seedling as early as 21day seedling stage (Fig. 2).

Conclusion: Anthocyanin can be used a morphological marker to select F_1 from pure breed seedling. Segregation of anthocyanin at F_2 between Basmati370 and 217, and V1PGMS, V2TGMS and V3PGMS experience segregation distortion and it is non-Mendelian. This may be an indicator of F_1 spikelet sterility.

Recommendation

Introgression of wide compatibility gene $S5^n$ in Basmati rice may be needed for its effectively used in hybrid seed technology.

References
Ahn SN, Bollich CN, Tanksley SD.1992. RFLP tagging of a gene for aroma in rice. Theoretic and Applied Genetics. **84,** 825–828.

AFIDEP. 2012. www.populationaction.org Population, Climate Change, and Sustainable Development in Kenya. African Institute for Development policy, Population Action International and the African Institute for Development Policy, Nairobi, Kenya.

Ali J, Siddiq EA, Zaman FU, Abraham MJ, Ahmed I. 1995. Identification and characterization of Temperature sensitive genic male sterile sources in rice (Oryza sativa .L). Indian Journal of Genetics **55 (3),** 243-259.

Bagali PG, Prabhu AHPD, Raghavendra K, Bagali PG, **Hittalmani** S, **Vadivelu JS**. *2010.* Application of Molecular Markers in Plant Tissue Culture. Molecular Biology and Biotechnology **18 (1),** 85-87.

Bert CYC, Casiana M, Vera C, Kenneth L, McNally, Parminder SV, Mackill DJ. 2008. Rice Molecular Breeding Laboratories in the Genomics Era: Current Status and Future Considerations. International Journal of Plant Genomics **208**, 1-25.

Bradshaw HD (jr) Stettler RF. 1994. Molecular growth of genetics and development of Papulus, II, segregation due to genetic load. Theoretical and Applied Genetics. **89**, 552-558

Dalmacio RD, Brar DS, Ishii T, Sitch LA, Virmani SS, Khush GS. 1995. Identification and transfer of a newcytoplasmic male sterility source form Oryza perennis into indica (Oryza sativa L.). Euphytica **82**, 221-225.

De Jong W.S, Eannetta N.T, De Jong D.M, Bodis M .2004. Candidate gene analysis of anthocyanin pigmentation loci in the Solanacea. Theoretic and Applied Genetics.. **108**, 423–432.

Edwards MD, Stuber CW, Wendel JF .1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116,**113–125.

FAO .2006. Prospects for food, nutrition, agriculture and major commodity groups. World agriculture: towards 2030/2050. Global Perspective Studies Unit Food and Agriculture Organization of the United Nations Rome, Italy.

FAO. 2008. Climate Change, Water and Food Security, FAO Water Reports 36. Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy.

GoK (Government of Kenya) .2007. Kenya Vision 2030. Ministry of Planning and National Development, Treasury Building, P. O. Box 30005,00200, Nairobi, Kenya.

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G,Herrmann RG (1991) Construction of an RFLP map of barley. Theoretic and Applied Genetics. **83**, 250–256.

IRRI (International Rice Research Institute) .2010. Accelerating Hybrid rice development. Edited by Xie F. and Hardy B. IRRI. Metro Manila, DAPO Box 7777, Philippines **2009**, p 3-8.

Guiderdoni E. 1989. Comparative gametic selection in sexual and anther culture derivatives from five distant F1 hybrids of rice. Rice Genetics Newsletter 6, 91-92.

Harushima Y, Kurata N, Nagamura Y, Sasaki T, Minobe Y, Nakagahra M.1996. Detection of segregation distortion in an *indica-japonica* rice cross using a high-resolution molecular map. Theoretic and Applied Genetics. **92**, 145-150.

Holton TA, Cornish EC. 1995. Genetics and Biochemistry of Anthocyanin Biosynthesis. The Plant Cell Vol.**7**,1071-1083.

Kanya JI, Njiruh PN, Kimani John M, Wajogu RKi, Kariuki SN . 2013. Evaluation of photoperiod and thermosensitive genic male sterile lines for hybrid rice seeds production in Kenya. International Journal of Agronomy and Agricultural Research (IJAAR) Vol. **3**, No. (2), p. 21-39.

Khush GS. 1994. Increasing the genetic yield potential of rice: prospects and approaches. International Rice commission Newsletter **43**, 1-7.

Kortstee AJ, Khan SA, Helderman C, Trindade LM, Wu Y. Visser RGF, Brendolise C, Allan A, Schouten HJ, Jacobsen E .2011. Anthocyanin production as a potential visual selection marker during plant transformation. Transgenic Reserves **20**, 253–1264.

Kreuzaler F, Ragg H, Fautz E, Kuhn, DN, Hahlbrock K. 1983. UV-induction of chalcone synthase mRNA in cell suspen- Sion cultures of fetmselinum hortense. Proceedings. of the National Academy of Sciences. USA. **80**, 2591-2593.

Kropff M, Cassman K, Peng S, Matthews R, Setter T. 1994. Quantitative understanding of yield potential. In Workshop on Rice Yield Potential in Favorable Environments. Edited by Cassman K. International Rice Research Institute, Los Ban˜os, Philippines **1994**, 21-38.

