STANDARDIZATION OF HYBRID RICE SEED PRODUCTION FOR KERALA USING THREE LINE SYSTEM OF BREEDING

ARUNKUMAR C.

(2018-11-173)

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

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by

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2020

DECLARATION

I, hereby declare that this thesis entitled **"STANDARDIZATION OF HYBRID RICE SEED PRODUCTION FOR KERALA USING THREE LINE SYSTEM OF BREEDING"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani | **Arunkumar C**
Date: | (2018 - 11-173)

CERTIFICATE

Certified that this thesis entitled **"STANDARDIZATION OF HYBRID RICE SEED PRODUCTION FOR KERALA USING THREE LINE SYSTEM OF BREEDING"** is a record of research work done independently by **Mr. Arunkumar C (218-11-173)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellayani

Date:

Dr. Jayalekshmy V. G.
(Major Advisor, Advisory Committee)
Professor and Head
Department of Seed Science and Technology
College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Arunkumar C (218-11-173)**, a candidate for the degree of **Master of Science in Agriculture** with major in Seed Science and Technology, agree that the thesis entitled **"STANDARDIZATION OF HYBRID RICE SEED PRODUCTION FOR KERALA USING THREE LINE SYSTEM OF BREEDING"** may be submitted by **Mr. Arunkumar C,** in partial fulfillment of the requirement for the degree.

Major Advisor

Dr. V. G. Jayalekshmy
Professor and Head,
Dept. of Seed Science and Technology,
College of Agriculture, Vellayani.

Dr. Beena R.
Assistant Professor,
Department of Plant Physiology,
College of Agriculture, Vellayani.

Dr. Jacob John
Professor and Head,
IFSRS, Karamana,
Thiruvananthapuram-695002

Dr. Sheeja K Raj
Assistant Professor,
Department of Agronomy,
College of Agriculture, Vellayani

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OFABBREVIATIONS AND SYMBOLS USED

^{0}C	Degree Celsius
%	Per cent
CMS	Cytoplasmic Male Sterile
CD	Critical Difference
cm	Centimetre
mg	Milligram
RBD	Randomised Block Design
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
Kg	Kilo gram
ha^{-1}	Per hectare
KAU	Kerala Agricultural University
t ha^{-1}	Tonne per hectare
kg ha^{-1}	Kilogram per hectare
kg $plot^{-1}$	Kilogram per plot
$Plant^{-1}$	Per plant
$Plot^{-1}$	Per plot
Via	Through
Mm	Millimeter
No.	Number
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely
d.f	Degrees of freedom
S. E	Standard Error

IRRI	International Rice Research Institute
WA	Wild Abortive
F_1	First generation
Gas	Gibberellins
GA_3	Gibberlic acid
NAA	Naphthalene Acetic Acid
R line	Restorer line
A line	Seed parent (Cytoplasmic Male Sterile line)
RRS	Rice Research Station
IFSRS	Integrated Farming System Research Station Kerala
ICAR	Indian Council of Agricultural Research
NATP	National Agricultural Technology Project
IARI	Indian Agricultural Research Institute
m.ha	Million hectare
m. tons	Million tons
UNDP	United Nations Development Programme
$Panicle^{-1}$	per panicle
FAO	Food and Agricultural organization
L.	Linnaeus
CGMS	Cytoplasmic Genetic Male Sterility system
am.	Ante meridiem
ml/l	millilitre per liter
g/L	gram per liter
DHECD	7,8-dihydro-8α-20-hydroxyecdysone
km hr^{-1}	Kilometer per hour
FYM	Farmyard Manure
Cm	Centimeter
BL	Brassinolide
BRs	Brassinosteroids
NRRI	National Rice Research institute

INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa. L*) is the important staple food for about one- third of the world's population. According to Yadav *et al.* (2007) "Rice is life'' for the people of India. Rice plays main role in national food security and is a means of livelihood for millions of households in rural areas. Asia accounts for about 90 per cent of world's area and production of rice. India stands first in area but second in production next to China, occupying 45.5 million hectares with a production of 99.18 million tons. Compared to other cereals and pulses, rice is the major food crop of our nation which accounts 37 percent of the area and 43 percent of total food grain production. However, productivity is much lower than the average productivity of the world (INDIASTAT, 2018).

The ever-increasing population and the decrease in cultivated area lead to food shortage and is a serious global problem in this century. A two per cent annual growth of population is estimated in most of the Asian countries. Babu *et al*. (2012), reported that area under rice is reducing and productivity is in plateau. Hybrid rice technology is an option to enhance the productivity of rice.

Hybrid rice production was started in India in the year 1954. The heterosis in rice was first documented by Sampath and Mohanty (1954) at Central Rice Research Institute, Cuttack, Odisha. Heterosis (hybrid vigour) is an important genetic tool that has an increased level of performance for certain traits above the average performance of their parents and also enriched with many other desirable quantitative and qualitative traits which facilitated a yield increase of 30 to 40 percent over the parent (Srivastava, 2000). The productivity should be enhanced and the production level needs to be elevated by two million tons every year in order to meet the additional food requirement of the growing population. The productivity enhancement and gain in food security, which can be attained via heterosis breeding and other efficient breeding approaches (Pandey *et al.*, 2010).

Under irrigated conditions, compared to conventional inbreds, hybrid rice contributed 10-30 percent yield advantage (Virmani and Kumar, 2004**).** In India, till now around 78 hybrids have been released using three-line system of breeding (A, B and R line system). Among the different approaches followed by breeders, the use of cytoplasmic male sterility (CMS) is the most relevant, convenient and cost effective in hybrid seed production. The planting ratio of pollen and seed parent and synchronization of flowering are the factors affecting the success of hybrid seed production. Government of India had set a target of expanding the cultivation of hybrid rice to 25 percent of the rice cultivating area by 2025 (Spielman *et al*., 2013) as a part of national food security**.** Hence, breeders are now trying to develop improved hybrids for various ecological conditions with an average yield of 6.3 t ha^{-1}.

In Kerala, even though rice being the staple food, area under rice has been declining due to the fragmentation of land holding and the conversion of wet land under rice cultivation which affects the food security and make Kerala highly depend on other neighboring states. The actual production of Kerala is around 6 lakh tons and which falls far behind the consumption requirement of 40 lakh tons (Abraham, 2019).

The Keralites are more preferred red kernelled rice varieties which are more nutritious, higher in carbohydrate and fiber content than white rice. Kerala is the only state which develops and release red kernelled rice varieties. Hence, the hybrid for Kerala must be red kernelled but so far no hybrid rice variety has been released from Kerala. One of the reasons is the lack of standardized seed production package for three line system of breeding. Row ratio of parental lines (CMS, to maintainer or restorer lines) plays a vital role in hybrid rice seed production. The suitability of the climate of Kerala for hybrid rice seed production using three line system also needs validation.

The planting ratio of male and female parents in hybrid seed production has positive effect on outcrossing rate. Hence, planting of pollen and CMS seed parents should be done such a way that it can utilize maximum pollen to obtain higher rate of seed set. The success of F_1 seed production depends on the outcross rate, panicle exsertion and flag leaf angle in seed parent. These can be achieved by using the plant hormones like gibberllic acid and brassinosteroids.

With this background the present study was proposed with the objective of standardization of gibberlic acid, brassinosteroid and optimisation of planting ratio in restorer line (R) and male sterile line (A) to get maximum hybrid seed yield under Kerala condition.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

India in order to meet demand of the growing population has unceasingly accelerated additional production. In small states like Kerala, where the population density is high the natural resources like land, water and scarcity of labour makes the task of attaining additional crop production more challenging. In the current scenario, Kerala rich in genetic diversity of the traditional rice varieties which are grown in various season and different climatic conditions is no longer self-sufficient to feed the rapidly growing population (Kumari *et al.,* 2010). Production can be enhanced via introduction of hybrids and high yielding varieties which play a crucial role. Breeders are now trying to develop improved hybrids for different ecological condition and find out suitable package of practices with adequate supply of seeds by way of producing quality seeds of concerned hybrids. Standard heterosis is vital over locally adopted high yielding check variety, planting ratio and grain parameters. The literature available on the aspect of hybrid rice seed production using three-line system of breeding is reviewed in this chapter.

2.1 HYBRID RICE

Hybrid rice research was initiated by China in 1964. Later a male sterile wild rice (wild abortive or W A type) plant was found by B.F. Li, in 1970. This discovery was a breakthrough in hybrid rice breeding. The first set of W A type cytoplasmic male sterile (CMS) lines and their maintainers was developed in 1972, through wide test crosses and successive backcrossing. In the next year, the first restorer lines were identified by screening existing varieties introduced from Southeast Asian countries. In 1974, some rice hybrids with strong heterosis were developed and a complete procedure of hybrid seed production technology was established in 1975. In 1976, hybrid rice in China was released for commercial use on a large scale.

The identification of MS lines resulted in yield advantage of 20% and is now commercially cultivating about 50% of the rice in China (Singh *et al*., 2015).

Various research works were carried out on hybrid rice by different group of scientists but they could not succeed in developing commercial hybrid. The first commercial hybrid was developed in China by Yuan Long Ping in 1974, which was a well performing, stable commercial cytoplasmic genetic male fertility restorer system from an accidently found cytoplasm of *Oryza sativa f. spontanea* (WA) (Lin and Yuan, 1980). Virmani (1981), confirmed that F_1 hybrids which are produced by exploiting the cytoplasmic genetic male sterility are superior and observed 20-30 percent yield advantage over varieties. They also suggested that the commercial exploitation of heterosis in rice is possible by the use of cytoplasmic genetic male sterility and fertility restoration system.

In 1989, the Indian Council of Agricultural Research (ICAR) launched a time-bound and goal-oriented project on the "Production and Use of Hybrid Rice Technology," which began in 1989 in collaboration with IRRI, and was reinforced in 1991 with financial support from the United Nations Development Programme (UNDP), Mahyco research Foundation, World bank funded National Agricultural Technology Project (NATP) and FAO technical cooperation. Within a short period of five years, India commercialized half a dozen rice hybrids each from the public and private sectors. During 1994 the country released the first four rice hybrids viz., APHR-1, APHR-2, MGR-1 and KRH-1. After which, two more hybrids were published *viz.*, CNRH-3 and DRRH-1. These projects were continued till 2005.

The hybrids had an average yield advantage of 15-20% over the pure line check varieties. In some leading Asian rice growing countries, a total area of 17.11 m ha was under hybrid rice during the year 2005-06. Akram *et al.* (2007), experimented on hybrid rice and reported that hybrid rice showed 37 % higher net return ha^{-1} than that of conventional varieties. Hybrid rice was planted in an area of 1.4 m ha during 2008 and through this technology an additional rice production of 1.5 to 2.5 m t was added to our food basket. More than 80 % of the total hybrid rice area is in eastern Indian states like Uttar Pradesh, Jharkhand, Bihar, Chhattisgarh, with some little area in states like Madhya Pradesh, Assam, Punjab and Haryana (Viraktamath, 2011).

2.1.1 Hybrid rice seed production technology

Three-line system of hybrid rice seed production technology involves two major steps viz., multiplication of CMS line and production of hybrid seed.

Extensive research in IRRI led to the identification of following guidelines for successful hybrid rice seed production (Virmani and Sharma, 1993)

1. Selecting seed parents with long exerted panicle, long duration and wider angle of floret opening and pollen parent with a high per cent of residual pollen per anther after anther exsertion with synchronized time of anthesis.
2. Sowing of 1kg seed per 20 m^2 and 5-10cm raised seed beds with approximately 1m width.
3. Synchronizing the flowering time of two parents by seeding them at different dates depending on their growth duration
4. Transplanting of 21 days old seedlings to ensure timely heading and flowering of parental lines.
5. Clipping of flag leaves when primary tillers are at booting stage
6. Practicing supplementary pollination on calm days when wind speed is very low (1-3 km hr^{-1}) and it disperses pollen grains uniformly over the seed parent plants.
7. Rouging is done at the most important stages such as at maximum tillering, flowering and just before harvest
8. Harvest when 90 percent of the grain in main panicles of A line plants are clear, firm and straw coloured and seed moisture is less than 20%. The A line and R line harvest must be kept separate from each other during harvesting, threshing, drying and bagging.

