BREEDING FOR DWARFISM AND AROMA ENHANCEMENT IN BASMATI 370 RICE

FAITH WARUCU GATERE

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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award

Signature _____ Date _____

Faith Warucu Gatere
Department of Water and Agricultural Resource Management
A506/1432/2019

This thesis has been submitted for examination with our approval as the University Supervisors;

Signature _____ Date _____

Prof. Paul Njiruh Nthakanio
Department of Water and Agricultural Resource Management
University of Embu

Signature _____ Date _____

Prof. James Ireri Kanya
Department of Biology
University of Nairobi

Signature _____ Date _____

Prof. Esther Edith Arunga
Department of Water and Agricultural Resource Management
University of Embu

DEDICATION

To my beloved father, mother, sister and husband, whose unwavering support, love, and encouragement have been the cornerstone of my journey and success.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	- Analysis of Variance
BC	- Backcross
BS	- Basmati
EGMS	- Environment Genic Male Sterile
GA	- Gibberellic Acid
IBPGR	- International Board of Plant Genetic Resources
IRRI	- International Rice Research Institute
KEBS	- Kenya Bureau of Standards
MoA	- Ministry of Agriculture
PGMS	- Photoperiod Sensitive Male sterile
SSD	- Single Seed Descent
SSRs	- Simple Sequence Repeats
SNPs	- Single Nucleotide Polymorphisms
QTL	- Quantitative Trait Loci
TGMS	- Thermo-sensitive Genic Male Sterile
PGMS	- Photoperiod Sensitive Genic Male Sterile
UNDP	- United Nations Development Programme

ABSTRACT

Basmati 370 rice (BS370) is popularly grown in many parts of the world for its good aroma and long, slender grains with excellent cooking and eating qualities. However, Basmati's tall stature and weak stem structure render it susceptible to lodging. This happens particularly when Basmati is subjected to high doses of nitrogenous fertilizer which leads to grain loss. Lodging reduces the efficiency of mechanized harvesting, which is commonly used for large-scale rice production, and further aggravates pre-harvest losses. A transformation of BS370 to a short-statured plant is crucial to address this challenge. This study's primary research objective was to create a semi-dwarf rice line of BS370 origin while retaining the Basmati-like aromatic quality. To realize this objective, Basmati 370, being the female parent, was crossed with a semi-dwarf variety, IR64 using the manual hybridization technique. For confirmation of successful hybrids, distinct anthocyanin pigmentation was used. Thereafter, pyramiding of aroma genes was performed where BS217 was crossed with BS370 to retain the aroma trait in BS370. This was followed by using unique anthocyanin pigmentation selection and molecular marker-assisted selection to distinguish successful from non-successful crosses. Molecular marker-assisted selection was performed to confirm whether the distinctive aroma gene in Basmati rice was retained. The F_1s from the cross between BS370/IR64 and BS217/BS370 were then crossed and the resulting semi-dwarf aromatic lines underwent backcrossing with Basmati 370 as the recurrent parent and selfing up to BC_1F_2. The BC_1F_2 populations and parents were evaluated for agronomic performance at KALRO, Mwea in a randomized complete block design replicated thrice. Data on agro-morphologic traits was collected and subjected to analysis of variance (ANOVA) on SAS 9.4 software. Tukey's HSD test at $P \leq 0.05$ was employed to compare the means of different traits among the genotypes. The findings from this study demonstrated the successful development of an aromatic, semi-dwarf BS370 rice line. The improved line exhibited significantly reduced plant height, shortened culm length, and moderately shorter leaf length. Specifically, plant height was reduced by an average of 34 cm, culm length by 7 cm, and leaf length by 27 cm, compared to the tall parent, BS370. The study was also able to select homozygous fragrant semi-dwarf plants confirming the successful retention of both the fragrance and semi-dwarfing gene in Basmati 370. This breakthrough offers promising prospects for increased food security through reduced grain loss, and improved efficiency in mechanized harvesting. The successful incorporation of the *sd-1* gene and aroma into Basmati 370 resulted in a significantly decreased plant height and aromatic breeding line. This breeding line can be further selected for better performance and released as a new cultivar. Once stabilized in advanced filial generations, the new semi-dwarf aromatic line will positively impact rice yield since the dwarf stature will reduce the plant lodging. This will ultimately benefit Kenyan Basmati rice farmers and satisfy consumers' preferences, which will expand the rice industry as a whole.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Rice (*Oryza spp.*) is the primary staple meal for over 50% of the world's population (Gadal et al., 2019). The main consumers of rice are in Asia, America and Europe. In Africa, rice consumption is increasing more than the production as food preferences change (Demont et al., 2017). With the increasing global population that currently stands at over 8.1 billion, rice demand is increasing (Worldometer, 2024). The semi-dwarfing gene is recessive and widely used to produce varieties with short stature that improve rice resistance to lodging thus increasing uptake of nitrogenous fertilizers. This has a direct correlation to yield increase and harvest index (Nagai et al., 2018).

The green revolution of 1966 released high-yielding varieties from a gene in the semi dwarf variety Dee-gee-won (Tomita & Ishii, 2018). Since the discovery of the *sd-1* gene, new dwarf rice lines such as IR36, IR50, and IR64 with resistance against disease and pests in addition to short maturity duration have been released. Combination of the dwarf gene and hybridization has increased rice yield from 1.7 tonnes/hectare before the green revolution to about 17.1 tonnes/hectare (Yuan et al., 2017).

Many of the high-yielding rice varieties lack good cooking qualities compared to Basmati (Kumar, 2019). Basmati rice commands high prices, which makes it most popular among farmers and customers in India, Pakistan, and Thailand. However, at roughly 4.5 t/ha, its yield has remained low. The major Basmati rice varieties grown in India subcontinent include Basmati 370 and Jasmine. Efforts to improve their yield include population improvement, hybrid development as well as production of dwarf rice variety (Khan et al., 2015).

Customers in Kenya favor Basmati rice, commonly referred to as Pishori, because of its exceptional cooking properties and appealing scent (Sharma et al., 2023). Basmati is a traditional paddy rice variety cultivated primarily under flooded field conditions in Kenya. According to Kenya Plant Health Inspectorate Service (2024), Kenya's Basmati yield is 3.5 t/ha – 4.5 t/ha, which is significantly less than the dwarf lines such as IR8 which have recorded yields up to 8t/ha (Peng et al., 2010). The main

Basmati types grown in Kenya are Basmati 370, Basmati 217, Pusa Basmati 1120 and Pusa Basmati 1121. Basmati 370 is mainly grown because it is slightly high yielding compared to other rice varieties, but with less aroma relative to Basmati 217 (Ngendo et al., 2022). Consumers have continued to demand better fragrant rice leading to a bigger acreage of land being put under Basmati by rice farmers. According to National Rice Development Strategy (2019), the rice area is projected to increase from 32,988 - 171,676 hectares by 2030. The land size under rice may increase but with 4. t/ha as the average production, it will be difficult to produce over 1.2 million tonnes projected for local consumption by 2030 (National Rice Development Strategy, 2019).

Kenya's rice consumption, through its Foreign Agricultural Service's grain and feed annual report, is estimated to be 825,000 metric tons against production of about 217,872 metric tons (Ministry of Water, Sanitation and Irrigation, 2024). By 2030, the consumption is expected to be 1,292,000 metric tons while production is estimated to expand to 846,000 metric tons (MoAL&F, 2020). Mwea produces 60% of rice in Kenya, majority of it being Basmati rice (Atera et al., 2018). The rest come from other areas that include Ahero, Perkerra, West Kano and Bunyala.

Aromatic levels in Basmati vary due to biotic and abiotic factors (Fitzgerald et al., 2008). Kenyan rice consumers have expressed their concerns about the fragrance variation in Basmati rice. Some of the factors leading to variation in aroma include blending rice that leads to adulteration of Basmati, which disadvantages consumer desire. The pressure to meet customers' demand has driven vendors to mix Basmati and non-Basmati rice and sell it as pure Basmati. The adulteration issue has resulted in KEBS introducing a standard for blended rice in the market (DKS 2086: 2020) and revising it to KS 2086:2024(KEBS, 2024). Breeding for high-yielding basmati can solve the issue of blending; however, it is frustrated by some inherent weaknesses, like a weak stem, which makes it lodge in cases of high nitrogenous fertiliser application and windy environment (Njiruh et al., 2013). The distinctive height of Basmati 370 causes 20% yield losses during the heading stage due to lodging. This research aimed at developing a novel rice line with a semi-dwarf gene, *sd-1* and high aroma levels by pyramiding aroma genes from Basmati 370 and Basmati 217 into the semi dwarf line using marker-assisted selection. It was conceptualized that the new

line would have the semi-dwarf gene, hence shorter than Basmati 370 and aroma genes retained.

1.2 Statement of the Problem

Basmati 370 farming faces significant agronomic challenges that hinder its productivity. One of the primary issues is its weak stem structure and medium height, which predisposes the plant to lodging. Lodging is the falling over of the plant due to environmental stress or excessive nutrient application, particularly nitrogen. While nitrogen is essential for rice growth and yield enhancement, excessive application leads to increased lodging incidents, severely impacting the crop's viability. Research indicates that lodging can cause pre-harvest grain losses of up to 20% (Shah et al., 2019). This not only results in reduced yields but also complicates mechanized harvesting processes, leading to further post-harvest losses. Current production levels are significantly lower, highlighting the urgent need for high-yielding rice varieties to meet this growing demand. Consequently, there are expressed concerns of low aroma in Basmati that have not been addressed. The cumulative effect of these challenges has resulted in a significant quality and yield gap for Basmati 370, with current average yields in Kenya standing at only 4.0 t/ha and the consumer concern of declined aroma. The persistent low yields of Basmati 370 contribute to increasing demand-supply deficit for rice in Kenya. The need for rice rises as the population keeps growing leading to higher consumer prices and exacerbating food insecurity (Gitonga, 2017). The inability to produce sufficient quantities of Basmati rice not only affects local consumers but also threatens the livelihoods of farmers who rely on this crop for their income.

1.3 Justification

The introduction of the semi-dwarfing gene into Basmati 370 will reduce its height and enable uptake of higher doses of nitrogenous fertilizer which is essential for raising production without lodging (Singh et al., 2020). About 20% pre-harvest and harvesting loss in rice is due to lodging (Singh et al., 2020). A variety with dwarf variety genes will enable the new lines to withstand environmental stresses such as wind and heavy rainfall. This resilience will enable farmers to apply higher nitrogen levels without the risk of lodging, thereby maximizing yield potential. Historical data from the Green Revolution indicates that the introduction of semi-dwarfing genes has

previously led to significant yield increases, tripling rice production in the 1960s (Gaur et al., 2020).

Secondly, pyramiding aroma genes in Basmati 370 is essential for retaining the aromatic qualities of the rice. Aroma is a significant determinant of consumer preference and market value for Basmati rice (Musila et al., 2018). As the demand for premium fragrant rice grows worldwide, preserving the aroma of Basmati 370 will make it compete more effectively in both domestic and international markets. Basmati with enhanced aroma will satisfy consumer taste and it is expected to increase demand and fetch premium pricing. The better farm gate price will leave more disposable income to the farmers thereby improving economic stability. Given that rice farming is associated with women, more income will significantly impact the livelihoods of farmers and gender equity.