Ku SJ, Cho KH, Cho YJ, Baek WK, Kim S, Suh HS, Chung YY. 2001. Cytological Observation of Two Environmental Genic Male-Sterile Lines of Rice. **Molecular Cell 12** (3), 403-406.

Landry BS, Kesseli RV, Ferrara B, Michelmore RW .1987. A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance, and morphological markers. Genetics **116**,331–337.

Lin SY, Ikehashi H, Yanagihara S, Kawashima K. 1992. Segregation distortion via male gametes in hybrids between *Indica* and *Japonica* or wide compatibility varieties of rice *(Oryza sativa* L.). Theoretic and Applied Genetics **84**, 812-818.

Lopez MT, Virmani SS. 2000. Development of TGMS line for developing two-line rice hybrids for the tropics. Euphytica **114**, 211-215.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics **76**, 815-829

Ministry of Agriculture. 2010. The ministry of agriculture at a glance. Office of the Permanent secretary, Kenya.

Nakagahra M. 1972. Genetic mechanism on the distorted segregation of marker genes belonging to eleventh linkage group in cultivated rice. Japanese Journal of Breeding **22**, 232–238.

Oka HI .1974. Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetices **77**, 521-534.

Reddy OUK, Siddiq EA, Sarma NP, Ali J, Hussein AJ, Nimmakayala P, Ramasamy P, Pammi S, Reddy AS .2000. Genetic Analysis of temperature-sensitive male sterility in rice. Theoretic and Applied Genetics **1000**, 794-801.

Roger D. 2000. The importance of red pigment to plant life: experiment with anthocyanin. Journal of Biological Education **34**(4), 2006-2010.

Sano Y. 1990. The genetic nature of gamete eliminator in rice. **Genetics 125**, 183–191.

Shanti ML, Shenoy VV, Devi GL, Kumar VM, Premalatha P, Kumar GN, Shashidhar HE, Zehr UB, Freeman WH. 2010. Marker-Assisted Breeding for Resistance to Bacterial Leaf Blight in popular cultivar and parental lines of hybrid rice. Journal of Plant Pathology **92** (2), 495-501.

Shi MS. 1981. Preliminary research report on breeding and utilization of the natural two-uses line in late japonica rice. *Scientia Agriculturae* Hubei **7**, 1-3.

Shi MS. 1985. The discovery and study of the photosensitive recessive male-sterile (Oryza sativa L. subsp. japonica). Scientia Agriculturae Sinica **19**, 44-48.

Shi MS, Deng JY. 1986. The Discovery, Determination and Utilization of the Hubei

Photosensitive Genic Male-sterile Rice (*Oryza sativa* subsp. *japonica*). Acta Genetica Sinica **13**(2), 107-112.

Virmani SS. 1994. Heterosis and Hybrid Rice Breeding. Monographs on Theoretical and Applied Genetics **22**, p 7.1 Springer-Verlag. Berlin Heidelberg, Germany.

Virmani SS. 1996. "Hybrid Rice", Advances in Agronomy.Vol. 57. Academic Press Inc. p 377-462.

Virmani SS, Sharma, HL. 1992. Manual for Hybrid seed production. International Rice Research Institute. DAPO Box 7777, Metro Manila, Philippines.

Virmani SS, Sun ZX, Mao TM, Jauhar Ali A, Mao CX. 2003. Two-Line Hybrid Rice Breeding Manual. p 4-85. IRRI. Manila, Philippines.

Wang B, Xu WW, Wang, JZ, Zheng HG, Yang ZY, Ray JD, Nguyen HT. 1995. "Tagging and mapping the thermosensitive genetic male sterile gene in rice (*Oryza sativa* L.) with molecular markers". Theoretical Applied Genetics **91**, 1111-1114.

Xie F, Hardy B. 2009. Accelerating Hybrid rice development. p 3-8. IRRI. Manila, Philippines.

Xu Y, Zhu L, Xiao J, Huang N, McCOuch S. R .1987. Chromosome regions associated with segregation distortion of molecular marker in F2, Backcross, doubled haploid and recombinant inbred lines in rice. Molecular General Genetics **253**, 535-545.

Xu YB, Crouch JH. 2008. Review and Interpretation, Marker-Assisted Selection in Plant Breeding: From Publications to Practice. Crop Science −USA. **48**: 391-407.

Yamagishi M, Takeuchi Y, Tanaka I, Kono I, Murai k, Yano M. 1210. Segregation distortion in f2 and doubled haploid populations of temperate *japonica* rice, Journal of Genetics. **89**, 237–241.

Yang SR, Zhang LB, Chen WF, Xu ZJ, Wang JM. 1996. Theories and Methods of Rice Breeding for Maximum Yied. Acta Agronomica Sinica **22** (3), 295-304.

Yuan LP. 1994. Increasing yield potential in rice by exploitation of heterosis. In: Virmani SS, editor. Hybrid rice technology: new developments and future prospects. Selected papers from the International Rice Research Confernce. Manila (Philippines): International Rice Research Institute. p 1-6.

Zhang QF, Zhen BZ, Dai XK, Mei MH, SaghaMaroof MA, Li ZB.1994. Using bulked extremes and recessive class to map genes for Photoperiod-sensitive genic male sterility in rice. Proceedings of the National Academy of Sciences USA. **91**, 8675- 86789.