2.2 PLANTING RATIO

Maruyama and Oono, (1983) proposed the use of a facultative female sterile line as pollinator for rice seed production involving mixed planting of seed and pollen parents. Sharma and Virmani, (1994) recorded a positive effect of row ratio on outcrossing rate. This expressed the importance of planting proportion of male parent and the CMS seed parent to obtain the higher rate of seed set per panicle. Mao *et al.* (1998), suggested that for the required planting ratio hybrid seed yield can be increased by increasing the pollen load of male parent and spikelet's number per panicle.

Seed yield obtained from a male sterile line used in a hybrid seed production plot is a function of yielding ability of the male sterile line (extrapolated from the yielding ability of its maintainer line), proportion of male sterile line to pollen parent and outcrossing rate of the male sterile line. Improvement in proportion of pollen parent and the seed parent can increase hybrid rice seed yield (Virmani and Kumar, 2004).

Ramos *et al.* (2004), conducted a study to determine the optimum row ratio for male and female parents in hybrid rice and reported that two row ratios (2:6 and 2:8) were the best performers in all the three seasons during which the study was conducted. The 2:8 row ratio produced the higher seed set percentage (15%) and seed yield (687.0 kg ha^{-1}) and 2:6 showed seed set percentage of 12.33% with seed yield of 640 kg ha^{-1}, whereas, 2:12 planting ratio produced least seed set percentage (7.67%) and seed yield (370 kg ha^{-1}) during 1999 dry season. The 2:6 row ratio produced higher seed set (27.97%) and seed yield (1391.7 kg ha-1) and 2:8 showed 25.33% seed set and 1117.3 kg ha^{-1} seed yield while, 2:12 row ratio produced 20.3% seed set with 803 kg ha^{-1} seed yield, in 2000 dry season. From the study he concluded that increasing female parent rows in the hybrid seed production with constant pollen parent rows reduced the seed set percentage and seed yield.

As reported by Hasan *et al.* (2010), the highest number of tillers per hill (18), panicle exsertion rate (76.67%) and seed yield (6.63 t ha^{-1}) was obtained in the row ratio 2:12.

Rahman *et al.* (2012), reported that the 2:12 row ratio resulted in highest number of grains per panicle (52.23) and higher seed yield (2.05 t ha^{-1}) in BRRI hybrid Dhan 2. They also reported that the highest number of effective tillers per hill (11.37) was produced with the row ratio 2:8.

Angamuthu (1996) conducted an experiment on Hybrid Seed Production and Storage Technology in Rice (*Oryza sativa*), to standardize optimum planting ratio of IR6289 A and IR 10198-66-2R line for CORH-1rice hybrid seed production and clearly indicated that a female to male ratio of 8:2 was found to be perfectly optimum to maximum seed set leading increased seed yield and quality.

In a **s**tudy conducted by Hamad *et al.* (2015), they reported that 2:4 row ratio resulted in a higher plant height (88.93cm and 90.35cm), panicle exsertion (69.15% and 70.15%) and seed set (34.1% and 34.82%) during 2013 and 2014 seasons respectively. However, the highest grain yield (1.80 t $ha^{-1}$and 1.90 t ha^{-1}) was reported with 2:8 row ratio in 2013 and 2014 seasons respectively.

Hasan *et al.* (2015), reported that 2:10 row ratio resulted in highest panicle exsertion rate (84.62%) and 2:14 row ratio resulted in higher seed yield of 3.30 t ha^{-1}.

Abo-Youssef *et al.* (2017), conducted an experiment on enhancing seed yield of hybrid rice through row ratio and gibberellic acid. The results of the study indicated that the 2:8 row ratio resulted in highest values of flag leaf area (37.64 cm^2 and 37.94 cm^2), panicle exsertion (80.38% and 81.38%), panicle length (24.20cm and 24.67cm) and seed set (23.72% and 36.63%) in 2013 and 2014 seasons respectively in hybrid rice.

Sushmita *et al.* (2019), reported that planting ratio between seed parent and pollen parent has a profound effect on hybrid seed setting and yield. In PRH 10 hybrid, 2:6 planting ratio resulted in significantly higher seed setting percentage (12%) and 2:8 planting ratio resulted in the highest seed yield (692.07 kg ha^{-1})

2.3 EFFECT OF BRASSINOSTEROID

Brassinosteroids (BRs) comprise a group of poly hydroxylated sterol derivatives and are ubiquitously distributed in the plant kingdom. BRs are structurally most similar to animal steroid hormone. Brassinolide (BL), the most biologically active and naturally occurring form of the BRs was first isolated and purified from

Brassica napus pollen as reported by Grove *et al.* (1979). Since then, more than 50 BL analog have been identified (Fujioka and Sakuri, 1997). In rice, BRs affect many agricultural traits that influence grain yield, including plant height, leaf angle, grain size and tiller number (Ikekawa and Zhao, 1991).

2.3.1 Plant height

Exogenous application of BRs had a dramatic effect on stem elongation, including promotion of epicotyls, hypocotyls, and peduncle elongation in dicots and enhanced growth of coleoptiles and mesocotile of monocots (Mandava, 1988).

A study was carried out by Farooq *et al*. (2009), in fine grain aromatic rice on which exogenous application of two BRs i.e. 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) was carried out which were given as foliar spray. The results of the study showed that height of the crop was increased in the treated plants with 45.27 cm in HBL applied plants and 49.41 cm in EBL applied plants which was greater than that of the control where the height of the crop is 39.17 cm.

2.3.2 Flag leaf angle

Flag leaves, are those that cover the panicle during booting stage and develop in to whole leaf. Thus, the leaf acts as a barrier against the exerted panicle and results in reduced rate of seed set. So, to overcome the barrier effect on out crossing, flag leaf clipping is the best method that is practiced with skilled workers.

Wada *et al*. (1981) carried out research on rice and found that auxin has an impact on lamina inclination. The inclination of the lamina joint contributes significantly to leaf angle formation. Rice lamina inclination assay is a highly specific and sensitive bioassay for brassinosteroids (Wada *et al*., 1981; Wada *et al*., 1984). The well-known BRs assay indicates that lamina joint bending is sensitive to exogenous 24-epibrassinolide (24-eBL) (Cao and Chen 1995; Wada *et al*., 1984). Correspondingly, BRs-deficient and BRs-insensitive mutants which inhibit BRs biosynthesis or disrupt the signal transduction pathway result in a decreased bending of the lamina joint. However, rice seedlings treated with BRs display a dose-dependent increase in the degree of bending of the lamina joint, i.e., the higher the concentration of BRs added, the greater the bending of the lamina joint (Hong *et al*.2005 ; Tanabe *et al*. 2005; Yamamuro *et al*. 2000).

BRs deficiency leads to erect leaf orientation, implying erect leaves indicate reduced expression of the BRs biosynthesis gene OsDWARF4 and reduced accumulation of brassinosteroids, suggesting that OsHAP3E can control the leaf angle by inhibiting the expression of OsDWARF4 (Ito *et al.*,2011).

2.3.3 Number of grains per panicle

Thussagunpanit *et al.* (2015) investigated the effect of exogenously applied brassinosteroid, 24-epibrassinolide (EBR), and brassinosteroid-mimic compound, 7,8-dihydro-8α-20-hydroxyecdysone (DHECD) on number of filled grains per panicle in rice cultivar Pathum Thani 1 and found that number of filled seeds and seed mass of rice was increased. The number of filled seeds per panicle in control was 78.36 which was lower than that of the plants on which exogenous application of EBR and DHECD was done where the number of filled seeds per panicle is 90.81 in plants applied with EBR and 98.73 in plants applied with DHECD.

2.3.4 Yield

Richards (2000), reported that the increase in the yield in plants occurred due to the increase in the photosynthetic rate per unit area and carbon partitioning from the source to the sink. BRs play an important role in maintaining the size and weight of the seed. The mechanism for this is unclear but it has been shown that BRs can regulate the initial carboxylation activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and thereby influence photosynthetic CO_2 assimilation (Yu *et al.*, 2004). Several researches reported that the activity of BRs could increase crop yield (Wu *et al*,. 2008; Divi and Krishna, 2009).

Rice plants deficient in or insensitive to BRs produce short and smaller seeds. This explains the role of BRs in enhancing the load of assimilates in rice seed to increase grain yield (Morinaka *et al.*, 2006). It functions in managing embryonic and post embryonic development and adult homeostasis. They are also involved in regulating the process that are more specific to plant growth including regulation of flowering time and cell expansion.

In rice cultivar Pathum Thani 1, EBR (brassinosteroid, 24- epibrassinolide), and DHECD (brassinosteroid - mimic compound, 7,8-dihydro-8α-20-hydroxyecdysone) were applied exogenously and found that the yield was increased in the EBR and DHECD applied plants. The seed yield is higher in treated plants with 504.40 g m^{-2} in EBR treated plants and 429.60 g m^{-2} in DHECD treated plants (Thussagunpanit *et al.,* 2015).

2.4 EFFECT OF GA_3

The application of GA_3 is successful in plant growth and promotes the elongation of cells. GA_3 has a key role in winning high seed yields in the cultivation of hybrid rice seeds. The main drawback of the three- line system of breeding is the low F_1 seed production level which is due to incomplete panicle exsertion and lack of outcrossing.

The panicle exsertion can be improved through GA_3 application and it depends on many floral traits such as number of opened spikelets, duration and angle of spikelet opening, pollen load, pollen longevity, length of style, panicle exsertion, flag leaf angle and length and stigma characters (length, width and receptivity) (Kato and Namai, 1987). Ponnuswamy *et al.* (1998), carried out research on application of GA_3 on plants and found that there is an increase in plant height, panicle exsertion, flag leaf angle, seed setting percentage and seed yield of hybrid rice.

The application of GA_3 enhances the panicle exsertion from the flag leaf, increase the rate of stigma exsertion, plant height, extend the duration of floret opening and make the crop taller and productive (Virmani and Sharma, 1993; Yuan *et al*., 2003; Viraktamath and Ilyas, y2005; Gavino *et al.*,2008).

2.4.1 Plant height

An experiment was conducted by Gavino (2008), in which five dosages of GA_3 i.e., 0, 75, 150, 225, 300 g ha^{-1}were applied and it was repeated three times. The parental lines were IR5825A and IR60819R for Mestizo 1, IR68888A and IR62161R for Mestizo 2 and IR68897and IR60819R for Mestizo 3. The plant height of the parental lines was increased with increasing dose of GA_3 and the higher plant height was recorded with 300 gha^{-1} of GA_3. They also reported that the sensitivity of R line to GA_3 is stronger than A line. The results showed that the plant height of R line of Mestizo 1 treated with 300 g ha^{-1} was over 30 cm higher than A line.

The study conducted by Niknejhad and Pirdashti, (2012) reported that application GA_3 increased the plant height from 75 cm in control to 93.31 cm in 100% of recommended dose of GA_3. Susilawati *et al.* (2014), reported that the plant height was greatly influenced by GA_3 spraying and with increase in concentration of GA_3 spraying the plant height was increased. The control treatment resulted in a plant height of 93.97cm and it was increased to 106.81cm, 115.21cm and 121.11 cm with 100ppm, 200ppm and 300ppm GA_3. The GA_3 dosage of 300 g ha^{-1} resulted in higher plant height of 102.44 cm and 104.32 cm compared to the control treatment without GA_3 which resulted in lowest values of plant height (Hamad *et al.,* 2015).

Pandey *et al.* (2017), found that GA_3 spraying has significantly influenced the plant height in female parent. The plant height was 73.33 cm in control and it was increased to 100.66 cm, 106.00 and 110 cm with GA_3 dosages 60g ha^{-1}, 90g ha^{-1} and 120g ha^{-1} respectively. Begum *et al.* (2018), reported that foliar spray of GA_3 @ 50ppm resulted in a significantly higher plant height (130.5 cm) in rice.

Pin *et al.* (2019), reported that the GA_3 treated plants increased plant height by 29.92%, 17.46%, 11.89% and 10.74% at flowering stage of 0%, 10%, 30% and 50% respectively, when compared to the control.

2.4.2 Flag leaf angle

Phytohormones, such as auxin (GA_3), gibberellins (GAs), and ethylene, are involved in leaf angle formation through adjusting the orientation of leaves resulting in higher rate of photosynthesis (Cao and Chen, 1995). Interestingly, current evidence indicates that most of these phytohormones like auxin (GA_3), gibberellins (GAs), and ethylene regulate the leaf angle through crosstalk with BRs (Cao and Chen, 1995; Takeno and Pharis, 1982; Wang *et al.,* 2019).