Grain yield increase from the new aromatic dwarf Basmati 370 variety is aligned with national goals for food security, as specified in Kenya's Big Four Agenda and the UNDP Sustainable Development Goal 2, which aims for zero hunger. With Kenya's population projected to grow at a rate of 2.7% per year, the demand for rice is expected to reach approximately 1,290,000 metric tonnes by 2030 (National Rice Development Strategy-2, 2019-2030). This research is not only a response to consumer preferences for high-quality rice but also a strategic move to enhance agricultural productivity and sustainability. By addressing the challenges of lodging and aroma, this new variety will help bridge the yield gap, increase farmers' incomes, and contribute to improved food security in Kenya. The integration of these genetic improvements is essential for the future of rice cultivation in the region, ensuring that it meets both market demands and the nutritional needs of the population. In light of these challenges, there has been a need to investigate and implement strategies to enhance the yield of Basmati 370, focusing on improving its resistance to lodging. Addressing these issues is crucial for bridging the yield gap, stabilizing market prices, and ensuring food security in Kenya.

1.4 Hypotheses

H_o1: The *sd-1* gene cannot be introgressed into Basmati 370 to produce a new line that can enhance resistance to lodging.

H_o2: Pyramiding of aroma genes into Basmati 370 cannot enhance its aroma.

H_o3: There is no significant difference in agro-morphological performance between semi-dwarf aromatic rice breeding lines and their parents.

1.5 Objectives

1.5.1 General objective

To develop a high-grain-yielding dwarf Basmati 370 rice variety with enhanced aroma traits.

1.5.2 Specific objectives

1. To introgress the *sd-1* gene into the Basmati 370 rice cultivar to enhance resistance to lodging.
2. To pyramid aroma genes into the Basmati 370 rice cultivar for improved aroma quality.
3. To evaluate the agro-morphological performance of rice breeding lines with *sd-1* and aroma genes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rice taxonomy and genome evolution

Rice (*Oryza sativa* L.) is an annual grass belonging to the kingdom plantae, division magnoliophyta, class liliopsida, order cyperales, family poaceae, genus *Oryza* and species *sativa*. The genus *Oryza* consists of twenty-five species, of which 23 are wild species and two; *O. sativa* and *O. glaberrima* are domesticated (Chen et al., 2017). The genus has six genome groups; A, B, C, D, E, and F with 12 chromosomes in its genome (2n=24). The cultivated species are *O. sativa* (2n = 2x =24) with the genome formula AA and *O. glaberrima* (2n = 2x = 24) designated as AgAg (Acquaah, 2007).

Oryza sativa has two primary subspecies; indica and japonica (Zhang, 2022). Indica varieties are non-sticky and primarily grown in lowland areas while japonica varieties are sticky when cooked and are mostly cultivated in upland areas in rain fed conditions (Hori et al., 2021). The two sub-species vary in grain size, production levels of phenols, awnness and chalkiness among other agronomic traits (Feng et al., 2017). Basmati 370 and IR64 are both indica with origin from indica and japonica sub-species (Kishor et al., 2020). Development of indica-compatible japonica lines which carry the wide compatibility genes is important in breeding (Guo et al., 2022)

2.1.1 Geographical origin and diversity

Archaeological evidence links the origin of *Oryza sativa* to the middle Yangtze and upper Huai rivers in China, whereas *Oryza glaberrima* was first discovered in the area surrounding the Niger River Delta in West Africa (Ndjiondjop et al., 2018). This was after the domestication of the wild rice *Oryza rufipogon* in 1926 (Wang et al., 2020). Padding and transplanting seedlings were done along the Pearl river influencing farmers to join in rice cultivation (Civet et al., 2015). This spread through Western India, Brazil, Greece and Africa (Sangeetha et. al., 2020).

2.2 Rice farming in Kenya

Rice ranks third among staple foods in Kenya, after maize and wheat (Gitonga, 2017). The Europeans brought rice cultivation to the Kenyan coast for the first time in 1907 (Uma, 2022). The annual production of rice in Kenya is 191,067 metric tonnes

compared to importation of 742,367 metric tonnes (Agriculture and Food Authority, 2025). Importation is done to make up for the enormous gap between supply and consumption. The rice is mostly imported from Pakistan and India. Kenyans grow the majority of their rice (Paddy rice) under irrigation. Mwea, Bunyala, Ahero, West and Kano are the four primary irrigation systems for rice cultivation (Muema et al., 2018). About 95% of all domestic production comes from these schemes, with the other 5% being cultivated in coastal regions, including Tana River, Kwale, and Kilifi, as well as in some areas of Western Kenya, under rain-fed conditions (Dianga et al., 2021). The largest irrigation scheme, Mwea, produces 95% of Kenya's Basmati rice, known locally as Pishori, and more than 86% of all rice under irrigation produced in the country (Gichuhi et al., 2019). In addition to Basmati rice, these irrigation systems are used to grow native landraces such as Komboka and NERICA (Denis et al.,2022). There is one main rice growing season in Kenya from August to January (Samejima et al., 2020). Farmers also take advantage of a complementary ratoon season instead of planting seed from the month of February (Kimari, 2019).

2.3 Aromatic rice production

2.3.1 Characteristics of Basmati rice

Around the world, Basmati rice varieties are highly renowned for their exceptional cooking and eating capabilities (Hinge et al., 2016). According to Slathia (2018), all of the classic Basmati cultivars have light green, with drooping leaves and are tall of 150–175 cm with weak stems (Plate 2.1). Additionally, their grains are long and thin, measuring 6.8 mm to 7.0 mm in length and having a length to breadth ratio of 3.5 to 3.7 (Akhter & Haider, 2020). Basmati 370 reaches its heading stage at 84 days. It matures at about 114 days and reaches optimal drying stage at 16% moisture content at 134 days. The latest rice varieties classification grouped Basmati as an indica rice (Luqman et al., 2023). Basmati varieties have high fragrance levels (Udhayakumar & Karunakaran, 2020). The aroma levels also determine levels of amino acids that are available to consumers as well as methionine, lysine and leucine content. With non-aromatic Basmati rice types, this is not the case (Gaur et al., 2016).

Source; Own

Plate 2.1: Lodging and weak stem observed in Basmati 370 rice fields in the current study

2.3.2 Agro-ecological conditions suitable for production of aromatic rice

The environment significantly affects plant growth, dispersal, and yield variations all around the globe (Tandzi & Mutengwa, 2020). Environmental changes contribute to climate change, which negatively and positively impacts crop production (Zhou et al., 2017). In rice production, growth and grain formation are influenced by rainfall, temperature, atmospheric carbon dioxide, and solar radiation. Aromatic rice, including Basmati, is particularly sensitive to environmental conditions, which may have a major impact on production and aroma quality (Ayut et al., 2024). Optimal conditions for Basmati rice include evenly distributed rains throughout the growth period, temperatures ranging from 20°C to 38°C, clear skies during the day, and low night-time temperatures (Sagar, 2024). The chemical makeup and aroma of fragrant rice are significantly influenced by temperature. Temperature influences the expression of rice aroma and the number and variation of volatile constituents, with 25°C being ideal for the highest aroma score (Prodhan et al., 2017). High temperatures

combined with elevated carbon dioxide (CO_2) levels reduce seed set and a 1000 grain weight in Pusa Basmati 1121 by affecting starch metabolism enzymatic activity (Chaturvedi et al., 2017). Anthesis is a crucial stage in crop development, and higher temperatures can enhance pollen sterility by reducing anther dehiscence, thus affecting pollen deposition on the stigma and subsequent germination (Cao et al., 2015). Rainfall is essential for rice production, but its impact varies at seedling stage, tillering, panicle initiation and grain filling stages. During heading and flowering stages rainfall can damage pollen grains and ovules, leading to a significant drop in productivity (Abbas & Mayo, 2021). High rainfall during maturity can discolor grains, darkening or rotting of spikelets.

Carbon dioxide enhances photosynthesis in C_3 crops such as rice, wheat, oats among others. Unfortunately, industrial activities have increased greenhouse gases, including CO_2. Myers et al. (2015) argued that higher CO_2 levels over the next 40 to 60 years could reduce essential elements like nitrogen, zinc, and iron in C_3 crops, impacting human nutrition. This concurred with findings showing that elevated CO_2 levels could cause nutritional deficiencies in rice grains (Ujiie et al., 2019). The higher CO_2 levels alter soil microbial composition, reducing microbes involved in polycyclic aromatic hydrocarbons (PAHs) degradation. In a study on the aromatic rice variety, elevated CO_2 and increased temperatures resulted in higher yields due to better shoot biomass and efficient source-sink transfer of photosynthates. Morita et al. (2016) found that chalky grains are more common during ripening when temperatures are high and sun radiation is low. Ishimaru et al. (2018) discovered that whereas milky-white grains did not positively correlate with temperature, white-back and basal-white grains did. Li et al. (2020) indicated that increased reduced solar radiation significantly lowers aromatic rice expression. Soil is a vital component for agricultural productivity because the microorganisms in soil determine the accessibility of vital nutrients for plants. The diversity and abundance of soil microbiota play a crucial role in nutrient cycling, making nutrients accessible for uptake by plant roots. Without a healthy soil microbiome, plants struggle to obtain the necessary nutrients for growth and yield.

Research has shown that soil texture influences the amount of water that soil can hold for plant use (Dou et al., 2016). Clay soils, with their small and tightly packed particles, have a higher surface area to volume ratio compared to sandy soils. This allows clay soils to hold more water molecules on their surfaces, increasing the soil's

water-holding capacity. Additionally, clay soils tend to have higher organic matter content due to the tight packing of clay particles, which helps retain water and nutrients more effectively. The greater water holding capacity of clay soils results in a higher available water capacity for plants to utilize. In aromatic rice varieties, such as Basmati, consistent water availability particularly during grain filling can influence the biosynthesis and accumulation of aroma compounds like 2-acetyl-1-pyrroline (2-AP). Therefore, the greater water holding capacity of clay soils may contribute to enhanced aroma expression and overall grain quality.

Flooded soil is used to grow rice, which produces an anaerobic environment that promotes the growth of particular rice cultivars. However, there are also upland rice varieties that are cultivated on well aerated soils in elevated areas. These upland rice varieties are adapted to grow in drier conditions and can thrive in well-drained, aerated soils which can enhance the synthesis of aroma related compounds such as 2-acetyl-1-pyrroline (2-AP). Furthermore, flood prone river basins are used to grow deep water rice since the plants can withstand extended durations of submersion (Oladosu et al., 2020). This can affect nitrogen availability and limit oxidative processes, potentially reducing aroma expression. Deep water rice varieties have evolved mechanisms to elongate their stems rapidly in response to rising floodwaters, allowing them to maintain contact with the atmosphere and continue photosynthesis.

2.4 Basmati rice breeding

2.4.1 Basmati hybrid rice breeding in Kenya

While consumers prefer Basmati rice to non-aromatic types, it has a number of agronomic weaknesses that are challenging to breeders. Some the weaknesses include low yield of 3.50 t/ha– 4.50 t/ha compared to 8.0 t/ha in high yielding varieties (KEPHIS, 2024) and propensity to lodging due to their weak stem. Basmati hybrid rice seeds' production has been championed by rice breeders. Kenya can produce hybrid rice, however before investing in the commercial production of hybrid seeds, a breeding program is required (Njiruh et al., 2013). Thermal-sensitive genic male sterile (TGMS) and photoperiod-sensitive genic male sterile (PGMS) lines have been used to create hybrid Basmati 370 (Nthakanio & Kariuki, 2019). Thermo-sensitive genic male sterile (TGMS) and photoperiod-sensitive genic male sterile (PGMS) rice lines have genes that cause male gametes to become sterile at high temperatures and

extended growth days, respectively (Nthakanio & Kariuki, 2019). Nonetheless, gametes may survive at temperatures between 19 and 35 degrees Celsius and for 12 hours of sunshine (Njiruh et al., 2013). Nyankemba et al. (2025) assessed the environment-genic male sterile (EGMS) spikelet fertility and investigated F_1 lines and their parents for adaption to Kenyan weather settings. When grown outside of a greenhouse, plants showed a notable degree of fertility, but when grown in a greenhouse with long daylight hours, they were entirely sterile. According to an evaluation of EGMS spikelet fertility, plants cultivated in environments with high temperatures and long daylight hours had pollen that stained yellow in over 99% of cases, and at maturity, the plants had a seed set rate of less than 1% (Nthakanio & Kariuki, 2019). This suggests that the panicles were sterile and the pollen was of the abortive kind, opening the door for Kenya to use a 2-line hybrid to improve basmati and other rice lines like Komboka, NERICA among others.