Hamad *et al.* (2015), reported that the flag leaf angle was increased with increased GA_3 dosage and 300 g ha^{-1} GA_3 resulted in highest flag leaf angle of 43.830˚ and 44.330˚ in 2013 and 2014 seasons respectively.

2.4.3 Flag leaf area

Different GA_3 such as 50%, 100%, 200% of recommended dose was applied and control without the application of GA_3 was maintained in Tarommahalli rice variety in which the flag leaf area was determined at flowering stage. The external GA_3 significantly increased the flag leaf area from control with 117.11 cm^2 to 173.65 cm^2, 221.15 cm^2 and 243.35 cm^2 at 50% and 200% recommended dosage of GA_3 respectively (Niknejhad and Pirdashti, 2012).

2.4.4 Number of leaves

Niknejhad and Pirdashti (2012) reported that the number of leaves was increased with the increased dosage of GA_3 and 200% of recommended GA_3 dosage recorded the highest number of leaves (2%) followed by 100% and 50% GA_3 recommended dosage with 1.62% and 1.40% number of leaves respectively.

2.4.5 Tiller number

Tillering of rice, determined the panicle number per plant, and is an important agronomic trait for grain. It is dynamic and adjustable. Plant hormones play important role in regulating the tiller occurrence (Liu *et al.,* 2012). Growth stimulators had a significant effect on tiller number. As reported by Niknejhad and Pirdashti (2012), GA_3 markedly increased the tiller number in ratoon. The results recorded showed an increase in number of tillers from control with 14 tillers plant^{-1} to treatments with 19 tillers plant^{-1}, 16 tillers plant^{-1} and 20 tillers plant^{-1} at 50%, 100% and 200% GA_3 dosage respectively.

Number of tillers per plant was increased by GA_3 application and 120 gha^{-1} GA_3 resulted in higher number of tillers per plant (13.82) (Pandey *et al.,* 2017).

2.4.6 Panicle Exsertion

Panicles should emerge completely from the flag leaf sheath, so that part of the internode below the panicle base is exposed. The lower panicle branches often remain enclosed because the upper internode is short. Such enclosed spikelets are sterile or partially filled and are often blackened by secondary pathogens, resulting in yield losses (Jennings *et al.,* 1979).

Owing to the stimulating effect of GA_3 on various physiological processes such as cell division and cell elongation in plants, the rate of panicle exsertion increases with increasing GA_3 level. Deshapande *et al.* (2003) found that the application of 300 g GA_3 ha^{-1} resulted in the best panicles exsertion of A-line plants. It enhanced the cross pollination in hybrid seed production. The application of 200 g NAA ha^{-1} resulted in the highest percentage of panicle exsertion (88%).

Rahman *et al.* (2012) conducted a study on effect of GA_3 and row ratio of restorer, CMS lines (a) on different traits and seed production of BRRI hybrid DHAN2 and reported that highest panicle exsertion rate (94.94%) was found at the dosage of 250g GA_3ha^{-1} and the lowest panicle exsertion (46.63%) was noticed with the control.

GA_3 spraying at 100, 200 and 300ppm significantly increased the panicle exsertion compared to control treatments. The panicle exsertion rate was increased from 83.93% with 100ppm GA_3 to 87.77% and 89.96% with 200ppm and 300ppm GA_3 respectively (Susilawati *et al.,* 2014).

The panicle exsertion rates increased from 24.90% in control treatment (without GA_3) to 73%, 81.87% and 91.28% with 150 gha^{-1}, 200 gha^{-1} and 300 gha^{-1} GA_3 dosage as reported by Hamad *et al.* (2015)

Abo-Youssef *et al.* (2017), reported that application of GA_3 @400g ha^{-1} recorded the highest values of panicle exsertion (83.64 and 87.09%) in 2013 and 2014 seasons, respectively. Application of GA_3 @ 60gha^{-1} resulted in panicle exsertion of 81.81% and it was increased to 83.47% with 90g ha^{-1} GA_3 and 89.90 g ha^{-1} with 120 g ha^{-1} GA_3 application (Pandey *et al.*, 2017).

A micro-crossing plot experiment was carried out by Pin et al. (2019) in Homchonsith rice variety (HCSA/HCSB) with five treatments, which include application of GA_3 at 0%, 10%, 30% and 50% panicle heading stages and a control without application of GA_3.The results of the study revealed that applying GA_3 at 0% heading to 50% flowering stage had significant effect on panicle exsertion rate of both hybrid parental lines. When GA_3 applied at 10% to 50% flowering stage in HCSA line, its panicle exsertion rate was increased significantly from 73.66% to 80.80% when compared to the control without effect on the panicle exsertion of the HCSB line. GA_3 application at 30% and 50% flowering stage gave higher panicle exsertion rate of A line when compared to the control. In contrast, the panicle exsertion rates of both parents were reduced slightly when the plants had GA_3 applied at 0% heading stage compared to others due to significant reduction to the length of the 1st uppermost internode.

2.4.7 Number of filled grains panicle^{-1}

Gavino *et al*. (2008) conducted a study with five dosages of GA_3 such as 0, 75, 150, 225, 300 g ha^{-1}. The results showed that the untreated control plots recorded the lowest percentage of seed set (6.26%) which was obviously lower than the mean of other treatments applied with GA_3 (20.86%). The optimal GA_3 dosage at which highest percentage of seed set was obtained and most economical were 225, 150 g ha^{-1} for hybrid rice Mestizo 1, Mestizo 2 and Mestizo 3, respectively.

Application of optimum dosages of GA_3 increased the photosynthetic capacity, delayed the leaf senescence and promoted the rate of rice seed-setting (Hasan *et al.,* 2015). Rahman *et al.* (2012) reported that minimum number of sterile spikelets per panicle (72.75) was produced at 250 g GA_3 ha^{-1}.

As reported by, Niknejhad and Pirdashti (2012), the grain number per panicle is higher with 100% GA_3 dosage (50%) followed by 200% and 50% GA_3 and the control treatment recorded 32.23% lesser grain number per panicle. Hamad *et al.* (2015), reported thatGA_3 @ 300 g ha^{-1} produced the highest number of panicles (23.65 panicles per hill) and highest seed set percentage (44.23%).

Application of 200ppm GA_3 resulted in highest seed setting (24.92%) compared to 300ppm (24.11%) and 100ppm (20.33%) (Susilawati *et al.,*. 2014). Hamad *et al.* (2015), found that the seed setting was increased with GA_3 application and 300 gha^{-1} resulted in higher seed set of 43.05% and 44.23% in 2013 and 2014 seasons respectively.

Abo-Youssef *et al.* (2017), reported that GA_3 played an important role in increasing the percentages of seed setting. The highest values of seed setting (27.99 and 45.94%) were recorded at 400 g ha^{-1} GA_3 in 2013 and 2014 seasons respectively.

Pandey *et al.* (2017), found that application of GA_3 resulted in increased number of filled grains per panicle and seed setting rate compared to the control treatment. The highest number of filled grains per panicle (49.41) and seed setting rate (36.61%) was resulted with the application of GA_3 @120 g ha^{-1}.

2.4.8 1000 Grain Weight

Niknejhad and Pirdashti (2012), reported that the 1000 grain weight was increased with GA_3 application with 24.23 g at 50% GA_3 dosage, 24.70 g at 100% GA_3 dosage and 23.78 g at 200% GA_3 dosage when compared with that of the control where the 1000 grain weight is less with 20.26 g.

In contrast, Abo-Youssef *et al.* (2017), reported that 1000 grain weight significantly decreased with increasing concentrations of GA_3 and the highest 1000 rain weight was observed under the control condition.

2.4.9 Yield

Suralta *et al*. (1999) also found that the optimum rate of 300 ppm of GA_3 with three splits at a ratio of 25:50:25, beginning at the 5-10% panicle heading stage increased seed yield of the A line.

Rice ratoon cultivar Tarom Mahalli when exposed to different GA_3 dosages such as 50%, 100%, 200% of recommended dose, the yield was increased. In control yield is less with 1192 kg ha^{-1} when compared with that of the treatments where it was increased to 1421 kg ha^{-1}, 1502 kg ha^{-1} and 1662 kg ha^{-1}at 50%, 100% and 200% GA_3 dosage respectively (Niknejhad and Pirdashti, 2012).

Susilawati *et al.* (2014), reported that the optimum rate of 200 ppm of GA_3 application with two splits at the 5-10% panicle heading stage increased the seed yield (1517.50 kg ha^{-1}) of A lines. Hamad *et al.* (2015) reported that application of 300 g ha^{-1} GA_3 resulted in 2.19 t ha^{-1}of grain yield and 19.25% of harvest index.

Application of GA_3 at 30% heading stage gave significantly higher seed yield (12.32 g per plant) of the A line when compared to the application at 0%, 10%, 50% flowering stage and the control (Pin *et al.*, 2019).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The project entitled "Standardization of hybrid rice seed production using three line system of breeding for Kerala" was conducted at College of Agriculture, Vellayani, Thiruvananthapuram and Integrated Farming System Research Station Kerala (IFSRS), Karamana, during 2019-2020 with an objective of standardization of GA_3, brassinosteroid concentration and optimization of planting ratio of restorer line (R) and male sterile line (A) to get maximum hybrid seed yield under Kerala conditions.The materials used and the methods adopted for the study are described in this chapter.

The study comprised of two experiments

Experiment I: Phenological studies in male sterile line (Aline).

Experiment II: Standardization of dose of GA_3 and brassinosteroid for the planting ratio of A, R line as 6:2 and 8:2.

3.1 EXPERIMENTAL DETAILS

3.1.1. Location

The first experiment was conducted at College of Agriculture, Vellayani situated at $8^0$5' N latitude and $76^0$9' E longitude with an altitude of 29m above mean sea level. The second experiment was done at Integrated Farming System Research Station (IFSRS), Karamana, geographically located at 8.4736^0 N latitude, 76.9614^0 E longitude and at an altitude of 5 m above mean sea level.

3.1.2. Experimental Material

The female seed parent, CRMS 31A was collected from National Rice Research Institute (NRRI), Cuttak, Orissa and the male pollen parent, Remya (MO10) was obtained from Rice Research Station (RRS), Mancombu. The variety Remya (MO10) was reported as a restorer parent for CRMS 31A in the three line breeding programme (Das, 2017). The parents were grown during March to July, 2020 (Virippu).

3.2. PHENOLOGICAL STUDIES IN MALE STERILE LINE (A LINE).

The male sterile plants were transplanted to pots after 24 days of sowing. Ten plants in ten pots were maintained under controlled condition to reduce the pest and disease incidence. The morphological and flowering related traits of sterile line CRMS 31A were recorded from each pot at tillering, booting, panicle primordial initiation and flowering stages.

3.2.1. Morphological observations of CRMS31A at vegetative stage

3.2.1.1 Tillering stage

The time of tillering was observed from germination to first tiller starts. It was recorded in days.

3.2.1.2. Plant height at maturity (cm)

The plant height was measured in centimeter from base of the stem to tip of the topmost leaf at 100 DAS.

3.2.1.3. Total number of tillers

Number of tillers were recorded from observational plants from each pot at harvest and mean value were calculated accordingly.

3.2.1.4. Total number of leaves

Number of leaves was recorded from observational plants at 100 DAS and expressed in numbers.

3.2.1.5. Flag leaf area (cm^2)

It was calculated from length and breadth measured from the leaves and expressed in cm^2. Leaf area was measured at 100 DAS.

3.2.2. Observations on floral characters of CRMS31A

3.2.2.1. Days to panicle primordial initiation

Number of days taken from germination to panicle primordial initiation was recorded from the plants and expressed in days. The main tillers were dissected at different days to find the pattern of panicle initiation.

3.2.2.2. Days to booting

Number of days taken from germination to booting stage was recorded and it is expressed in days.

3.2.2.3. Days to panicle emergence

Days from germination to panicle emergence was recorded from the plants. The results were expressed in days.

3.2.2.4. Flag leaf angle

It was recorded by measuring the angle between the flag leaf blade midrib and the rachis holding the spikelets within the inflorescence. With the help of a protractor the flag leaf angle was measured by keeping the main panicle axis as basal line, from this basal line to the flag leaf position was measured and expressed in degrees.

3.2.2.5. Days to anthesis

Number of days taken from sowing to anthesis was recorded and expressed in days.

3.2.3. Statistical Analysis

Descriptive statistical analysis was performed to analyze the characters. The statistical package GRAPES from Kerala Agricultural University was used for the analysis.

3.3 STANDARDIZATION OF DOSE OF GA_3 AND BRASSINOSTEROID FOR THE PLANTING RATIO OF A, R LINE IN THE RATIO OF 6:2 AND 8:2

The second experiment was conducted with A and R line in open field condition at IFSRS, Karamana to standardize the dose of GA_3 and brassinosteroid and to analyze the seed set efficiency in 6:2 and 8:2 planting ratio during hybrid rice seed production programme.

3.3.1. Planting Material

The A and R line seeds were germinated on water soaked tissue paper placed in petriplates. The germinated seeds were sown in dapog trays of 60 cm x 30 cm filled with field soil at a spacing of 5 cm between plants. Irrigation was done as required (Plate 1).

3.3.2 Transplanting

Transplanting of 24 days old seedlings of female parent along with 21, 24, 27 days old R line seedlings were done on the 24th day in the main field at a spacing of 20 cm x 15 cm to get maximum pollen for hybrid production. The staggered sown R lines were maintained in a sequence of 21, 24, 27 days old seedlings in a single line.

3.3.3. Planting ratio

The A and R lines were planted in the ratio of 6:2 and 8:2 with a spacing of 20 cm x 15 cm. A total of 96 and 128 plants were maintained in the main field of 9 m^2 plot size with 6:2 and 8:2 planting ratio respectively (Plate 3).

3.3.4. Layout of the experiment

Design: RBD

Treatment: 7

Replication: 3

Plot size: 3m x 3 m

Spacing: 20 cm x 15 cm

Season: March to July 2020 (Virippu)

Location: IFSRS, Karamana

3.3.5. Desirable characteristics of CMS lines

In hybrid seed production, seed yield depends on desirable panicle, floret and stigma characteristics of the CMS line. The panicle should be completely exerted from the flag leaf and should have at least 100 widely opened spikelets. The florets should remain open for at least 45 minutes or longer with stigma receptivity of 5-7 days.

3.3.6. TECHNIQUES FOR HYBRID RICE SEED PRODUCTION

For seed production, the techniques standardized by IRRI (Virmani and Sharma, 1993) was followed,

3.3.6.1. Staggered sowing

The CMS line CRMS 31A and restorer line Remya were sown in staggered dates. The R line was sown in three staggered dates. i.e., one at three days before the sowing date of CMS lines, the second set along with CMS line and the third set were sown three days after the sowing of CMS lines. The synchronized flowering dates were recorded.

3.3.6.2. Isolation

The space and time isolation was practiced in the experimental field by selecting the area 100m away from other rice seed production plots and avoiding planting at the same time in nearby plots.

3.3.6.3 Chemical spraying

Separate spraying of GA_3 and brassinosteroid was done at two stages *viz.*, 15-20% heading and 35-40% panicle emergence. The concentrations of both the chemicals are detailed in Table 1. The required amount of GA_3 and water for spraying was calculated and computed amount 100% GA_3 powder was dissolved in 100% ethanol first and then mixed with required amount of water (Virmani and Sharma, 1993). Liquid form of brassinosteroid was mixed with calculated amount of water for spraying. Both the solutions were prepared in ppm for 2 litres per plot.

Table 1: Concentration of hormones and planting ratio in the treatments

Treatment	Planting ratio	GA_3 concentration (ppm)	Brassinosteroid concentration (ppm)
T_1	6:2	50	2
T_2	8:2	50	2
T_3	6:2	50	6
T_4	8:2	Control	Control
T_5	6:2	70	2
T_6	8:2	70	2
T_7	6:2	Control	Control

3.3.6.4. Hybridization technique

CRMS 31A was used as seed parent so there was no need of manual hybridization (or emasculation and bagging) MO10 (Remya) had provided to supplement enough quantity of pollen grains for pollination.

3.3.6.5. Supplementary pollination

Supplementary pollination was done with the help of rod driving (Plate.4). This operation was done 4-5 times (every 30 minute) between 9 am to 11.30 am on calm days when there was no movement of canopy ie, when the wind velocity is between 1-3 km $hr^{-1}$and continued in every 30 minutes until all blooming florets in the pollen parents were closed. Wooden rods of 10 cm diameter were moved towards the CMS line rows from both sides. Supplementary pollination continued even after the floret of the seed parent has closed because exerted stigmas were still receptive to pollen.

3.3.6.6. Flag leaf clipping

Clipping of flag leaf was done when the primary tillers were at booting stage. It was practiced to enhance the pollen dispersal and to ensure higher seed set. The flag leaves were clipped by holding the upper leaves just above the flag leaf joint of the main tiller. Half to two third areas of the flag leaf were removed.

3.3.6.7. Roguing

To ensure high genetic purity, periodical roguing was done in the seed production plots during the maximum tillering stage, at flowering and just before harvesting. Care was taken to ensure that all the off types from the seed parent were removed before flowering.

3.3.7. Agronomic Practices

3.3.7.1. Field preparation and layout

Field was thoroughly ploughed with power tiller and was uniformly leveled. The experimental plot of 3 m x 3 m was made with bunds of 30 cm width on all the four sides and an irrigation channel of 30 cm was provided between them. Later the plot was leveled and maintained 2 cm of water up to transplanting (Plate.2).

Plate 1. Nursery Preparation

Plate.2. Field preparatio

Plate.3. Field view

Plate 4. Supplementary pollination

3.3.7.2. Application of manures and fertilizers

The crop was uniformly fertilized with farm yard manure @5t ha^{-1} and chemical fertilizer 150:75:75 kg N: $P_2O_{5:}$ K_2O (Mohan *et al*., 2014). The entire dose of FYM was incorporated at the time of ploughing; one third N and K and full P at 15 days after sowing, one third N and K at 35 days after sowing and remaining one third N and K at 55 days after sowing.

3.3.7.3. Water management

Water management was carried out as per Package of Practices Recommendation: crops (KAU, 2016)

3.3.7.4. Weed management

Weeds were controlled by hand weeding. First hand weeding was carried out at 20 days after sowing and second hand weeding at 40 days after sowing.

3.3.7.5. Harvesting

Manual harvesting was done. All the R line rows were first harvested by cutting them off at the base with a sickle or scythe. Removed the harvested R lines and stored it in threshing yard. Threshed seeds from the net plot area of seed parent were dried under sun and reduced the moisture percentage to 13 per cent and yield was expressed in gram per plot.

3.3.8. Observations

3.3.8.1. Morphological observations taken for different treatments

3.3.8.1.1. Flag leaf angle (degree)

It was recorded by measuring the angle between the flag leaf blade midrib and the rachis holding the spikelets within the inflorescence. With the help of a protractor the flag leaf angle was measured by keeping the main panicle axis as basal line, from this basal line to the flag leaf position was measured and recorded.

3.3.8.1.2. Panicle exsertion (cm)

The panicle exsertion was measured as the distance from the flag leaf ligules to the panicle basal node and expressed in cm. Positive values were indicated as complete exsertion and negative values were indicated as incomplete exsertion.

3.3.8.1.3. Plant height (cm)

The plant height was measured in centimeter from base of the stem to tip of the topmost leaf at 100 DAS.

3.3.8.1.4. Productive tiller

Number of productive tillers were recorded from observational plants from each pot at harvest and mean value were calculated accordingly.

3.3.8.1.5. Total tillers

Number of total tillers were recorded from observational plants from each pot at harvest and mean value were calculated accordingly.

3.3.8.1.6. Number of leaves

Number of leaves was recorded from observational plants at 100 DAS and expressed in numbers

3.3.8.1.7. Flag leaf area (cm^2)

It was calculated from length and breadth measured from the leaves and expressed in cm^2. Leaf area was measured at 100 DAS.

3.3.8.2. Yield related observations taken on different treatments

3.3.8.2.1. Thousand grain weight (g)

Thousand grains were selected randomly from each plot and measured the weight by using an electronic weighing balance and expressed in grams.

3.3.8.2.2. Number of filled grains per panicle (g)

Individual panicle was harvested from the five observation plants and number of filled grains per panicle was counted on working board.

3*.3.8.2.3. Number of unfilled grains per panicle (nos)*

The number of unfilled grains per panicle were recorded in the individual plants and expressed in numbers.

3.3.8.2.4. Spikelet fertility percentage (%)

Five panicles of A line from each plot were selected randomly and the spikelet fertility percentage was calculated and expressed as percentage.

Spikelet fertility percentage $= \frac{\text{Number of filled grains}}{\text{Total number of florets}} \times 100$

3.3.8.2.5. Seed yield per plot (g)

The grains were harvested from each plot separately and dried under sun to a moisture content of 13 per cent and the weight was recorded and expressed kilogram.

3.3.9. Statistical Analysis

The data obtained was analyzed using analysis of variance (ANOVA) technique in GRAPES software.

RESULTS

4. RESULTS

The present investigation entitled "Standardization of hybrid rice seed production using three line system of breeding for Kerala" was carried out to standardize the GA_3, brassinosteroid concentration and to optimize planting ratio in restorer line and male sterile line to get maximum hybrid seed yield under Kerala condition. The first experiment was conducted under polyhouse condition at College of Agriculture, Vellayani during December 2019 – March 2020. Second experiment was raised under rainfed low land condition at IFSRS, Karamana during March – July, 2020. Various traits were studied at vegetative and reproductive stages and respective observations were recorded in both the experiments. The results obtained are presented with suitable plates and tables separately for both the experiments in this chapter.

4.1 EXPERIMENT I - PHENOLOGICAL STUDIES IN MALE STERILE LINE (A-LINE).

The study on phenology of CRMS31 A consist of several parameters under the following subheadings viz.,

4.1.1 Morphological characters of CRMS 31 A at vegetative stage
4.1.2 Floral characters of CRMS 31A

4.1.1 Morphological characters of CRMS 31 A at vegetative stage

The five morphological characters of 10 CRMS 31A were taken and is given in table 2.

4.1.1.1 Tillering stage

Tillering stage started twenty two days after transplanting. The basal dose and the first split dose of fertilizer had triggered the speed of vegetative growth. Tillering stage initiated with the appearance of the first tiller from the axillary bud from one of the lowermost nodes. The male sterile seed parents showed less response to fertilizer compared to KAU variety (male parent-Remya) hence the number of tillers were less in male sterile seed parent than that of KAU variety.

4.1.1.2 Plant height at maturity (cm)

The plant height of male sterile seed parent (CRMS 31 A) was almost similar to that of male pollen parent (Remya).The average height of the CRMS 31 A was 123.23 cm.

4.1.1.3 Total number of tiller and productive tiller

The male sterile parent significantly scored more productive tillers compared to male parent i.e., 18.10 for CRMS 31 A and nine for Remya. The difference between total number of tiller and productive tiller was minimum for CRMS 31 A and few tillers had more than one panicle, the secondary and tertiary tillers were also productive with small panicles.

4.1.1.4 Total number of leaves

The total number of leaves produced depends on the number of tillers and number of leaves per tiller. Then numbers of leaves produced by the main culm of CRMS 31 A was recorded and the average numbers of leaves was 91.0.

4.1.1.5 Flag leaf area

The average flag leaf area of observation plants was 38.97.

Table 2: Morphological traits of the male sterile line-CRMS 31A

Variables	**Mean**	**SD**	**CV**	**Range**
Tillering stage	23.30	1.34	0.06	21.00-25.00
Plant height at maturity (cm)	123.23	1.34	0.05	116.20-136.70
Total number of tiller	18.10	1.45	0.08	16.00-20.00
Total number of leaves	91.00	6.55	0.07	81.00-101.00
Flag leaf area (cm^2)	38.97	3.27	0.08	34.44-44.08

4.1.2 Floral characters of CRMS 31A

The five floral characters of 10 CRMS 31A were taken and recorded in Table 3.

4.1.2.1 Days to panicle primordial initiation

Initiation of the panicle was observed on 58.90 days old plants. The panicles developed between internodes of the first stem. The panicle initiation was associated with the beginning of stem internodes formation and the developing panicle was microscopic in size (2-4 mm) with a fuzzed tip inside the main culm (Plate 5 and 6). Later those panicles were developed completely inside and visible as minute panicle. The boot stage initiated with the development of panicle primordial inside the culm.