2.4.2 Advances in Basmati rice breeding

Through breeding, abiotic challenges including drought, high salinity, and flooding, as well as for disease and insect resistance and improved nutrition have been resolved (Dixit et al., 2020). Research on Basmati rice mutation breeding has also focused on creating mutants with a broad range of phenotypic diversity, such as large panicles, earliness, and more grains per panicle, among other mutant features that probably improve yields and quality (Hameed et al., 2023). Further, there have been efforts to increase nutrition through bio-fortification with micronutrients such as zinc to solve the need of zinc deficiency especially in children and expectant mothers (Swamy et al., 2016).

Breeding for early maturing Basmati rice has been performed by a group of rice breeders in India. As a result, IET 11348, a new Basmati variety, was released (Chandanshive et al., 2020). With similar yield and quality, this variety matured 30–35 days before Basmati 370, taking 115–120 days to reach maturity (Pillai et al., 2020). In 1991, the rice variety IET 10367 (HKR 228/Haryana Basmati), known for its dwarfism, high yield, and blast resistance was introduced (Sharma et al., 2023). In 1992, Haryana released Purified HBC 19 (Karnal Local), a traditional export quality rice with high demand, as Taroari Basmati. Semi-dwarf, high-yielding Basmati

varieties, Pusa Sugandh 2 and Pusa Sugandh 3, were introduced in 2001 (Kota et al., 2017).

High-yielding short-statured indica rice types and Basmati rice variants differ genetically. As a result, different degrees of F_1 sterility brought on by incompatibility are seen when Basmati varieties cross with other high-yielding cultivars (Nyankemba et al., 2025). Due to its low yield, susceptibility to pests and diseases like blast, and poor response to nitrogen fertilizer, Basmati 370 also has a limited genetic foundation because all Basmati lines created by breeders from one source (Akhter & Haider, 2020). This brings about a problem of compatibility when it comes to breeding with other rice cultivars. Basmati rice has exhibited segregation distortion when crossed with other lines (Njiruh et al., 2013). This distortion has been linked to genes located on chromosomes 3 and 5, which affect male gametes, and on chromosome 6, which affects female gametes. These genetic factors act during hybridization and contribute to its instability, even in advanced generations (Zhou et al., 2022). Furthermore, when high-yielding non-Basmati types are crossed with varieties with plants with dominant genes like tallness, qualitative features like aroma are suppressed in F_1 due to recessive gene regulation. In addition, introduction of genetic clones into Basmati by transformation is best realized by use of calli tissue. However, the recalcitrant nature of Basmati rice calli to induction has made its genetic transformation difficult (Njiruh et al., 2013).

2.4.3 Genetic control of aroma gene in rice

Aroma genes in rice especially in Basmati cultivars have been realized to have different variations from being monogenic to being polygenic and controlled by environmental growth factors (Daygon et al., 2017). It has also been recorded that there is significant haplotype variation in rice aroma, both in japonica and indica species (Shao et al., 2013). Genetic characteristic analysis performed on Barth Marty aromatic rice brought about realization that apart from the *Badh2* gene, 2 more quantitative trait loci (QTLs) were noted to regulate the rice grain's aroma. According to reports, the *Badh1* gene is connected to the quantitative trait loci on the fourth chromosome (Gaur et al., 2016). Although the genetic basis of rice aroma appears to be complicated, most experts concur that a significant recessive gene (*fgr*), located on the eighth chromosome of the rice genome, controls aroma (Golestan et al., 2015).

Aroma gene expression has also been attributed to a deletion mutation in a locus that does not happen in non-aromatic rice varieties. The deletion occurs at 8-bp in the aroma characterizes *badh2* gene of rice. It appears that this deletion results in the production of betaine aldehyde dehydrogenase, which is biologically inert and gives rice its aroma (Chakraborty et al., 2016).

2.4.4 Marker-assisted breeding in rice

Conventional rice breeding has become more efficient through the use of molecular markers. Novel rice cultivars have previously been bred utilising molecular markers to boost their accuracy in breeding for resistance to biotic and abiotic stress (Das & Rao, 2015). One example is the SSR marker RM589, which is strongly associated with the *Bph3* gene and improves rice resistance to brown plant hopper disease. It was employed to check the genotype of the BC_3F_2 population that had the relevant gene (Badhini & Ulanagathan, 2019). To produce BC_3F_3, only offspring with the pertinent gene were chosen. Among other molecular markers, single-nucleotide polymorphisms, simple sequence repeat markers, and single target site markers have been employed in rice breeding (Wijerathna, 2015). The rice variety WH421 has been bred with the dwarf Indonesian local rice cultivar Siputeh using STS markers (Luo et al., 2014). To encourage molecular breeding of rice aroma by marker-assisted selection, two SNP molecular markers (SNP_badh2-E2 and SNP_badh2-E7) were designed in 2020 (Li et al., 2020). In addition to SNPs and SSR markers, Bradbury et al. (2005) created allele-specific amplification (ASA), a method that separates alleles that differ by SNPs, insertions, or deletions in a single polymerase chain reaction at a time, improving the efficiency of both aromatic and non-aromatic variety identification and selection.

2.4.5 Gene pyramiding to improve aroma in rice

Breeders frequently aim to integrate two or more important, unlinked features from outstanding rice genotypes into a single background through a process known as gene pyramiding (Haque et al., 2021). The gene pyramiding techniques have been used to improve a number of crop traits. One of the remarkable ones is the use in diseases and pests' resistance. This has been done by combining multiple resistance genes into one background thus reducing the likelihood of pathogens or pests breaking the plant

defense mechanisms. Success has been recorded in rice where *Pi9* and *Pi54* genes have been pyramided for durable blast resistance (Kiran, 2020).

Rice varieties such as Basmati and Jasmine are preferred by consumers and thus fetch premium prices due to their aroma. The aroma trait is primarily linked with the presence of 2-acetyl-1-pyrroline (2-AP) compound that has a popcorn fragrance (Wei et al., 2021). Different types of rice have varying degrees of aroma. The variation has been associated to, environmental, processing and genetic factors. A rice variety like PTB-33 has been produced from pyramiding superior traits like resistance to the brown plant hopper, incorporating fgr genes and aroma QTLs on the fourth and eighth chromosome (Logeshwari et al., 2024). During pyramiding, a three-way cross is commonly used to bring all genes of interest under one background (Mundt, 2018). Following crossing, pyramiding performance is assessed to determine the appropriate genotypes for the following stage and to verify that desirable genes are successfully passed down to the succeeding generation. Molecular markers have been utilized in rice breeding by pyramiding gene conferring broad spectrum durable resistance traits bacterial leaf blight caused by *Xanthomonas oryzae pv. Oryzae* (Chukwu et al., 2019).

Aroma in Basmati and related rice is caused by the accumulation of 2-acetyl-1-pyrroline (2-AP) (Katiyar et al., 2016). Varieties including Basmati, Jasmine, Paw San Hmwe, Ambemohar, Kalijira, Wayanad Jeerakasala, Wayanad Gandhakasala, Chak Hao Amubi, Tulaipanji and Chinigura vary in terms of aroma levels (Vemireddy et al., 2021). There has been a challenge of differentiating the level of aroma at marker-assisted level. Alternative methods that have been used to detect aroma in rice are the Potassium Hydroxide (KOH) method and the manual chewing method (Zheng et al., 2022). However, these have proved to be non-satisfactory to be incorporated in aroma screening processes and hence the need to combine aroma marker-aided breeding with the conventional aroma evaluation methods for best breeding for aroma through gene incorporation analysis (Alrufaye et al., 2018). Attempt to incorporate aroma traits into one background has been made using molecular-aided backcrossing where aroma traits from Basmati 370 and Basmati 217 have been pyramided under one background. Similar attempts have been done by Nyankemba et al. (2025) through pyramiding aroma traits into a photo/thermos-sensitive genic male sterile rice line background.

According to Bradbury et al. (2005), the cause of fragrance in Basmati and Jasmine rice is linked to deletion of eighth base pair and three SNPs in exon 7 of the gene encoding betaine aldehyde dehydrogenase on chromosome 8 of *Oryza sativa*. This is different from non-fragrant rice varieties which have a uniquely fully functional copy of the gene hence resulting to frame shift that generates a stop codon that disables the *BADH2* enzyme (Withana et al., 2020). This polymorphism provides a basis for construction of a marker for fragrance in rice (Bradbury et al., 2005).

Marker assisted aroma breeding has also been used to detect aroma levels with the recent use of External Sense Primer (ESP), External Antisense Primer (EAP), Internal Fragrant Antisense Primer (IFAP) and Internal Non fragrant Sense Primer (INSP) (Gatere et al., 2025). External Sense Primer and External Antisense Primer work such that they anneal to sequences common to both fragrant and non-fragrant varieties and that are external to the area where the mutation occurs (Nyankemba et. al., 2025). Three bands can be observed upon amplification. The 580 bp band represents a positive control, amplified using both external primers (ESP and EAP). The 355 bp band indicates a PCR product amplified from the non-fragrant allele, using the internal non-fragrant sense primer (INSP) and the external antisense primer (EAP). Meanwhile, the 257 bp band corresponds to a PCR product amplified from the fragrant allele, using the internal fragrant antisense primer (IFAP) and the external sense primer (ESP). Internal Fragrant Antisense Primer and INSP anneal only to their specified genotype producing DNA fragments with their corresponding external primer pair, ESP and EAP. Simple sequence repeat markers (SSR) markers have been successfully used to accelerate precision of aroma genes in rice include; RM3459, RM23120, Aro7, RM7049, RM7356 and RM7556.

2.4.6 Dwarf aromatic rice

To meet the demand for high yielding and premium rice with enhanced agronomic qualities, a number of dwarf aromatic rice lines have been effectively created. This has been made possible using marker-assisted selection and traditional breeding techniques. One such example is the pyramiding of the *sd-1* gene for semi-dwarfism and the *badh2* gene responsible for fragrance into popular aromatic varieties. Gaur et al. (2020) reported the successful development of a Thai fragrant rice line (T5105) by incorporating semi-dwarf stature, submergence tolerance, and resistance to major

diseases while retaining the characteristic aroma of the KDML105 variety. Similarly, there was another line identified with dwarf, aromatic, and photo-insensitive segregants in a cross between Katarni and Rajendra Sweta using molecular markers (Bharti et al., 2020). These progenies demonstrated homozygosity for both fragrance and semi-dwarf traits, confirming the effectiveness of combining traditional selection with molecular tools in developing improved Basmati-type rice lines. Induced mutation and genome editing have further contributed to the development of dwarf aromatic rice. Early efforts using gamma irradiation on KDML105 variety led to the creation of photoperiod-insensitive, dwarf mutants that retained strong aroma (Jayasri & Chakraborty, 2024). In recent years, elite semi-dwarf cultivars have had the *OsBADH2* gene directly blasted out using CRISPR/Cas9 technology, adding aroma without sacrificing plant height or other desired characteristics (Hui et al., 2022). These precise edits allow breeders to maintain yield potential and stress resilience while enhancing grain quality through aroma. Such advances represent a significant step forward in aromatic rice improvement, providing cultivars that meet both farmer preferences for manageable plant height and consumer demand for aromatic grain. In a broader context, Chakraborty (2020) highlighted the additional utility of genome editing particularly CRISPR/Cas9 to directly target *OsBADH2*, generating novel fragrant alleles in japonica and indica backgrounds. These precise edits enabled creation of fragrant lines without linkage drag from donor parents, while retaining elite plant architecture.