4.1.2.2 Days to booting

The increase in size of the panicle primordia and its upward extension inside the leaf sheath were detected inside the rapidly elongated culm (Plate.7). The booting stage started at an age of 66.20 days.

4.1.2.3 Days to panicle emergence

The first panicle emerged on 74.10 days old plant and fifty percentages of panicles emerged within a week (Plate.9).

4.1.2.4 Flag leaf angle (degree)

The average flag leaf angle of CRMS 31 A was 15.60^0 which was very less when compared to traditional varieties.

4.1.2.5 Days to anthesis

The CRMS 31 A took 85.50 days to anthesis. That had taken 9 days to finish the anthesis of whole spikelets on panicle. Microscopic view of a male sterile spikelet is shown in Plate 8.

Table 3. Flowering related traits of male sterile plant-CRMS 31A

Variables	**Mean**	**SD**	**CV**	**Range**
Days to panicle primordial initiation	58.90	2.13	0.04	56.00-63.00
Days to booting	66.20	4.16	0.06	55.00-70.00
Days to panicle emergence	74.10	1.79	0.02	72.00-77.00
Flag leaf angle (°)	15.60	2.63	0.17	12.00-20.00
Days to anthesis	85.50	2.27	0.03	83.00-89.00

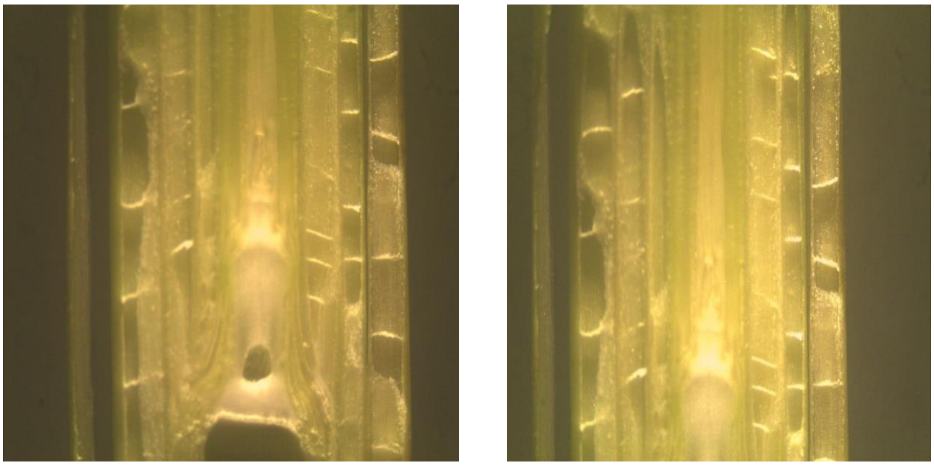

Plate 5 : Microscopic view of panicle initiation

Plate 6: Initiation and development of panicle primordial, in 58 days' old main tillers

Plate 7: Panicle emergence, heading of panicle from the boot stage

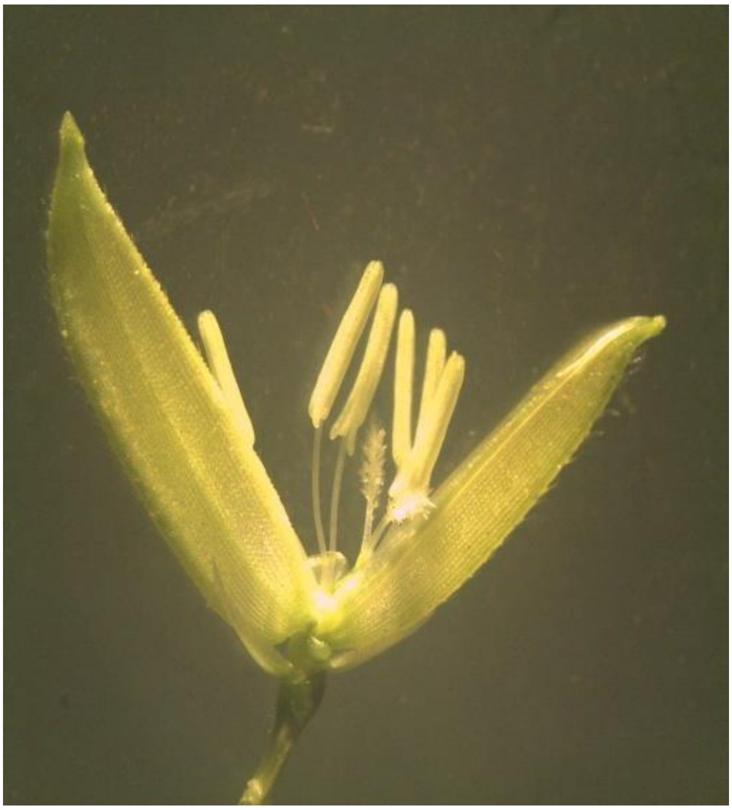

Plate 8: Microscopic view of a male sterile spikelet

4.2 STANDARDIZATION OF THE DOSE OF GA_3 AND BRASSINOSTEROID FOR PLANTING RATIO OF A AND R LINE IN THE RATIO OF 6:2 AND 8:2

4.2.1 Morphological parameters

The observations on seven morphological parameters were recorded at different growth stage and depicted in Table 4. The data on morphological parameters in the seven treatments in Fig.1.

4.2.1.1 Leaf angle (˚)

The measure of leaf angle varied from 15.267˚ to 26.600˚. The treatment T_6 recorded maximum leaf angle (26.600˚) which is on par with T_5 (26.400˚). The minimum was recorded for the control (6:2 planting ratio) treatment T_7 (15.267˚) it was on par with control (8:2 planting ratio) treatment T_4 (15.467˚).

4.2.1.2 Panicle exsertion (cm)

The panicle exsertion was more in the treatment T_5 (8.707 cm) and it was low in both the control plants T_4 (-16.447 cm) and T_7 (-14.460 cm).

4.2.1.3 Plant height (cm)

Each treatment showed varied plant height ranging from 111.007 cm to 145.653 cm. The highest plant height was recorded for the treatment T_5 (145.653 cm) and the control plant T_4 showed lowest (111.07 cm) plant height.

4.2.1.4 Productive tillers

The treatment T_6 recorded maximum (25.00) productive tillers and the control plant T_4 recorded minimum number (11.00) of productive tillers.

4.2.1.5 Total tillers

The total tillers had the range from 18.00 to 27.00. The maximum tillers were produced in the treatment T_6 (27.00) and the minimum tillers were produced in the control plants T_4 (18.00).

Table 4: Morphological parameters recorded in the different treatments

Treatments	Leaf angle (^{0})	Panicle exsertion (cm)	Plant height (cm)	Productive tiller	Total tiller	No. of leaves	Flag leaf area (Cm^2)
T1	17.100	4.520	116.600	16.00	20.00	100.00	59.460
T2	17.600	5.473	117.093	17.00	21.00	106.00	55.143
T3	18.733	5.627	117.433	18.00	23.00	115.00	42.767
T4	15.467	-16.447	111.007	11.00	18.00	88.000	35.683
T5	26.400	8.707	145.653	22.00	25.00	125.00	64.747
T6	26.600	8.233	144.160	25.00	27.00	135.00	60.307
T7	15.267	-14.460	112.253	13.00	20.00	101.00	45.650
Mean	19.595	0.236	123.457	17.42	22.00	110.00	51.965
CD (0.05):	1.410	0.647	2.9360	1.153	2.188	10.939	9.2310
SE±(m):	0.457	0.210	0.9530	0.374	0.710	3.5500	2.9960
CV	4.043	153.99	1.3380	3.728	5.590	5.5900	9.9850

Table 5: Yield parameters recorded in different treatments

Treatments	1000 grain weight (g)	Number of filled grains per panicle	Number of unfilled grains per panicle	Spikelet fertility percentage (%)	Seed yield per plot (g)
T1	14.963	22.00	169.00	11.653	647.299
T2	17.200	17.00	179.00	9.250	714.981
T3	19.400	28.00	175.00	16.140	961.779
T4	13.733	13.00	182.00	7.127	350.182
T5	17.267	33.00	170.00	19.507	1,355.74
T6	16.500	24.00	178.00	13.787	1,489.32
T7	13.900	13.00	183.00	7.397	316.757
Mean	16.137	21.428	176.571	12.123	833.723
CD (0.05):	2.625	3.162	N/A	2.100	153.290
SE±(m):	0.852	1.026	6.801	0.682	49.7420
CV	9.656	8.250	6.676	9.738	9.7870

4.2.1.6 Number of leaves

The treatment T_6 produced maximum number (135) of leaves and the treatment T_4 produced least number of leaves (88.00).

4.2.1.7 Flag leaf area (cm^2)

The measure of flag leaf area was varied in each treatment from 35.683 cm^2 to 64.747 cm^2. The treatment T_5 (64.747 cm^2) showed highest flag leaf area where as, the control treatment T_4 (35.683 cm^2) recorded the lowest flag leaf area.

4.2.2 Yield parameters

The five yield parameters recorded are given in Table 5. The yield parameters recorded in the five treatments is compared in Fig.2.

4.2.2.1 1000 grain weight (g)

The highest 1000 grain weight was observed in the treatment T_3 (19.400g) and the lowest was recorded in the treatment T_4 (13.733g). The control plants under 8:2 planting ratio (T_4) was on par with the treatment T_7 (13.900).

4.2.2.2 Number of filled grains per panicle

The treatment T_5 produced more number of filled grains per panicle (33.00) as compared to the control plants T_4 (13.000nos) and T_7 (13.400).

4.2.2.3 Number of unfilled grains per panicle

The least number of unfilled grains per panicle was observed in the treatment T_1 (169.00) which is on par with the treatments T_5 (170.00) and T_3 (175.00). The control treatment T_7 recorded highest number of unfilled grains per panicle and it was on par with the treatments T_4 (182.00) and T_6 (178.00).

4.2.2.4 Spikelet fertility percentage (%)

Spikelet fertility percentage was found to be maximum in the treatment T_5 (19.507%) and the minimum was in the control treatment T_4 (7.127%). The treatment T_4 was on par with the control T_7 (7.397%).

4.2.2.5 Seed yield per plot (g)

The seed yield per plot was highest for the treatment T_6 (1489.320 g) followed by T_5 (1355.746 g) and lowest for the control treatments T_7 (316.757 g) and T_4 (350.182 g).