Beyond Asia, Africa Rice developed the ORYLUX aromatic lines derived by crossing WITA 1 with Pusa Basmati. These lines combined fragrance, early maturity, and moderate resistance to local stresses (Africa Rice, 2022). Although primarily conventional in methodology, there was aroma, taste and long grain introgression toward semi-dwarf stature aligned with market preferences. These methodologies highlight the feasibility and value of developing fragrant rice cultivars with semi-dwarf, farmer and market preferred plant architecture. Future breeding efforts will likely focus on integrating improved grain quality with resilience traits, using both conventional and next generation genomic approaches. One major hurdle in breeding for dwarf aromatic rice is linkage drag and incompatibility in combining the semi-dwarf *sd1* allele with various *badh2* fragrance alleles from different subspecies. For instance, most fragrant alleles stem from japonica backgrounds, while many high-

yielding semi-dwarf varieties are indica, leading to sterility and poor recombination (Huang et al., 2024). When fragrance and dwarfism are successfully introgressed, breeders must contend with extended linkage disequilibrium around the *badh2* locus, reflecting limited historical recombination and challenging fine mapping without disrupting aroma pathways.

2.4.7 Semi-dwarfing gene in rice (*sd-1*)

Among the most crucial genes used in rice breeding is the gene for semi-dwarfism *(sd-1*) which is used to significantly increase rice yield (Bhuvaneswari et al., 2020). It is recessive in nature and found in chromosome 1 (Cheng et al., 2022). Presence of the *sd-1* gene in rice produces dark green leaves, an upright, shorter culm, and a high tolerance to lodging, all of which increase the plant's ability to withstand high nitrogen fertilizer dosages and boost production per hectare. Nitrogen in rice promotes rapid plant growth, improves grain yield and grain quality (Zhou et al., 2017). This is through increased tillering ability, leaf area development, grain formation and filling, and protein synthesis (IRRI Rice Knowledge Bank, 2016).

The *sd-1* gene was initially described in the dwarf Chinese variety known as Dee-geo-woo-gen (DGWG) by Priyadarshan (2019). It was subsequently crossed with the tall rice variety Peta in the early 1960s to create the semi-dwarf cultivar IR8 in 1966. All across Asia, the IR8 rice cultivar produced exceptional yields. As such, it served as the foundation for the creation of new semi-dwarf, high-yielding rice varieties such as IR36 and IR64. The IR8 rice variety, however, had a number of defects including decreased yield levels due to environmental effect, susceptibility to diseases and pests, late maturity in addition to low grain quality (Peng et al., 2010). This led to release of a new improved variety from IR8 called IR36 in the early 1980's which had disease and insect resistance, early maturity and relatively high yields. In 1985, another variety named IR64(Plate 2.2) was released by IRRI, Philippines as an improvement to IR36 that was dwarf in stature, had disease and insect resistance, was early maturing and now had excellent palatability when cooked. This variety out yielded IR36 and was widely spread in rice growing areas (Mackill & Khush, 2018).

Lack of the inactive gibberellin (GA) hormone has been found to be the cause of semi-dwarfism (Lu et al., 2021). Changes in GA content brought on by mutations in the semi-dwarf gene are consistent with a block at this point in the GA production

pathway (Bhuvaneswari et al., 2020). The semi dwarf gene contains gene mutations that lead to changes in GA content that are consistent with a block at this step in the GA biosynthetic pathway (Ferrero-Serrano et al., 2019). Numerous species have been shown to exhibit GA-responsive dwarf mutants, which are frequently caused by abnormalities in the genes encoding GA biosynthesis enzymes (Hu et al., 2020). These proteins are responsible for leaf sheath growth through cell elongation and promotion of carbohydrate, protein and photosynthetic metabolism (Chen et al., 2020).

Plate 2.2: Images of the semi-dwarf rice variety IR64 used as a male parent in the current study at KALRO-Mwea

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site description

The research was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO)-Mwea, Kirinyaga County, Kenya. The site is located on Latitude 0°37′S, and Longitude 37°20′E at an elevation of 1,159 m above sea level (World elevation map finder, 2025). The site has vertisols characterized by very deep, dark grey, imperfectly drained clays that are susceptible to cracking (Sombroek et. al., 1982). The study was carried out over the course of four growing seasons, with mean temperatures of 22°C and mean humidity levels of 57%, and temperatures ranging from 15.6°C to 28.6°C. With a bimodal trend of light rains from October to December and an extended wet season from March to June, the region receives 500 mm to 850 mm of rainfall yearly.

3.2 Plant materials

Rice cultivar IR64 was used as the *sd-1* gene donor and was sourced from IRRI through KALRO –Mwea. Basmati 370 and Basmati 217 are medium tall with long slender grains and has soft and fluffy texture and lengthwise elongation on cooking and pleasant aroma. Both Basmati 370 and Basmati 217 share a common genetic background but have some differences as in Table 3.1. Seeds of Basmati 370 and Basmati 217 were provided by the National Irrigation Authority, Kenya. Variety IR64 is a high-yielding dwarf short-grain non-aromatic rice genotype developed by IRRI, Philippines with some disease and pest resistance, early maturity and has good cooking traits (Mackill & Khush, 2018).

TRAIT	BASMATI 370	BASMATI 217	IR-64
Pedigree	Traditional Basmati variety from India	Traditional fine Basmati variety from Punjab	Cross between IR5657-33-2-1 and IR2061-465-1-5-5 developed by IRRI
Plant height	160cm-175cm at maturity	160-175cm at maturity	Approximately 100 cm at maturity
Year released	1933	1969	1985
Ecological Requirements	Prefers warm, sub-tropical climates with well-drained loamy soils	Prefers warm, sub-tropical climates with well-drained loamy soils	Adaptable to tropical and subtropical climates with a range of soil types
Maturity	Matures in approximately 145-150 days	Matures in approximately 145-150 days	Matures in about 117 days
Grain Type	Extra-long, slender, aromatic grains with long awns	Extra-long, slender, aromatic grains without awns	Medium-long, slender, non-aromatic without awns
Potential Yield	4.6 mt/ha/ha	4.5 mt/ha	8.28 - 8.76t/ha
Rain-fed or Irrigated	Mostly irrigated	Mostly irrigated	Grows well under irrigated & rain-fed lowland areas
Tolerance to Biotic Stresses	Susceptible to bacterial leaf blight, blast, sheath blight and false smut. Susceptible to yellow stem borer, leaf folder and brown plant hopper	Highly susceptible to blast	Tolerant to blast, bacterial leaf blight, and pests like brown and green plant hopper
Tolerance to Abiotic Stresses	Low tolerance to drought and salinity	Low tolerance to drought and salinity	Moderate tolerance to submergence, drought and salinity
Grain Quality	Aromatic, flavorful, and fluffy texture when cooked	Stronger aromatic compared to BS370 and fluffy texture when cooked	Non-aromatic, good grain quality and soft texture

Table 3.1: Characteristics of plant materials used in this study

3.3 Introgression of *sd-1* gene into Basmati 370

3.3.1 Experimental design and layout

In order to break seed dormancy, Basmati 370 and IR64 seeds were soaked in 30% hydrogen peroxide for 24 hours before being incubated for 48 hours at 35°C in an oven. Prior to transplanting, the pre-germinated seeds were placed in plastic trays with immuno-boosting biochar as the seedling medium and given 21 days to grow. Transplanting was done in 10-liter polyethylene pots of 28 cm diameter having 2 seedlings each at a spacing of 15 cm. Soils used to fill the pots were of vertisol type. There were four planting staggers, each spaced apart by a seven-day difference. Each experimental stagger comprised of 20 polythene 10-liter pots for both Basmati 370 and IR64 varieties**.** Staggering helped in synchronizing flowering between two varieties is crucial for successful hybridization. Basmati 370 and IR64 differ in their flowering times due to genotypic differences in phenology. By planting in staggered intervals, it was possible to align flowering windows to maximize the chances of viable cross-pollination. Plant staggering also accounted for fluctuations in environmental conditions such as temperature, humidity, light intensity, and soil moisture over time. These factors could influence plant growth and development, especially in pot experiments where the environment is not fully controlled.

3.3.2 Development of F_1 hybrids

Basmati 370 was the female parent and IR64 was the male parent in this cross. Cross-breeding was done in an open field to generate the F_1 breeding stock as described by Nthakanio & Kariuki (2019). The crossing involved a random sampling of plants during heading stage, when BS370 panicles had extended approximately one-third beyond the flag leaf. The flag leaf of Basmati 370 was carefully removed to expose the florets, after which a third of the upper and lower parts of the panicle of the panicle was cut off using clippers without injuring the stigma of the pistil - gynoecium, which could lead to self-pollination. Using clippers, the remaining florets were cut exposing the spikelet. Sharp sterilized forceps were used to carefully pick out the pollen without injuring the

gynoecium. Mature pollen from IR64 was collected in warm daytime between 9:00 am and 11:00 am, when Basmati is most receptive to pollination and tapped gently onto the emasculated Basmati 370. The emasculated and dusted flower was then covered with parchment paper to avoid unwanted cross-pollination. Unbagging of the covered panicles was done after ten days and the hybrid seeds were left to mature till harvesting at physiological maturity. The period of ten days was long enough to prevent unwanted cross-pollination, but short enough to avoid bagging-induced stress on the panicle and developing seeds.

3.3.3 Morphological selection of Basmati 370/IR64 F_1 hybrids

Anthocyanin pigmentation manifested at the base of the stem, leaf sheath or tips of seeds of the plant where Basmati had been used as one of the parents in the cross-breeds (Njiruh et al., 2013). Individuals from the F_1 population were planted in 20-litre plastic buckets with a circumference of 94.2 cm, filled with well-decomposed paddy field soil. Seeds were thereafter sown at a spacing of 15 cm between plants. Phenotypic selection was done at the onset of physiological maturity when the panicles turned from green to straw-colored, and the husks lost their green pigment. Anthocyanin pigmentation was observed at the base of the stem, leaf sheath or tips of seeds.

3.3.4 Molecular marker assisted selection for aroma genes in Basmati 370/IR64 F_1 hybrids

Young leaves of F_1 from section 3.2.4 were picked at 21 days old in sterile conditions and transported from KALRO-Mwea to University of Embu laboratory in a cool box containing ice. DNA was extracted using a modified (Mahuku, 2004) protocol in Appendix 1. Key reagents used in this DNA extraction protocol played critical roles in ensuring high-quality genomic DNA recovery. The TES extraction buffer (Tries-EDTA-Sodium chloride) maintained a stable pH (via Tris-HCl), chelates divalent ions to inhibit DNases (via EDTA), and facilitated protein removal and cell lysis (via NaCl and 1% SDS). SDS (Sodium Dodecyl Sulfate) acted as a strong detergent to disrupt cellular and nuclear membranes while denaturing DNA-binding proteins, thereby releasing intact DNA. Ice-cold isopropanol and 70% ethanol was used to precipitate and purify DNA by reducing its solubility.

For PCR amplification, DNA, 1 µl-10 µM of each primer(F+R), 12 µl molecular grade water, 1 µl-5mM dNTPS, 4 µl Accuris™ Taq-buffer and 0.2 µl of 5 units Accuris™ Taq polymerase were used. Internal fragrant antisense primer and external sense primer were used for fragrance gene amplification whereas internal non-fragrant sense primer and external antisense primer for non-fragrant gene amplification (Table 3.2). The PCR profile was as follows: an initial denaturation stage of 2 minutes at 95° C; followed by 30 cycles of 95°C for 20 seconds, annealing temperature at 58°C for 30 seconds, an elongation step of 72°C for 30 seconds, a final extension phase at 72°C for 5 minutes and maintained at 4°C. The amplified PCR products were resolved in 1.5% agarose gel pre-stained with 10,000X SYBR green 1 dye in 1 × Sodium borate buffer. Manual scoring of the amplicons' size for the fragrance gene was done as follows: homozygous fragrant (257bp), homozygous non-fragrant (355bp) and heterozygous individuals with three bands (580bp+355bp+257bp) according to Bradbury, 2005.