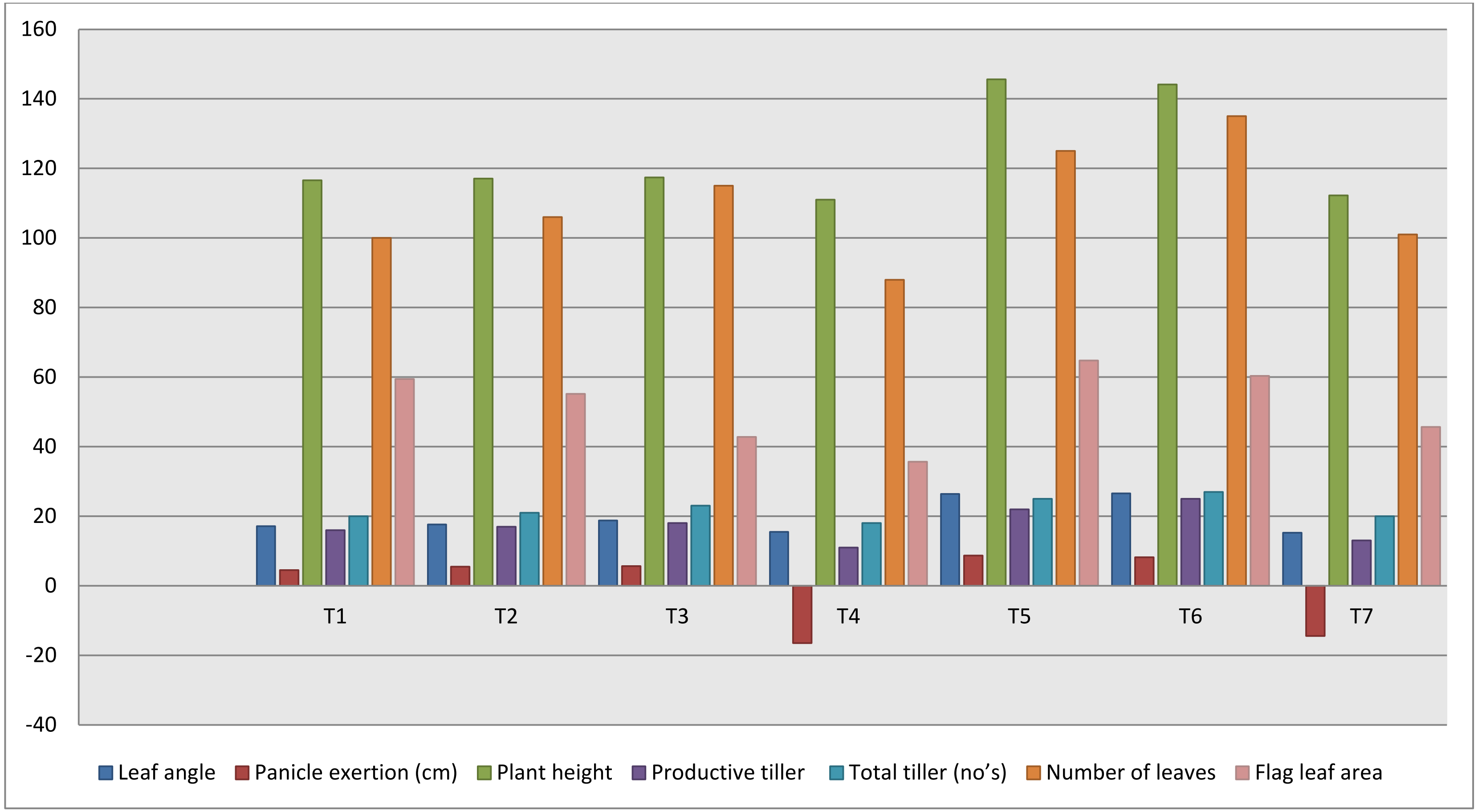

Fig. 1 Comparison of morphological traits recorded in different treatments

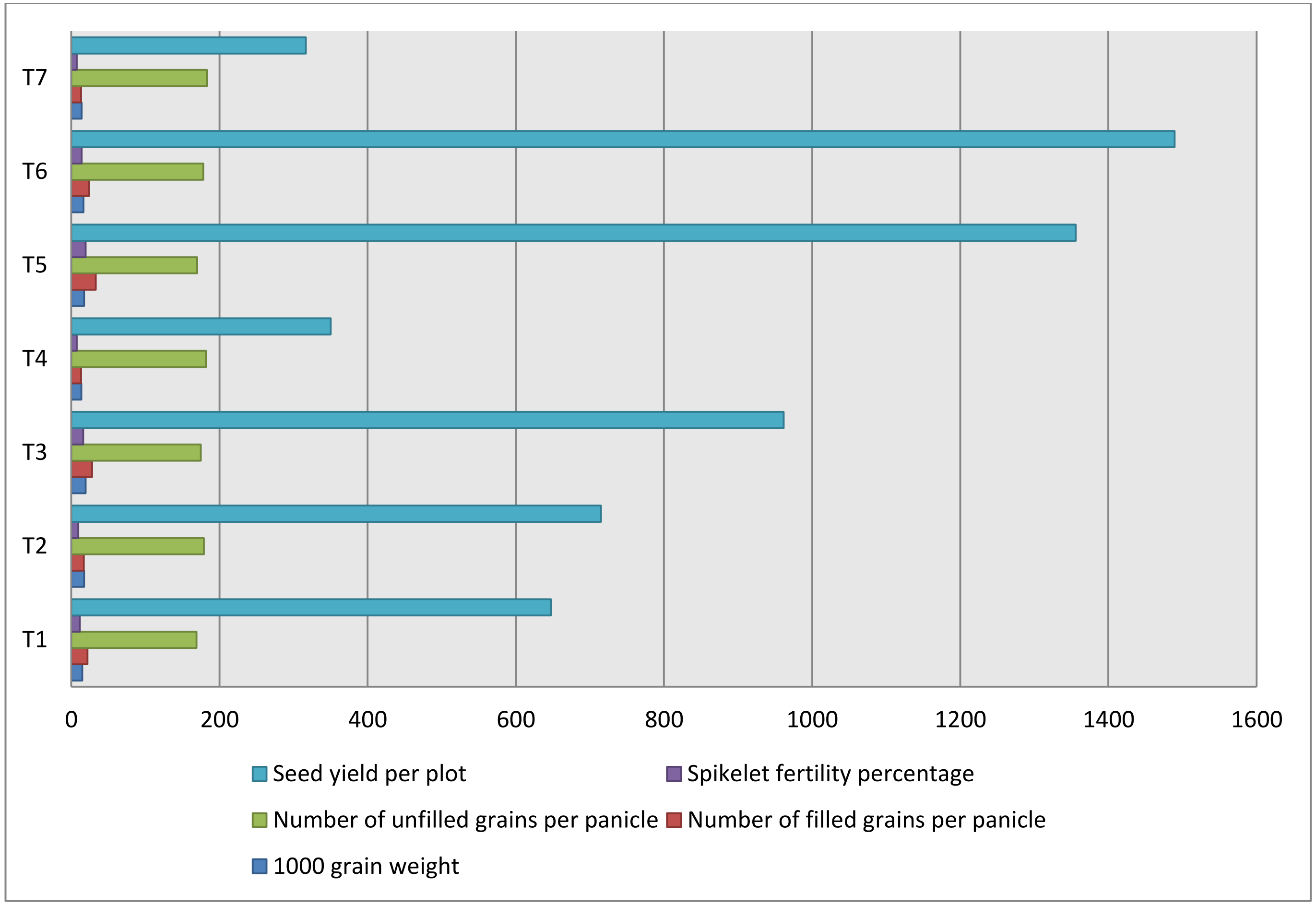

Fig 2. Comparison of yield traits recorded in different treatments

DISCUSSION

5. DISCUSSION

Rice (*Oryza sativa* L.) is the major food grain crop and staple food of Kerala. The requirement of the growing population can only be met through two ways: expanding the rice planting area and increased production, or both. But in the future, expansion will be more difficult and expensive due to the population growth and a substantial improvement can be done through the adoption of hybrid rice (Nguyen 2010). So, the development of hybrid rice is of prime importance in future existence. The hybrid production and hybrid seed production are difficult process hence in order to develop and produce F_1 rice seeds an effective male sterile system must be needed. The advancement and utilization of varieties of hybrid rice on a large scale is done by exploiting cytoplasmic male sterility- fertility restoration. In India more than fifty rice hybrids have been released during the past two decades by utilizing three-line system of breeding. In Kerala, so far, no hybrid rice has been released; the major reason is the non-availability of a hybrid combination with more than 15% yield advantage over the commercially accepted varieties. To attain a promising level of area and production of hybrid rice, the following problems need to be solved: An inadequate and sustainable supply of commercial cytoplasmic male sterile (CMS) and restorer lines, no synchronization of parental lines, low out crossing rate, lack of proper facilities for pilot seed production for conducting on-farm trials, and front-line demonstrations in farmers' fields are the main hurdles in hybrid seed production in Kerala. Seed production research should consider, increasing the outcrossing potential of CMS lines and seed yield per unit area. Seed production technology should be improved to attain higher and higher seed yields. Ponnuswamy *et al.* (1998) carried out research on application of GA_3 and found that there is an increase in positive effect on morphological and yield parameters. Gibberellic acid (GA_3) is the costliest input in seed production in India, use of this, increases the cost of production and seeds. To overcome this problem, it is necessary to determine appropriate doses of GA_3, brassinosteroid and optimization of row ratio for a promising hybrid rice combination for its commercial feasibility during seed production.

So, in view of the importance and demand for hybrids with good yield and grain quality, this investigation was carried out to Standardize $GA_{3,}$ brassinosteroid and optimisation of planting ratio in restorer line (R) and male sterile line (A) to get maximum hybrid seed yield under Kerala condition.

The present investigation was conducted in two experiments

1. Phenological studies in male sterile line (A line).
2. Standardization of the dose of GA_3 and brassinosteroid for the planting ratio of A, R line in the ratio of 6:2 and 8:2.

The observations and results from the experiments are discussed in subheads below.

5.1 PHENOLOGICAL STUDIES IN MALE STERILE LINE (A LINE).

Phenological studies were carried out during March to July 2020 (*Virippu,* 2020) in CRMS31A the male sterile parent of the hybrid. CRMS31A is the male sterile line developed by CRRI Cuttack. The objective of this experiment was to study the expression of different phonological traits of CRMS31A under Kerala conditions. Different observations of male sterile line CRMS 31 A were recorded which include days to panicle primordial initiation, days to booting, days to panicle emergence, flag leaf angle and days to anthesis. The phenological studies were done on pot plants of CRMS 31 A along with staggered sown pollen parent 'Remya'. Synchronized flowering was obtained for male parent which was sown as three sets first three days before CMS line, second along with CMS line and finally last set was sown three days after the CMS. Virmani (1987) had stressed the necessity for staggered sowing of the male parents in order to get synchronization of flowering for effective seed production in his manual of Hybrid Rice Breeding Technology.

The phenology was studied to perceive various growth and development stages in the CMS line. Days to panicle primordial initiation, days to booting, days to panicle emergence were noted and was found to be similar to the pollen parent but flag leaf angle and panicle exsertion were very less, these two results reduced outcrossing rate. It's the major constraint in hybrid rice seed production. That can be managed by use of gibberellic acid brassinosteroid and standardized planting ratio. In the second experiment after the application of GA_3 and brassinosteroids the flag leaf angle and the panicle exsertion were found to be increased when compared to that of the first experiment. So, it is necessary to determine appropriate doses of GA_3, brassinosteroid and row ratio for a promising hybrid rice combination for its commercial feasibility during seed production.

5.2. STANDARDIZATION OF THE DOSE OF GA_3 AND BRS FOR THE PLANTING RATIO OF A, R LINE IN THE RATIO OF 6:2 AND 8:2

Hybrid rice seed production was carried out during March to July 2020. The experiment could standardize the concentration of GA_3, brassinosteroid and optimize the plating ratio. Parental lines were planted with different planting ratio and evaluated in Randomized Block Design (RBD) with three replications. Observation on seven morphological traits and five yield contributing traits were recorded and the mean performance of hormones and planting ratio on hybrid seed production was evaluated.

Observation regarding planting ratio showed a positive effect on out crossing, seed set, spikelet fertility percentage and the finding were in close agreement to Ramos *et al.* (2004) who conducted a study to determine the optimum row ratio for male and female parents in hybrid rice and reported that 6:2 and 8:2 ratios were the best performers in all the three seasons. Sushmita *et al.* (2019) reported that planting ratio between seed parent and pollen parent has a profound effect of hybrid seed setting and yield. In PRH 10 hybrid, 2:6 planting ratio resulted in significantly higher seed setting percentage and 6:2 planting ratio resulted in the highest seed yield which is in agreement with our present study where 6:2 planting ratio (T_5) had higher spikelet fertility percentage and yield was higher with 8:2 planting ratio (T_6).

Kumar *et al.* (2010) carried out an experiment in wheat and found that exogenous application of GA_3 increased the height of the plant and this increase was more profound with the increased concentration of GA_3 and it was also higher when compared with that of the control. In another study in rice different GA_3 concentrations increased the height of the plant and it was found the greater the dosage of GA_3 higher is the difference in plant height among the treatments (Gavino *et al.*, 2008). Our present findings were in line with the earlier literature which showed that a higher concentration of GA_3 (70ppm) resulted in greater increase in plant height when compared with low dosage of GA_3 (50ppm) and with high concentration of GA_3 greater difference in plant height persisted among treatments. This increase in plant height is due to promotion of vegetative growth by GA_3 through active cell division, cell enlargement and cell elongation and thus helped in improving growth characteristics and also facilitated reproductive growth (Pareek *et al.*, 2000). Wada *et al.* (1984) stated that application of some of the brassinosteroids such as Stigmosterols in plants enhanced the growth parameters of the plants such as height of the plants. This study supported our present findings which showed that plant height of rice increased in the BRs treated plants.