Table 3.2: Molecular markers linked to rice *fgr* genes used in this study

Primer name	Primer sequence
External Sense Primer(ESP)	TTGTTTGGAGCTTGCTGATG
Internal Fragrant Antisense Primer (IFAP)	CATAGGAGCAGCTGAAATATATACC
Internal Non-Fragrant Sense Primer (INSP)	CTGGTAAAAAGATTATGGCTTCA
External Antisense Primer (EAP)	AGTGCTTTACAAAGTCCCGC

3.3.5 Experimental design and layout for evaluation of F_2 hybrids

The F_1s from section 3.3.5 were first incubated in an oven at 35°C for 48 hours to break seed dormancy. Altogether, the hybrid seeds and the parents (Basmati 370 and IR64) were soaked in 30% hydrogen peroxide for 24 hours to break dormancy and initiate germination. The pre-germinated seeds were then sown in plastic trays filled with biochar-media, a type of seedling media known for its ability to enhance seedling growth

and development. The seedlings were allowed to grow for 21 days in the trays before transplanting. This period was crucial for the seedlings to develop a robust root system and to be well established before being transplanted into larger pots. The transplanting process involved placing four seedlings each in 20-liter polythene pots of 40 cm height and 32 cm width. The seedlings were transplanted at a spacing of 15 cm between the seedlings. This spacing allowed for adequate air circulation and minimized competition for resources. The soils used to fill the pots were of the vertisol type, which is characterized by a high content of clay and a high water-holding capacity. This type of soil is well-suited for rice cultivation, as it provides a stable environment for the roots and helps maintain a consistent water supply. The experiment was laid out in a completely randomized design in an open field. A total of 240 BS370/IR64 F_2 seeds were harvested at physiological maturity when the panicles had turned from green to straw-colored, and the husks lost their green pigment.

3.3.6 Data collection

Data from each of the four plants at maturity within a pot were collected at physiological maturity as follows; Plant height, productive tillers, culm length, awnness and apiculus pigmentation. Plant height was measured in cm from ground level to the tip of the flag leaf at flowering stage once the rice plant had reached its maximum height. Number of productive tillers was counted. Culm length was measured in cm using a ruler from the pot's soil surface to the panicle base. Presence of awns was scored as following; long awns were scored as 2, short awns were scored as 1 while absence was scored as 0 while presence of apiculus pigmentation was scored as 1 and absence was scored as 0. Leaf length was measured in cm from the base of the flag leaf to the tip of the flag leaf at flowering stage once the rice plant had reached its maximum height. Leaf width was measured in cm at the widest portion of the flag leaf's blade using a 30 cm ruler.

3.3.7 Data analysis

Quantitative data was subjected to one-way analysis of variance (ANOVA) using SAS 9.4 statistical software version. Mean separation was determined using Tukey's studentized Range (HSD) at a 95% confidence level. The degree of relationship between

the quantitative characters was assessed using the correlation coefficient (r). The F_2 hybrid height segregation analysis was carried out using the chi-square test for goodness of fit. Classification of the plants according to International Board of Plant Genetic Resources (IBPGR, 1980) standards was applied as follows; >110 cm were classified as tall and semi-dwarf plants were those of heights <110 cm. This was used to statistically assess whether the segregation of plant height phenotypes in the F_2 population, specifically tall and semi dwarf individuals**,** follows the expected mendelian ratio based on the genetic model hypothesized for height inheritance. Pearson's correlation was done to compare the degree of association between the traits analyzed.

3.4 Pyramiding aroma genes into Basmati 370

3.4.1 Experimental layout, design and crop management

Basmati 370 and Basmati 217 are tall aromatic indica rice varieties. They were used in this study due to preference in cooking and eating qualities among the Kenyan consumers. The seeds were acquired from the National Irrigation Authority, Kenya. The experiment was laid as in section 3.3.2. Water management was carefully maintained throughout the growth cycle, with daily watering of 500 ml per pot during the critical stages of growth and the water drained prior to maturity to facilitate harvesting. Fertilizer management involved the application of basal fertilizer, Di Ammonium Phosphate at transplanting, followed by topdressing Calcium Ammonium Nitrate at tillering and panicle initiation. Weed control was achieved through manual weeding. Pest and disease management practices included regular field scouting for stem borers, blast, sheath blight, and brown spot, among others, with control achieved through integrated pest management strategies and pesticides. Other crop management practices included gap filling to replace missing plants after transplanting.

3.4.2 Development of F_1 hybrid seeds

Basmati 370 was crossed with Basmati 217. A reciprocal cross was also performed. Crossing was as described in 3.3.2 to produce the first filial generation when the panicle had grown about one-third past the flag leaf during the heading phase. Covering of the

emasculated Basmati 217 flowers was done using khaki bags to prevent unwanted pollination. After the development of F_1 seeds, they were selfed to generate the second filial generation.

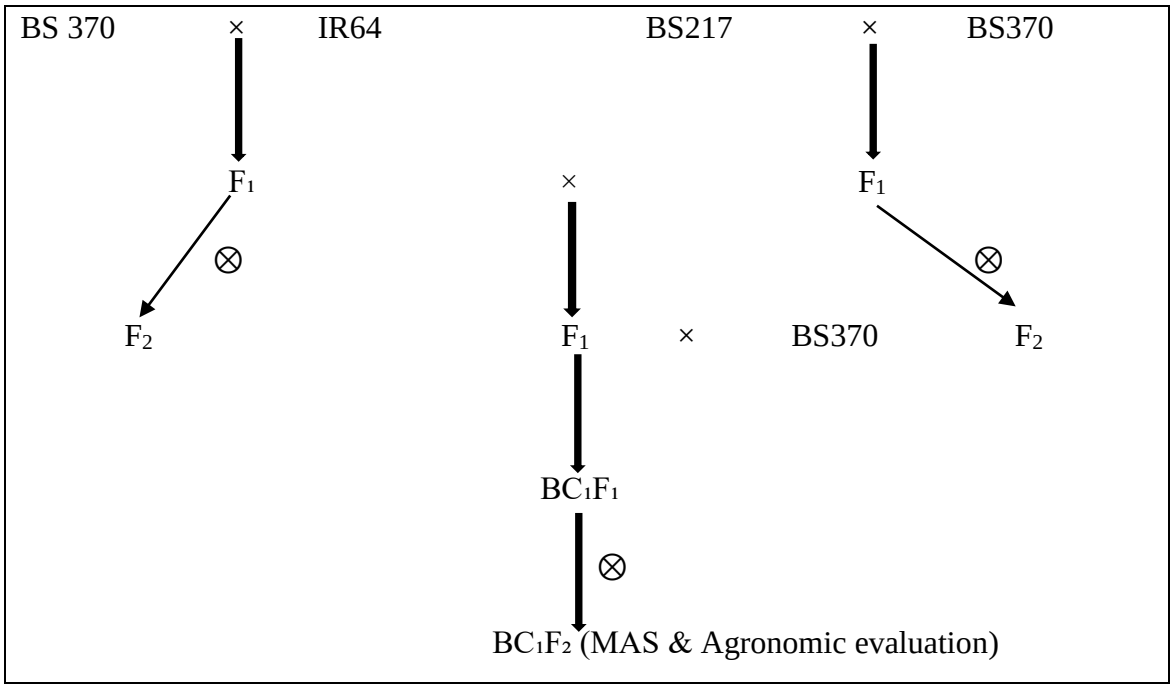
3.4.3 Selection of successful hybrids

Marker-aided selection for the fragrance gene(*fgr*) was achieved using ESP/EAP/IFAP/INSP markers that are linked with the fragrance genes (Carsono et al., 2020). DNA of the BS217/BS370 hybrids and a control non-aromatic line V1(IR-73827-23-76-15-7S) was extracted and amplified as per the protocol outlined in section 3.3.5 and scored for presence (1) or absence (0) of *fgr* genes for all seeds obtained from section 3.4.2.

3.5 Development and evaluation of BC_1F_2 breeding lines

3.5.1 Crossing and backcross generation

The F_1 seeds from BS370/IR64 and BS217/BS370 were incubated in an oven at 35°C for 72 hours to break seed dormancy. Basmati 370 seeds were soaked in 30% hydrogen peroxide for 24 hours and thereafter incubated in an oven at 35°C for 48 hours to break seed dormancy. They were sown in plastic trays with biochar as the seedling media and allowed to grow for 21 days before transplanting. Transplanting and hybridization of BS370/IR64 and BS217/BS370 was performed as in section 3.2.3 and harvesting of the seeds was done at physiological maturity. Backcrossing was thereafter done with Basmati 370 as the recurrent parent (Figure 3.1).



MAS –marker assisted selection, × - crossing, ⊗ - selfing

Figure 3.1: Marker-assisted backcross breeding scheme to introgress *sd-1* and fragrance genes in Basmati 370 rice

3.5.2 Selection generation

Phenotypic selection for height was done after vegetative growth using IBPGR standards for height. Anthocyanin pigmentation on the base, leaf sheath and seed tips was used as a morphological marker to discriminate successful from unsuccessful crosses. After elimination, molecular selection of plants for *fgr* genes was done using ESP/EAP/IFAP/INSP markers that are linked to fragrance genes. The successful selected semi-dwarf and fragrant genotypes were given optimum growth conditions to self and the BC_1F_2 seeds were harvested at physiological maturity stage.

3.5.3 Agro-morphological performance of the parents and BC_1F_2 hybrids

From each of the chosen BC_1F_2 plants, one panicle was harvested, threshed, and bulked. After pre-germination and sowing, 215 BC_1F_2 seedlings were transplanted in concrete

troughs at KALRO-Mwea for evaluation for agronomic performance as in Figure 3.2. Three replicates were sown in a randomized complete block design (RCBD) with 15 cm between plants and 20 cm between rows. Each replicate had 75 seedlings of BC_1F_2, BS370 and IR64. Basmati 370 and IR64 were planted as controls.

Figure 3.2: An experiment laid out in a trough for evaluation of rice breeding lines for agro-morphological traits

3.5.4 Data collection

Data of the parents (BS370 and IR64) and BC_1F_2 was collected as follows: Plant height, number of productive tillers, and culm length. Plant height was measured in cm from the ground to the top of the flag leaf at flowering stage once the rice plant had reached its maximum height. Number of productive tillers was physically counted. Culm length was measured in cm from the soil surface to the panicle base. Leaf length was measured in cm from the base of the flag leaf to the tip of the flag leaf at flowering stage once the rice plant had reached its maximum height. Leaf width was measured in cm at the widest portion of the leaf blade just below the flag leaf measured using a ruler. The frequencies of awnness and anthocyanin pigmentation were recorded.

Twenty panicles were randomly selected from 20 plants in each of the three replicate and the following data was collected: Number of panicles, panicle length, grain length, grain width kernel length, kernel width and 1000 grain weight. Total panicle number was physically counted. Panicle length was measured in cm from the panicle base to the tip using a ruler. Grain length, grain width, kernel length and kernel width were measured

using a vernier caliper. Grain length was measured in mm as the distance from the base, where the grain detaches from the pedicel to the tip of the lemma while excluding any awns. Further, grain width was measured in mm as the distance across the fertile lemma and the palea at the widest point. The panicles were threshed and the grains dehusked. This was followed by measuring kernel length in millimeters, that is the distance from the base to the tip of a dehulled rice grain (milled rice), excluding the husk and awns. Kernel width which is the maximum width across the broadest part of the dehulled rice grain was measured in millimeters using a venier caliper. In addition, 1000 grain weight was measured in grams by weighing 10 samples of dehusked 1000 grains from each treatment using an electronic weighing scale. The 1000 grains were counted using an electronic seed counter to ensure accuracy.

3.5.5 Data analysis

Data was recorded in spreadsheet in a Microsoft excel and exported to SAS 9.4 software package and was subjected to analysis of variance (ANOVA) using the statistical model:

$Y_{ijk} = \mu + G_i + \beta_j + \varepsilon_{ijk}$

Where; Y_{ijk} = individual observation

μ = overall mean

G_i = effect due to the i^{th} genotype

βj = effect due to j^{th} replicate

ε_{ijk} = estimate of experimental error.

Mean separation on all component traits was done using least significant differences at p=0.05.

CHAPTER FOUR

RESULTS

4.1 Introgressing *sd-1* gene into Basmati 370

4.1.1 Selection of Basmati 370/IR64 F_1 hybrids

Fifty-three F_1 seeds were obtained from the cross between BS370/IR64 cross and nine F_1s were selected as hybrids based on anthocyanin markers on base of stem, leaf sheath and tips of seeds. Successful hybrids had anthocyanin pigmentation at the base of their stems, leaf sheath and tips of seeds (Plate 4.1), unlike the parents and the unsuccessful crosses.

(a) (b) (c-1) (c-2) (c-3)

Plate 4.1: Anthocyanin pigmentation as a morphological marker for selection of successful crosses of BS370/IR64 (a) and (b) represent Basmati 370 and IR64 parents, respectively, while c-1, c-2 and c-3 are crossbreeds with anthocyanin evident at the base, leaf blade and tips of seeds, respectively

4.1.2 Marker assisted selection for *fgr* genes in Basmati 370/IR64 F_1 hybrids

DNA from five out of nine selected F_1 hybrids was successfully amplified using the ESP/IFAP/EAP/INSP fragrance molecular marker producing two bands 355bp and 257 bp (Plate 4.2). The PCR products of BS370 showed three bands after gel electrophoresis (580 bp +355 bp + 257 bp), whereas IR64 had only two bands of 355 bp + 257 bp. Out of the nine F_1s, only five showed two bands of 355bp and 257bp. The hybrids exhibited non-fragrant heterozygote nature similar to IR64, while Basmati 370 with three bands was fragrant heterozygote.

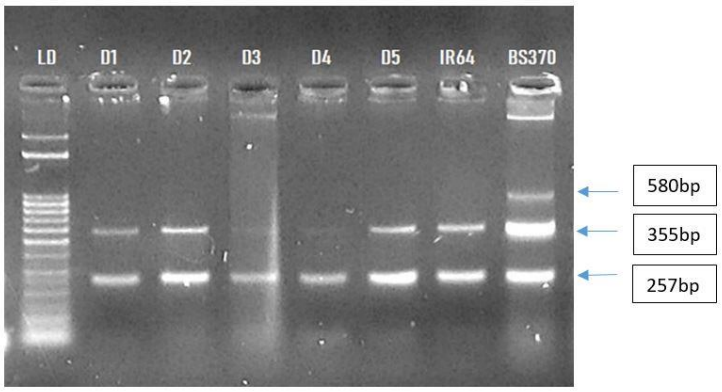


Plate 4.2: Amplification of genomic DNA of BS370/IR64 F_{1s} using ESP/IFAP/EAP/INSP fragrance markers electrophoresed on 1.5% agarose gel.
LD-50bp ladder. Entries D1-D5 are F_1 hybrids, IR64 and BS370 are the parental lines.

4.1.3 Morpho-agronomic characterization of Basmati 370/IR64 F_2 progenies

The mean height of the F_2 population was significantly lower ($p < 0.0001$), 115.49 cm than that of the female tall parent, BS370 which measured 147.66 cm (Table 4.1). The semi-dwarf male parent, IR64 had a mean height of 64.84 cm. Significant differences ($p < 0.0001$), were observed in productive tillers, with a notable decrease in tillers from the F_2 hybrids which recorded a mean of 11 tillers per hill compared with 18 tillers and 27 tillers per hill for BS370 and IR64, respectively. The mean length of the F_2 hybrid culms, 86.17 cm was notably reduced compared to that of the female tall parent which recorded a mean culm length of 90.10 cm, and was longer than the male shorter parent, IR64, with a mean of 37.90 cm.

Further, a significant difference ($p < 0.0001$), was observed in leaf length as seen in hybrids where the F_2 hybrids and IR64 had mean recordings of 29.16 cm and 28.37 cm respectively, while, BS370 had mean leaf length of 56.29 cm. There was no significant difference in leaf width ($p=0.3497$). The F_2 hybrids had a mean leaf width of 1.45 cm while BS370 and IR64 had a mean leaf width of 1.37 cm and 1.43 cm, respectively.

Table 4.1: Means of agronomic traits of BS370/IR64 F_2s and parents

Genotype	Parameter[a](Means + SE)				
	PH (cm)	PT	CL (cm)	LL (cm)	LW (cm)
F_2 hybrids	115.49^{b}+ 15.37	11.60^{c} +7.89	86.17^{b} +12.46	29.16^{b} +11.81	1.45^{a} + 0.89
BS370	147.66^{a} +11.27	18.73^{b} + 6.23	90.10^{a} +16.51	56.29^{a} +12.59	1.37^{a}+ 0.07
IR64	64.84^{c} +3.81	27.02^{a} +4.36	37.90^{c} +4.43	28.37^{b} +4.59	1.43^{a}+ 0.70
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.3497

(PH) Plant Height, (PT) Productive tillers, (CL) Culm Length, (LL) Leaf Length, (LW) Leaf width. Means sharing the same superscript letter within a column do not show significant differences at a 5% level.

4.1.4 Characterization of plant height of BS370/IR64 F2s and parents grown at KALRO Mwea

The frequency distribution of plant height among the BS370/IR64 F_2 population and its parental lines, Basmati 370 and IR64 revealed clear phenotypic variation (Figure 4.1). The tall parent, Basmati 370, exhibited a narrow range of plant heights, with the majority of individuals clustered around 140 cm – 150 cm. In contrast, the semi-dwarf parent, IR64, displayed a compact distribution, with a pronounced peak in the 60 cm –70 cm range, indicating limited variation in height. The F_2 population exhibited a broader and more continuous distribution of plant height, ranging from approximately 80 cm to 160 cm, forming a bell-shaped curve indicative of quantitative inheritance.

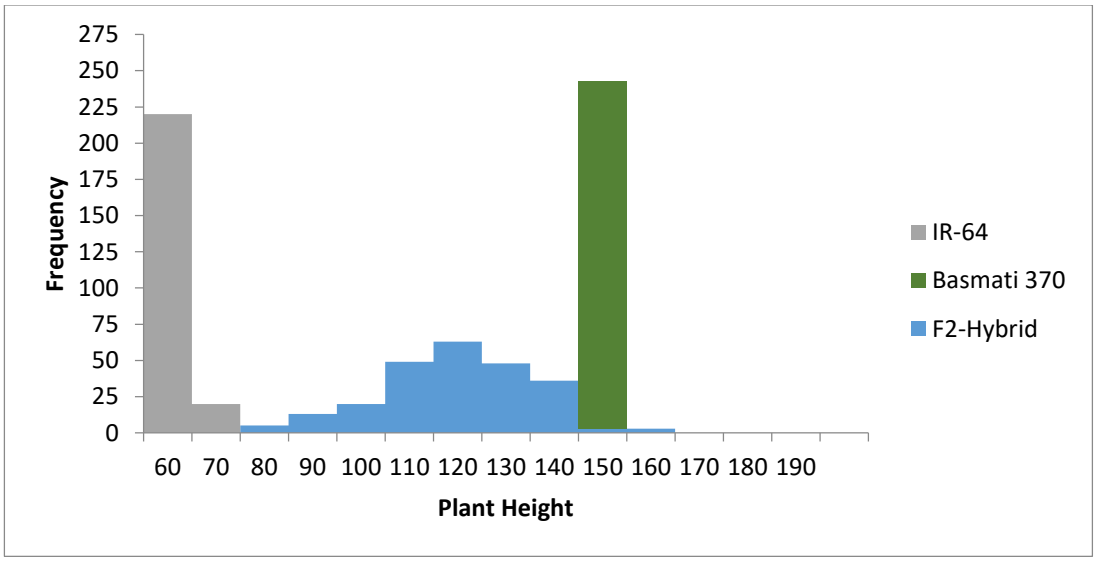


Figure 4.1: Frequency distribution of height of IR64, Basmati 370 and F_2 rice populations

Phenotypic observation among the three rice genotypes (Figure 4.2), highlights differences in plant stature and tiller characteristics. IR64 exhibits a relatively short stature with compact tillers and moderate panicle development, reflecting its semi-dwarf architecture typical of high-yielding varieties. In contrast, the F_2 hybrid (center) shows intermediate height and tiller size, combining traits from both parents, with moderately tall culms and more open, elongated panicles. Basmati 370, on the right, is distinctly taller with slender and erect culms, along with longer and more open panicles, a characteristic of traditional aromatic landraces.

Figure 4.2: Phenotypic observation of IR64, F_2 hybrid and Basmati 370 rice respectively, from the left

4.1.5 Inheritance of plant height based on Chi-Square analysis of F_2 progenies obtained from BS370/IR64 cross

The distribution of plant height in the 240 F_2 plants was distinctly bimodal, with 176 tall and 64 semi-dwarf plants (Table 4.2). Plant height segregation exhibited a strong fit, demonstrating a 3:1 ratio (χ 2 value of 0.36) between tall and semi-dwarf categories, with a p-value of 0.55.

Table 4.2: Chi-square distribution of plant height of BS370/IR64 F_2 hybrids

	Observed genotype	Expected genotype	O-E	$(O-E)^2$	$(O-E)^2/E$	χ 2	P
Tall	176	180	-4	16	0.09	0.36	0.55
Semi-dwarf	64	60	4	16	0.27		
Total	240						

χ2 = Chi-Square; P = P value

4.1.6 Pearson correlation between quantitative variables

Positive correlations were observed (Table 4.3) between plant height and awnness (r = 0.85***), plant height and apiculus pigmentation (r = 0.12**), plant height and culm length (r = 0.88***), plant height and leaf length (r = 0.66***), culm length and apical pigmentation (r = 0.34***), culm length and awnness (r = 0.67***) and culm length and leaf length (r = 0.46) and awnness and leaf length (r = 0.67***). Negative correlations were observed between plant height and productive tillers (r = -0.43***), and apiculus pigmentation correlated negatively to leaf length (r = -0.30***). Consequently, productive tillers correlated negatively to apiculus pigmentation (r = -0.49***), culm length (r =-0.54***), and awnness (r = -0.29***).

Table 4.3: Correlation coefficients of agronomic traits of BS370/IR64 F_2s rice grown at KALRO Mwea

	PH	PT	AP	AW	CL	LW	LL
PH	1						
PT	**-0.43*** **	1					
AP	**0.12** **	**-0.49*** **	1				
AW	**0.85*** **	**-0.29*** **	-0.06	1			
CL	**0.88*** **	**-0.54*** **	**0.34*** **	**0.67*** **	1		
LW	-0.04	-0.02	0.05	-0.03	-0.02	1	
LL	**0.66*** **	-0.03	**-0.30*** **	**0.67*** **	**0.46*** **	-0.04	1

*, ** and *** represent correlation coefficient significance at P<0.05, 0.01 and 0.001 respectively. (PH)Plant Height, (PT) Productive tillers, (AP) Apiculus Pigmentation, (AW) Awnness, (CL) Culm Length, (LW) Leaf width, (LL) Leaf Length

4.2 Pyramiding of aroma genes in rice

4.2.1 Morphological selection of BS370 and BS217 rice hybrids

A total of 16 seeds were harvested from the direct cross between BS370/BS217 and 27 seeds from the reciprocal cross BS217/BS370. These seeds were germinated to evaluate anthocyanin pigmentation as a morphological marker for confirming successful hybridization. Seven BS370/217 seeds germinated and 26 BS217/BS370 seeds germinated. In the BS370/BS217 cross, none of the germinated plants (zero out of seven) exhibited anthocyanin pigmentation at the leaf sheath or basal stem. In contrast, among the 26 seeds of the BS217/BS370 reciprocal cross, nine individuals showed clear anthocyanin pigmentation at the leaf sheath and base (Plate 4.3), which was used to identify them as successful hybrids.