The increase in yield with the application of plant growth regulators might be due to increased yield attributes, which in turn resulted from effective translocation of photosynthates. Plant growth hormones also increase the mobilization of reserve food materials to the developing sinks through increasing hydrolyzing and oxidizing enzyme activities and lead to yield increase. Liu *et al.* (2012) from his experiment in rice stated that with the application of $GA_{3,}$ the growth of productive tillers was promoted at early and late growth stages through inhibition of unproductive tillers and production of heavy panicles was increased thus resulting in increased yield. Goufo *et al.* (2011) conducted a study in two aromatic rice cultivars namely Guixiangzhan and Peizaruanxiang and found that with the application of GA_3 there is a significant increase in yield of the grain at both early and late growing seasons when compared with that of the control. These studies were similar to our present study which showed an increased grain yield with increased dosage of $GA_{3.}$

Morinaka *et al.*, (2006) observed that brassinosteroids play an important role in controlling seed size and weight, when applied externally in rice and it tends to increase seed yield per plant. This findings supported our present results which reported that higher yield was recorded with the treated plants and with increased concentration of BR the yield also increased and was highest (T_3) when compared with remaining treatments (T_1 and T_2) . An extensive study was conducted at Karnal to assess the effect of 12 row ratio combinations and found that row ratio 6:2 gave highest yield followed by 8:2 (NSP, 1994). Our present study was in agreement with earlier findings and it revealed that yield was highest at a planting ratio of 6:2.

In the present study the number of filled grains per panicle increased with higher dose of GA_3 and BRs when compared to lower dosage and also with control. The highest number of filled grains were recorded in the treatment T_5 (Planting ratio-2:6, GA_3-70 ppm, BRs-2 ppm) and these results are in agreement with the later findings. Application of GA_3 in hybrid rice seed production leads to increase in the percentage seed set which is a result of increase in number of filled grains per panicle (Devi *et al.,* 2010). Chenniappan *et al.* (2004) reported that foliar application of GA_3 in rice resulted in increased number of filled grains. Vardhini and Rao (2005) conducted an experiment on the influence of brassinosteroids on germination, growth and yield parameter of sorghum and reported that brassinosteroids are highly effective in increasing the number of filled grains by inhibiting the number of unfilled grains.

Incomplete panicle exsertion is a drawback with CMS line. Due to incomplete panicle exsertion 10-15 % spikelets are enclosed in the flag leaf and are not available for outcrossing (Ramesha *et al*., 1998). Gavino *et al.* (2008) observed that in rice, foliar application of GA_3 caused an increase in plant height, enhanced the exsertion of panicle and stigma resulting in increased seed set percentage and seed yield. This increase in plant height is due to GA_3 which is the effective and efficient plant growth regulator that is involved in stimulating cell elongation which further result in enhanced panicle exsertion in CMS lines (Tiwari, 2002). In a pot culture experiment two forms of brassinosteroids BL (Brassinolide) and HBL (Homobrassinolide) at 5ppm and 1ppm respectively were used to study the effect of panicle exsertion and found that the panicle exsertion was improved resulting in enhanced cross pollination leading to higher seed set (Vivency, 1998). These earlier reports support our present results which recorded greater exsertion of panicles in GA_3 and BR treated plants with the highest panicle exsertion recorded in T_5 (Planting ratio-6:2, GA_3-70 ppm, BR-2 ppm) treatment followed by T_6 (Planting ratio-2:8, GA_3-70 ppm, BR-2 ppm) treatment.

Present investigation in rice revealed that the exogenous application of GA_3 and BRs resulted in increased number of productive tillers with the greater number of productive tillers recorded in the treatments T_6 (Planting ratio-8:2, GA_3-70 ppm, BRs-2 ppm) followed by T_5 (Planting ratio-6:2, GA_3-70 ppm, BRs-2 ppm) and this findings are in line with the later studies. Since the productive tillers has direct contribution towards seed yield, the increase in yield was expressed through this character. Vasudevan *et al*. (2008) stated that application of GA_3 in rice resulted in increase in the number of productive tillers that cause an increase in seed yield. Wu *et al.* (2008) evaluated the effect of brassinosteroid application in rice plants and found that the treated plants showed increase in productive tillers per plant as compared to the control plants. This increase in productive tillers per plant might be attributed `to synergistic action of brassinosteroid with indigenous auxin in cell elongation and cell proliferation of meristematic tissue (Sairam, 1994).

Elankavi *et al.* (2009) proposed that exogenous application of GA_3 in rice caused an increase in the number of tillers. Application of GA_3 in rice produced seedlings with vigorous growth and caused two times higher seedling emergence as compared to control and it also showed an increase in number of tillers in the treated plants (Takahashi, 1976). In rice even though the branches develop from the axils on the main stem and the tillers develop from bases of the culms, application of brassinosteroids exogenously, results in increased number of tillers number (Doust, 2007). All these above literatures supported our recent results which recorded the greater number of tillers with the application of GA_3 and Brassinosteroids. Treatment T_6 (Planting ratio-8:2, GA_3-70 ppm, BRs-2 ppm) followed by T_5 (Planting ratio-6:2, GA_3-70 ppm, BRs-2 ppm) recorded highest number of total tillers.

Emongor (2007) carried out a field experiment in two cowpea cultivars namely Blackeye and Tswana to evaluate the effect of GA_3 on growth and development and reported that exogenous application of GA_3 increased the 1000 seed weight causing an increase in seed yield**.** Brassinosteroids play a vital role in regulating grain filling in rice, therefore application of brassinosteroids exogenously result in increase in 1000 seed weight (Chuan *et al.,* 2008). Highest 1000 grain weight was recorded by T_3 (Planting ratio-6:2, GA_3-50 ppm, BRs-6 ppm) treatment in this study and it was in line with earlier literature.

In rice spikelet fertility percentage is most crucial in hybrid breeding programme and it is closely associated with high yield per plant. Spikelet fertility percentage recorded highest with T_5 that is with higher concentration of GA_3 (70 ppm) and this results were supported by previous studies. Tiwari *et al.*, 2011 and Tian and Zhou (1991) conducted an experiment in rice hybrid by applying GA_3 exogenously and reported that GA_3 greatly influenced the panicle and spikelet exsertion which further improved the spikelet fertility percentage resulting in increased seed set percentage. This increase in number of spikelets per panicle with GA_3 could be due to role of gibberellic acid in flower induction, flower and seed development (Davies, 2005; Fleet and Sun, 2005). The action of BRs resulted a positive effect on the spikelet fertility percentage in treatments, the higher effect was with T_5 (2ppm BRs and GA_3 70 ppm with 6:2 planting ratio) but the spikelet fertility percentage for other treatments registered a lower value. The similar result was reported by Thussagunpanit *et al*. (2015). They investigated the effect of exogenously applied brassinosteroid and found that spikelet fertility percentage was increased.

Action of GA_3 and BRs yielded highest flag leaf area with T_5(2ppm BRs and GA_3 70 ppm with 6:2 planting ratio) treatment and these results were confirmed with investigations carried out by different scientists. Ibrahim *et al.* (2007) exogenously applied GA_3 in *Vicia faba* and the results showed an increase in flag leaf area when compared with that of control which is due to role of GA_3 in promoting cell division and cell elongation. Sairam (1994) reported that application of Homobrassinolide in wheat resulted in increase in the flag leaf area. Wada *et al.* (1984) found that application of brassinosteroids resulted in significant increase in the vegetative growth characters of yellow maize plants and also increased the physiological parameters such as flag leaf area which is due to cell elongation, source sink relation and photosynthetic activity.

Foliar spray of GA_3 in soybean resulted in increased number of leaves as reported by Kalyankar *et al.* (2008). Niknejhad and Pirdashti (2012) reported that external application of GA_3 significantly increased the leaf number in rice. Pipattanawong *et al.* (1996) recorded the effect of brassinosteroids on vegetative and reproductive growth in two-day neutral plants and reported that the number of leaves in treated plants were increased by 110-140% over the untreated plants. Earlier investigations confirmed the present findings which state that number of leaves increased with the application of GA_3 and BRs and the highest number of leaves were recorded with the treatment T_6 (Planting ratio-8:2, GA_3-70 ppm, BRs-2 ppm).

Leaf angle increased with the higher dosage of GA_3 and the greater value was recorded in the treatment T_6 (Planting ratio-8:2, GA_3-70 ppm, BRs-2 ppm). Hamamura (1981) conducted a trial in rice by applying GA_3 and found that application increased the leaf angle and with the increase in concentration of GA_3 the leaf angle also showed a greater value. Hong *et al.* (2002) studied BRs dose and the effect on rice seedlings and found that BRs dose-dependent increase in the degree of bending of the lamina joint, i.e., the higher the concentration of BRs added, the bending will be greater. The use of 2 and 6 ppm had a significant effect on leaf angle, the concentration also affecting on the leaf angle and observed erect leaf orientation. While comparing with observations of control plot, BRs showed much positive effect on the rice plants and this finding in the present study is supported by earlier literature.

Standardization of hybrid seed production technique suitable to a particular area is the prerequisite for the release of hybrid rice in that particular area. In Kerala so far no hybrid variety has been released. One major constraint is the lack of economic seed production package. In this study an attempt is made to derive a package for economic hybrid rice seed production for Kerala. The highest per pot yield was reported for treatment T6 (1599.503g/plot) on par with treatment T5 (1465.127g/plot). The plot size being $9m^2$ the per hectare yield can be projected as 1.77tons/ha and 1.66 tons/ha respectively. Viramani, 1966 had reported that the average seed production in the three line system of hybrid seed production in rice is 2ton/ha.

Weather parameters prevailing in the area of seed production highly influences the seed yield in the three line system of seed production. Temperature humidity and prevailing wind velocity highly influences the transfer of pollen from the restorer line to the malesterile line. Viramani, 1996 had reported that highest seed yield was obtained when seed and pollen parents flowered when RH was 50-60% max. Temp was 28-30 ^{0}C and min temp. 21-22^{0}C and wind velocity was above 2.5m/s. The weather data during the period of flowering and pollination in this study is given in the Table .6. The data shows that the average humidity in the morning is 80% and wind velocity during the period is 3.3km/hr *ie.,* 0.88m/s. High humidity hinders the free pollen flow and the low wind velocity do not help in the free transfer of pollen to the male sterile line. Above all there was intermittent rain also during the flowering period. Ponnuswamy *et al*. (1998) had reported that during the period of flowering there should not be continuous rain, which may cause poor seed setting due to washing of pollen, sufficient sunshine with wind velocity 2-3m per sec favours high seed yield. RH within a range of 70-80% prevent desiccation of stigma and favours opening of spikelets for longer period and higher RH favours high percentage of seed set and seed yield. So seed yield can be increased by changing the seed production period to a more favourable season.

In this study the doze of Brassino steroids 2 ppm and GA 70 ppm in both planting ratios of 6:2 and 8:2 had given substantially higher yield over the control. So hybrid rice seed production in Kerala can be enhanced by spraying of GA_3 70ppm and brassinosteroid 2ppm at 15-20% heading of tillers and 35-40% panicle emergence. The row ratio of the female parent to the restorer parent can be 8:2 considering the increased seed yield realized. More seasons and locations have to be tested to identify the best season and location for hybrid rice seed production in Kerala.

Table.6.Weather data recorded during flowering period

Date	Temperature (°C)		RH (%)		Wind Vel. (km/h)	Rain (mm)
	Max.	Min.	Morning	Afternoon		
01-May	34.3	25.8	85	58	3.0	0.0
02-May	35.1	26.2	79	61	5	0.0
03-May	34.3	26.3	82	66	4	18.0
04-May	33.6	26.2	86	66	2	19.4
05-May	33.8	26.8	85	65	3	0.0
06-May	34.2	26.0	86	65	2.0	5.6
07-May	34.3	26.5	83	74	5.0	0.0
08-May	32.8	25.1	90	73	4.0	27.6
09-May	32.8	25.8	90	77	3	13.2
10-May	32.7	25.6	92	71	4	7.0
11-May	32.6	25.1	93	88	1	47.3
12-May	31.7	25.8	93	85	0	0.0
13-May	31.6	25.7	95	83	2.0	10.6
14-May	31.8	26.2	87	77	3.0	0.0
15-May	32.6	25.2	95	77	7.0	31.4
Average	**33.2**	**25.86**	**88.0**	**72.4**	**3.2**	**12**

SUMMARY

5. SUMMARY

The study entitled "Standardization of hybrid rice seed production using three line system of breeding for Kerala" was carried out to standardize the GA_3, brassinosteroid concentration and to optimize planting ratio in restorer line and male sterile line to get maximum hybrid seed yield under Kerala condition. The first experiment was the phenological studies in male sterile line (A-line) and it was conducted under polyhouse condition at College of Agriculture, Vellayani during December 2019 – March 2020. Second experiment was the standardization of the dose of GA_3 and brassinosteroid for planting ratio of A, R line in the ratio of 6:2 and 8:2 which was conducted under rainfed low land condition at IFSRS, Karamana during March – July, 2020. The experiment was conducted in Randomized Block Design (RBD) with three replications. The parental lines were sown in staggered dates according to the duration of the CMS line and the restorer line. The sowing of male and female parents were planned to achieve the synchronization in flowering. The R line was sown in three staggered dates. i.e., one at three days before the sowing date of CMS lines, the second set along with CMS line and the third set were sown three days after the sowing of CMS lines. The sterile R line and pollen parent were sown in the planting ratio 6:2 with GA_3 and brassinosteroids in different concentrations (50 ppm GA_3 +2 ppm brassinosteroid, 50ppm GA_3 + 6ppm brassinosteroid, and 70 ppm GA_3 + 2 ppm brassinosteroid) and in 8:2 planting ratio with 50ppm and 70 ppm of GA3 along with 2 ppm brassinosteroid were sprayed at 15-20% heading and 35-40% panicle emergence. Two control treatments without chemical spraying were maintained at 6:2 and 8:2 planting ratio. Various traits were studied at vegetative and reproductive stages and respective observations were recorded in both the experiments. The salient findings of this study are summarized below.