A B C D E

Plate 4.3: Anthocyanin pigmentation as a morphological marker for selection of successful crosses BS370/217 and BS217/BS370 F_1 lines. (A) Basmati 217, (B) Basmati 370, (C) BS370/BS217 and (D&E) BS217/BS370

4.2.2 Marker assisted selection for aroma genes in Basmati 217/Basmati 370 F_2 hybrids

The parental lines, Basmati 370 and Basmati 217 had three bands of 580 bp +355 bp + 257 bp, whereas a control non-aromatic line, V1(IR-73827-23-76-15-7S) exuded one band of 355 bp. The hybrids from the cross between Basmati 217 and Basmati 370 exhibited heterozygous fragrant nature similar to the parental lines (Plate 4.4).

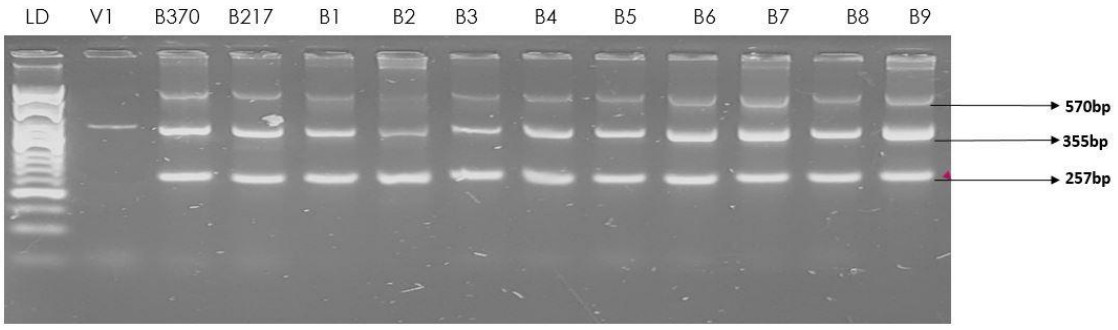


Plate 4.4: Amplification of genomic DNA of BS217/BS370 F_2 breeding population. IR-73827-23-76-15-7S (V1- non-fragrant control), B370 & B217 (parents) and B1 to B9 are F_2 hybrids. LD - 100bp ladder

4.3 Backcross lines with aroma and semi-dwarfing genes

4.3.1 Molecular marker selection for BC_1F_1 and BC_1F_2 individuals

The nine genotypes that were mentioned in section 4.1.1 were crossed to the nine individuals from section 4.2.1. Thereafter, the F_1s obtained were crossed to the recurrent parent BS370 and 82 seeds were obtained. Out of those seeds, 55 plants were selected according to semi-dwarfism, that is plants with height measuring below 110 cm. According to IBPGR, 1980, plants with height >110 cm were classified as tall and semi-dwarf plants were those of heights <110 cm. Out of these 55 plants, 36 plants were selected for molecular-marker assisted selection for the *fgr* gene. Fifteen genotypes were successfully amplified and six genotypes (3,21,22,30,31,35) were selected for their homozygosity for fragrance gene in the BC_1F_1 generation (Plate 4.5). The six genotypes were selected and selfed. The six genotypes that had fragrance markers had 257 bp implying a homozygous fragrant nature and the nine individuals with a two band profile of 257 bp and 355 bp were heterozygous non-fragrant. There was no observation of three bands (580bp+355bp+257bp) for hybrids that are heterozygous.

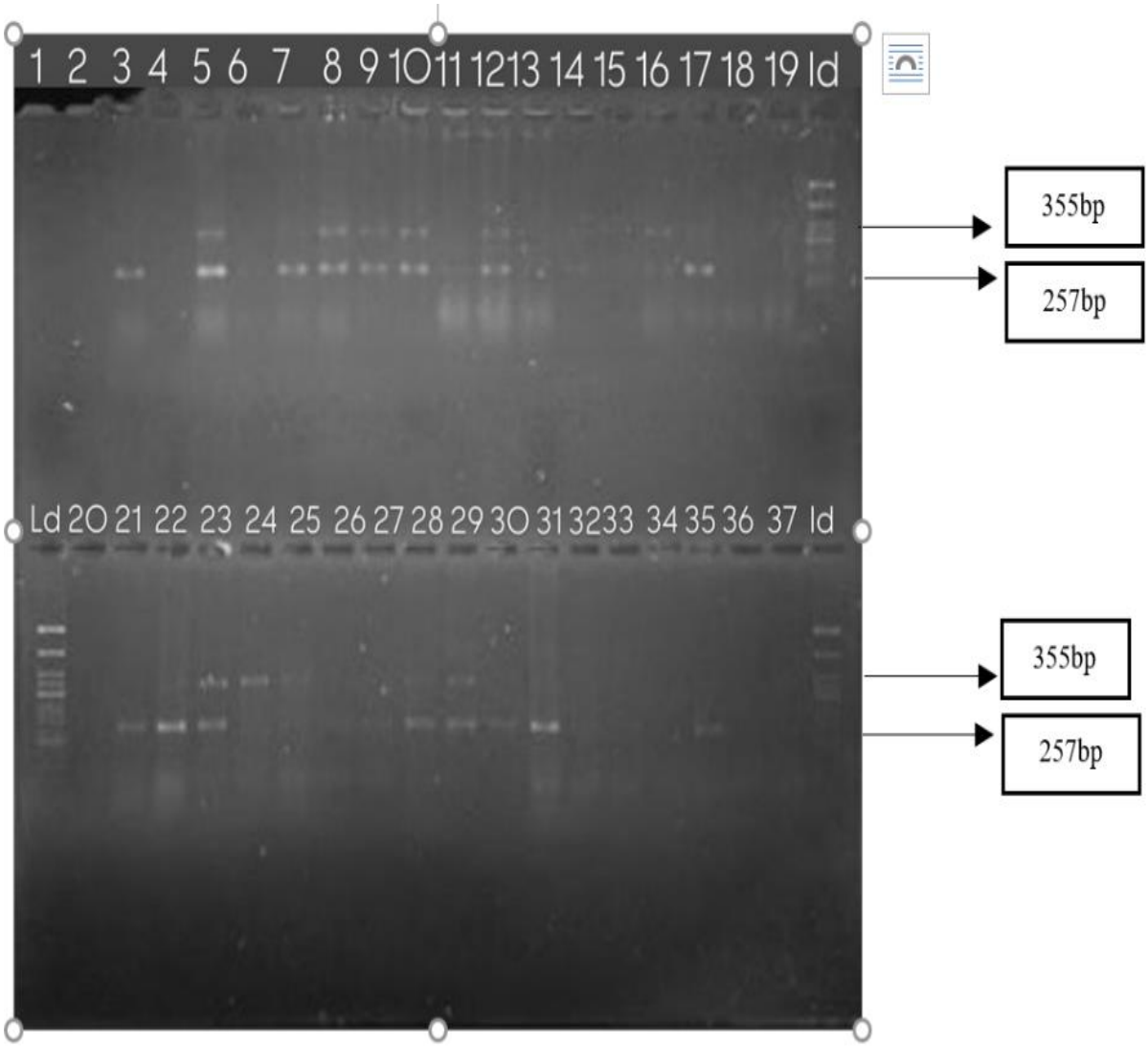


Plate 4.5: Amplification products of genomic DNA of BC_1F_1 hybrids using ESP/IFAP/EAP/INSP fragrance marker electrophoresed on 1.5% agarose gel. Ld= 100 bp ladder, 1-37 = BC_1F_1 hybrids

Seeds from the six BC_1F_1 populations were harvested and planted for selfing. A total of 66 seeds were planted. After physiological maturity, 42 plants were selected after scoring height below 110 cm. Out of the 42, 25 plants were selected based on anthocyanin pigmentation at the plant base, leaf sheath and tips of seeds. These 25 plants were screened using ESP/IFAP/EAP/INSP markers and 18 genotypes amplified (Plate 4.6). Three individuals (5,19,20) exhibited a distinct banding pattern at 257 bp, indicating a

homozygous state for the fragrant trait. Two individuals exhibited a heterozygous fragrant nature while five individuals exhibited a homozygous non-fragrant nature.

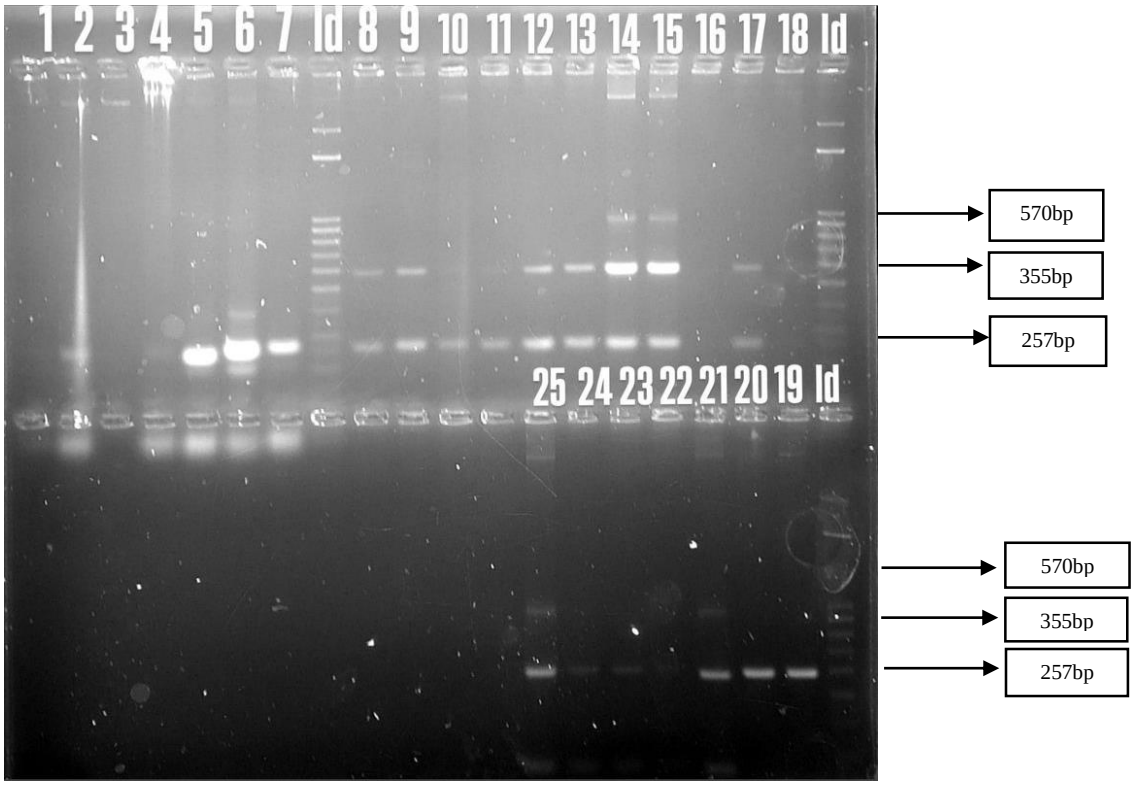


Plate 4.6: PCR amplification products of genomic DNA BC_1F_2 hybrids using ESP/IFAP/EAP/INSP fragrance marker electrophoresed on 1.5% agarose gel. Ld = 100 bp ladder, 1-25 = genotype serial numbers

4.3.2 Morpho-agronomic characterization of successful BC_1F_2 hybrids

The ANOVA results presented in the Table 4.4 provided a statistical comparison of various agronomic traits among the three genotypes; BC_1F_2, IR64, and Basmati 370. The analysis revealed significant differences ($p < 0.0001$) for most traits, except leaf width which shows no significant differences among the genotypes. The genotype, BS370 exhibited the tallest plant height (146.01 cm) and longest culm length (55.96 cm) compared to the other two genotypes. The genotype, BC_1F_2 had medium plant height (114.64 cm) and culm length (30.54 cm). For tillering ability, IR64 had the highest total number of tillers (27) and productive tillers (26), while BC_1F_2 has the lowest with 11 total

tillers, all of which were productive. Regarding anthocyanin pigmentation on leaves, base of stem and tips of seeds. The hybrids, BC_1F_2 recorded the highest score and presence of anthocyanin compared to the parents that had no apiculus pigmentation with scores of 0.00. Basmati 370 and BC_1F_2 registered presence of awns with BS370 having longer awns and scoring a mean of 1.81 while BC_1F_2 scored 0.75 and IR64 had no awns. Leaf length was significantly longer ($p<.0001$) in BS370 (54.91 cm) compared to BC_1F_2 (29.54 cm) and IR64 which recorded length of 28.69 cm. There was no difference observed for leaf width among the genotypes ($p=0.2116$). The mean number of panicles was highest in Basmati 370, recording an average of eight panicles per stem, followed by the BC_1F_2 population and IR64 with a mean of seven panicles per stem.