The phenological study of CRMS 31 A consisted of several parameters viz., morphological characters of CRMS 31 A at vegetative stage and floral characters. The tillering of CRMS 31 A started at 23.3 days after transplanting. The number of tillers was less in male sterile seed parent than KAU variety (male parent-Remya) since it showed less response to fertilizer compared to KAU variety. The plant reached a height of 123.33 cm at maturity and produced 18.1 number of total tillers which was more compared to the KAU variety. The sterile plant produced total of 91 leaves and the flag leaf had 39.01 cm^2 area.

The panicle initiation was observed on 58.9 days old plants and panicle initiation was associated with the beginning of stem internodes formation and the developing panicle was microscopic in size (2-4 mm) with a fuzzed tip inside the main culm. The booting stage was started at an age of 67.2 days and the first panicle was emerged on 74.1 days old plant and fifty percentages of panicles were emerged within a week. The average flag leaf angle of CRMS 31 A was 15.6^0 which was very less when compared to traditional varieties and flag leaf angle play a vital role in the percentage of seed filling (fertility percentage) by acting as a barrier between pollen from the male parent and the CMS plants spikelets. The CRMS 31 A started anthesis at 85.5 days and the whole spikelets anthesis of a panicle was completed within 9 days.

In the second experiment better performance of CRMS 31 A with respect to morphological and yield parameters were observed with 70ppm GA_3. The highest plant height (145.653cm) panicle exsertion (8.707cm) and flag leaf area (64.747 cm^2) was recorded for the treatment T_5 (GA_3-70 ppm, BRs-2ppm with 6:2 planting ratio) followed by T_6 (GA_3-70 ppm, BRs-2ppm with 8:2 planting ratio) with plant height (144.160), panicle exsertion (8.233 cm) and flag leaf area (60.307 cm^2). The control plots of 8:2 planting ratio (T_4) and 6:2 planting ratio (T_7) recorded lowest values of panicle exsertion (-16.447 and -14.460 respectively) and T4 recorded lowest values of plant height (111.007 cm) and flag leaf area (35.683 cm^2).

The treatment T_6 (GA_3-70 ppm, BRs-2ppm with 8:2 planting ratio) produced highest number of total tillers (27.00) and maximum productive tillers (25.00). The minimum number of productive tillers (11.00) and total tillers (18.00) were produced in the control treatment T_4 (Control of 8:2 planting ratio) the treatment T_6 produced maximum number of leaves (135) and the treatment T_4 produced least number of leaves (88.00). The highest flag leaf angle (26.600˚) was recorded in T6 and it was on par with T_5 (Planting ratio of 6:2 with GA_3 70ppm and BRs 2ppm) with flag leaf angle 26.400˚. The minimum leaf angle was recorded for the control (6:2 planting ratio) treatment T_7 (15.267˚) and it was on par with control (8:2 planting ratio) treatment T_4 (15.467˚).

The highest 1000 grain weight (19.400g) was recorded in the treatment T_3 (Planting ratio of 6:2 with GA_3 50ppm and BRs 6ppm) followed by T_5 (Planting ratio of 6:2 with GA_3 70ppm and BRs 2ppm) and T_2 (Planting ratio of 8:2 with GA_3 50ppm and BRs 2ppm) with 1000 grain weight 17.267g and 17.200g respectively. The lowest 1000 grain weight was recorded in the treatments T_4 (13.733g) and T_7 (13.900g). The highest number of filled grains per panicle (33.00) and lowest number of unfilled grains per panicle (13) were observed in T_5 (GA_3-70 ppm, BRs-2ppm with 6:2 planting ratio), T_4 and T_7. The control plants (T4 and T_7) recorded the highest number of unfilled grains per panicle. The spikelet fertility percentage was more in T_5 (19.507%), followed by T3 (16.140%), and less fertility percentage was observed in control plants T4 (7.127%) and T_7 (7.397%). Highest seed yield per plot (1489.320g) was recorded in T_6 (Planting ratio of 8:2 with GA_3 70ppm and BRs 2ppm) followed by T_5 (1355.746g) lowest seed yield was observed in control plants T_4 (350.182g) and T_7 (316.757g).

The present investigation for standardization of GA3 and brassinosteroid dosage at different row ratio in hybrid rice seed production revealed that the concentration of 70 ppm GA_3, 2 ppm brassinosteroid in 6: 2 planting ratio had highest morphological traits, spikelet fertility percentage, number of filled grains and lowest number of unfilled grains. Reduced yield per plot might be due to the decreased planting population in 6:2 ratio. The hormonal effect greatly influenced on the hybrid seed yield and panicle exsertion. GA_3 resulted higher seed set and seed yield by improving the panicle exsertion and flag leaf angle. The 50 ppm GA_3, 6ppm brassinosteroid in 6:2 planting ratio decreased flag leaf area, it might be due to the effect of BRs.

Lack of panicle exsertion and reduced seed filling are the main constraints in hybrid rice seed production in Kerala. The drawback of CMS line could be minimized by the application of hormones and improve seed set can be obtained. Humidity and the wind velocity prevailing in the location was not favorable for free flow of pollen and better seed set. So a more favorable season has to be tested for better yield.

In this study the doze of Brassinosteroids 2 ppm and GA 70 ppm in both planting ratios of 6:2 and 8:2 had given substantially higher yield over the control. So hybrid rice seed production in Kerala can be enhanced by spraying of GA_3 70ppm and brassinosteroid 2 ppm at 15-20% heading of tillers and 35-40% panicle emergence. The row ratio of the female parent to the restorer parent can be 8:2 considering the increased seed yield realized. More seasons and locations have to be tested to identify the best season and location for hybrid rice seed production in Kerala.

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STANDARDIZATION OF HYBRID RICE SEED PRODUCTION FOR KERALA USING THREE LINE SYSTEM OF BREEDING

By

ARUNKUMAR C

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Abstract of the thesis

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VELLAYANI, THIRUVANANTHAPURAM-695522

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ABSTRACT

The present study entitled "Standardization of hybrid rice seed production for Kerala using three line system of breeding" was carried out in the College of Agriculture, Vellayani and Integrated farming system research station (IFSRS), Karamana during 2019-2020, with an objective to standardize GA_3 and brassinosteroid and to optimize the planting ratio in restorer line (R line) and male sterile line (A line) to get maximum hybrid seed yield under Kerala condition. The study comprised of two experiments, the phenological studies in male sterile line (Aline) and standardization of GA_3 and brassinosteroid dose for the planting ratio of A, R line in 6:2 and 8:2.

Phenological studies of the CMS line CRMS31A were taken under protected condition during different growth stages. The production of tillers on male sterile line started at 23.3 days after sowing. At maturity, the plant height reached up to 123.33 cm length with 18.1 total numbers of tillers. The sterile plant produced total of 91 leaves and the flag leaf had 39.01 cm^2 area. The initiation of the panicle was observed on 58.9 days old plants and panicles were developed between internodes of the first stem. The panicle initiation was associated with the beginning of stem internodes formation and the initiated panicle was microscopic in size (2-4 mm) with a fuzzed tip inside the main culm. The increase in size of the panicle primordia and its upward extension inside the leaf sheath were detected inside the rapidly elongated culm. The booting stage was started at an age of 67.2 days then the first panicle was emerged on 74.1 days old plant and fifty percentages of panicles were emerged within a week. Flag leaf angle play a vital role in the percentage of seed filling (fertility percentage) by acting as a barrier between pollen from the male parent and the CMS plants spikelets. The average flag leaf angle of CRMS 31 A was 15.6^0 which was very less when compared to traditional varieties. The CRMS 31 A had taken 85.5 days to anthesis and taken 9 days to finish the anthesis of whole spikelets on panicle.

In the second experiment, field evaluation of hybrid seed production in the cross between CRMS 31A and the identified restorer 'Remya' was done in the fields of IFSRS Karamana for standardising the planting ratio and the doze of growth

hormones. This experiment was laid out in Randomized Block Design (RBD) with three replication during March-July 2020 (Virippu). The parental lines were sown in staggered dates according to the duration of the CMS line and the pollen Restorer line. The sowing of male and female parents were planned to achieve the synchronization in flowering. The R line was sown in three staggered dates. i.e., one at three days before the sowing date of CMS lines, the second set along with CMS line and the third set were sown three days after the sowing of CMS lines. The sterile R line and pollen parent were sown in the planting raio of 6:2 and 8:2 and two doses of GA_3 (50 and 70 ppm) and brassinosteroid (2 and 6ppm) were sprayed at 15-20% heading and 35-40% panicle emergence. Observations on eight morphological and five yield traits were recorded during the crop production.

All the traits used in the study showed significant variations. The mean performance of CRMS 31 A observed better performance in morphological and yield parameters at 70 ppm GA_3. The treatment T_5 (GA_3-70 ppm, BRs-2ppm with 6:2 planting ratio) recorded the highest panicle exsertion (8.707 cm), plant height (145.653 cm) and flag leaf area (64.747 cm^2) followed by T_6 (GA_3-70 ppm, BRs-2ppm with 8:2 planting ratio). The highest productive tiller (25.00), total tiller (27.00), number of leaves (135.00) and leaf angle (26.600º) were observed in T_6 (GA_3-70 ppm, BRs-2ppm with 8:2 planting ratio) and followed by T_5. The higher concentration of BRs reduced the flag leaf area (42.767) in T_3 (GA_3-50 ppm, BRs-6ppm with 6:2 planting ratio). The morphological traits of the male sterile line was improved with respect to panicle exsertion, flag leaf area, productive tillers and leaf area index by the application of GA_3 70ppm and BRs 2ppm.

The 1000 grain weight was higher in T_3 (19.400g) followed by T_5 (17.267g) and T_6 (16.500g) and the least was in control treatment T_4 (13.733g) and T_7 (13.900g). The highest number of filled grain per panicle (33.00 nos) and lowest number of unfilled grains per panicle (17 nos) were observed in T_5 with 70ppm GA_3. The highest number of unfilled grains per panicle was found in control plants (T_4 and T_7). The Spikelet fertility percentage was more in T_5 (19.507%), followed by T_3 (16.140%) and less fertility percentage was observed in control plants T_4 (7.127%) and T_7 (7.397%).

Highest seed yield per plot was recorded in T_6 (1489.320g) followed by T_5 (1355.746g) lowest seed yield was observed in control plants T_4 and T_7.

The drawback of CMS line could be minimized by the application of hormones and improve seed set can be obtained. Humidity and the wind velocity prevailing in the location was not favorable for free flow of pollen and better seed set. So a more favorable season has to be tested for better yield.

In this study the doze of Brassino steroids 2ppm and GA 70 ppm in both planting ratios of 6:2 and 8:2 had given substantially higher yield over the control. So hybrid rice seed production in Kerala can be enhanced by spraying of GA_3 70ppm and brassinosteroid 2ppm at 15-20% heading of tillers and 35-40% panicle emergence. The row ratio of the female parent to the restorer parent can be 8:2 considering the increased seed yield realized. More seasons and locations have to be tested to identify the best season and location for hybrid rice seed production in Kerala.