Table 4.4: Agronomic characters of BC_1F_2 in comparison with parents (BS370 & IR64) rice grown at KALRO-Mwea

	BC_1F_2	BS370	IR64	P-value
Plant height(cm)	114.64^b	146.01^a	64.81^c	<.0001
Culm length(cm)	30.54^b	55.96^a	28.36^b	<.0001
Total tillers	11.68^c	22.05^b	27.97^c	<.0001
Productive tillers	11.04^c	18.07^b	26.97^c	<.0001
A. pigmentation	0.73^a	0.00^b	0.00^c	<.0001
Awnness	0.75^b	1.81^a	0.00^c	<.0001
Leaf length(cm)	29.54^b	54.91^a	28.69^b	<.0001
Leaf width(cm)	1.46^a	1.36^a	1.38^a	0.2116
Panicle number	7.42^b	8.30^a	7.11^b	<.0001

Means with different superscript letter along a row are significantly different ($P\leq0.05$)

4.3.3 Seed characteristics

The parent, Basmati 370 had the longest mean grain length of 6.74 mm followed by IR64 with mean grain length of 6.45 mm and BC_1F_2 hybrid seeds with mean grain length of 5.98 mm. There was a significant difference ($p<.0001$) observed in kernel length. The variety, IR64 had the longest kernel length of 5.81 mm followed by Basmati 370 with a mean of 5.80 mm and lastly BC_1F_2 hybrid seeds with mean kernel length of 5.76 mm

(Table 4.5). The results also showed there were no significant difference (p=0.2116) among the genotypes in the grain width and kernel width. However, there was significant difference (p<.0001) observed in 1000 GW, with BC_1F_2 hybrids which registered a mean of 20.06 grams which was slightly higher than BS370 and IR64. Basmati 370 recorded a mean of 19.74 grams and IR64 recording a mean of 18.33 grams.

Table 4.5: Means of seed parameters of parents (BS370 & IR64) & BC_1F_2 grown at KALRO-Mwea station

Parameter	BC_1F_2	BS370	IR64	p-value
Grain length(mm)	5.98[a]	6.74[a]	6.45[a]	0.5254
Grain width(mm)	2.52[a]	2.52[a]	2.54[a]	0.9775
Kernel length(mm)	5.76[b]	5.80[a]	5.81[a]	<.0001
Kernel width(mm)	2.18[a]	2.18[a]	2.18[a]	0.9199
1000GW(gm)	20.06[a]	19.74[b]	18.33[b]	<.0001

Means sharing the same superscript letter within a column do not show significant differences at a 5% level. GW- grain weight

The visual comparison of seed morphology (Plate 4.7) among BS370**,** BC_1F_2, and IR64 rice genotypes, highlighted observable differences in grain length and awn presence. Basmati 370 exhibited slender, elongated seeds with long awns, while IR64 exhibited shorter, broader, and awnless seeds. The BC_1F_2 hybrid displayed intermediate morphology, with grains resembling IR64 in size and shape.

Plate 4.7: Sample seeds showing seed size from the three genotypes from the left, BS370, BC_1F_2 and IR64

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Introgressing semi-dwarfing genes into rice is a complex process and especially Basmati rice which is highly recalcitrant. This introgression of semi-dwarfing genes is often aimed at improving yield, nutrient utilization, and resistance to environmental stresses. Semi-dwarfism in rice, induced by specific genes, has been a subject of extensive research due to its potential to revolutionize rice cultivation. Some of the notable effects of introgressing semi-dwarfing genes include improved lodging resistance. Dwarf rice plants are less prone to lodging under heavy grain loads, strong winds and rainfall, hence improving harvest efficiency and reducing crop losses (Yadav et al., 2024). Superior resource allocation and nutrient usage results in higher grain yield per unit area hence plants have a higher adaptability for high-density planting, optimizing land use and increasing overall productivity (Sher et al., 2019).

In this study, where Basmati 370 (female) and IR64 (male) were crossed, the first notable observation was anthocyanin pigmentation at the base, leaf sheath and tips of seeds of some F_1 plants. This was an indicator of successful crossing. Research has previously shown that the expression of anthocyanin on the base, leaf sheath and tips of seeds may be influenced by other epistatic genes that interact with the genes responsible for anthocyanin production (Rahman et al., 2016). In the F_1 generation, the combination of alleles from the two parents may result in a genetic interaction that amplifies the expression of anthocyanin. Environmental elements including temperature, light, and the availability of nutrients can also affect anthocyanin expression (Enaru et al., 2021). In this study, the environmental conditions in the experimental setup were optimally the same hence ruling out the effect of the environment in anthocyanin expression.

Anthocyanin pigmentation in BS370/IR64 hybrids concur with previous studies that have showed similar observations when Basmati 370 and Basmati 217 were crossed with EGMS lines (Njiruh et al., 2013). In addition, anthocyanin was used to identify off types from genetically pure rice varieties in experiments (JICA, 2010). Given that both Basmati

370 and IR64 exhibited no anthocyanin pigmentation at the base, leaf sheath, or seed tips, the presence of anthocyanin pigmentation is likely due to a non-allelic two-gene interaction and a complex genetic organization (Ahirrao & Desale, 2019). After anthocyanin selection, individuals from the F_1 population were observed to have non-fragrant nature similar to IR64 through fragrant molecular aided selection. This assessment was crucial to verify the retention or loss of the recessive *fgr* genes in Basmati 370 as it is the characteristic most favored by consumers. A single gene controls the aroma in crops, and a mutation diminishes or stops the activity of amino aldehyde dehydrogenase (AADH) or betaine aldehyde dehydrogenase 2 (BADH2) which leads to the synthesis of 2 Acetyl Pyrolline (Golestan et al., 2018).

Notable effects of introgression of *sd-1* genes into BS370 included shorter F_2 hybrids than BS370. They were recorded to be shorter than Basmati 370 and taller than IR64. Considering that a single gene with a dominant allele for semi-dwarfism predominantly controls the condition in rice and a recessive allele for tallness, these findings are at par with the findings of Parimala et al. (2023). One of the most important genes used in rice breeding is the semi-dwarfing gene whose initiatives have been used to substantially boost rice yield (Nagai et al., 2018). It is positioned at 38.38 Mb on chromosome 1's long arm, it encodes GA20 oxidase 2 (OsGA20ox2) and is recessive (Andrew-Peter-Leon et al., 2021). Expression of semi-dwarfing gene in rice results in an erect, shortened culm, dark green leaves, and a high tolerance to lodging. This increases tolerance to high doses of nitrogen fertilizers, leading to increased yield per hectare.

The significant difference recorded in culm length as well as the positive correlation between plant height and culm length resonates with findings by Lv et al. (2022) that reduced plant height and leaf length conferred in plants is as a result of reduced cell elongation in the culm and leaves. In this study, reduced cell elongation was observed more in the leaves with a mean reduction of 27 cm compared to a reduction in culm length by only 7 cm. Basmati 370 naturally has long awns and IR64 has no awns while the hybrids registered short, long awns and some had no awns. Awnness is an extension of the lemma tip and plays a role in seed protection by irritability to predators. Its significant presence and positive correlation to plant height and culm length are as a result of the

dominant alleles, *Awn3-1* and *Awn4-2*, that are dominant with additive effects that confer awnness in Basmati rice (Priyanka et al., 2019). The negative correlation between tillering and plant height, culm length, apiculus pigmentation and awnness can be attributed to low plant vigor that arises from genotype combination. Tillering ability is essential for improving rice yield and can be enhanced by increased nitrogen levels. The effect of fewer the tillers is noticeable in decreased panicles per plant. On the other hand, excessive increased tillering may cause tiller death and reduced productive tillers because of higher nutrition competition, incomplete grain filling, and diminished yield (Wang et al., 2017).

Plant height segregation exhibited a strong fit, demonstrating a 3:1 ratio between tall and semi-dwarf categories. This indicated that plant height inheritance in Basmati rice is governed by a single major gene, *sd-1*. The semi-dwarfing gene dominance completely covers the effect of its recessive allele preventing the recessive allele from phenotypic display. This resonates with Bhuvasneswari et al. (2020) on a study were characterizing the *sd1-bm* allele in Pusa 1652. Results after gene analysis showed that inheritance of semi-dwarfism in rice is controlled by a monogenic and recessive.

Two historic Basmati rice cultivars that are close genetically are Basmati 217 and Basmati 370. After crossing and marker assisted identification, there was similarity in the two parental lines with both exuding fragrant heterozygosity. This was also observed in the all-other hybrids tested for fragrance genes. A study using 304 KASP markers found that these two varieties are highly similar, with low probability of being differentiated by chance (Steele et al., 2021). This suggests that Basmati 217 and Basmati 370 share a significant portion of their genetic makeup. The main gene contributing to 2-acetyl-1-pyrroline is *badh2*, located on chromosome 8. The BADH2 protein becomes truncated and non-functional when this gene is deleted (Imran et al.,2023). The mutant *badh2* transcript leads to 2-acetyl-1-pyrroline accumulation in Basmati rice. Furthermore, compared to non-aromatic rice cultivars, the *badh2* allele in all aromatic rice cultivars is characterized by 8 bp deletion and 3 SNPs in the seventh (7th) exon and 7 bp deletion in the second exon. These events suggest that the fragrance and non-fragrance genotypes diverged from the common ancestor (Vemireddy et al., 2021).

Both genetic and environmental variables influence the Basmati aroma, which is present in practically every aerial plant part except roots during every stage of crop growth (Zakaria, 2016). Although a primary gene controls scent, environmental factors and field growing methods also affect 2AP content and aroma intensity (Zakaria, 2016). Moreover, because *BADH* is closely linked to stress tolerance, plants subjected to water stress have larger concentrations of 2AP (Bradbury et al., 2005). The aroma of the Basmati type is stronger when the temperature is between 25°C and 32°C throughout the day, as opposed to 20°C to 25°C at night. When grown in the rainy season, Pusa Basmati rice had 0.03 ppm 2AP, but when grown in the summer, there was no discernible 2AP (Sagar, 2024).

Basmati 370 as the maternal parent yielded no hybrids. Basmati 217 as the female parent yields different results where successful crosses were observed from anthocyanin pigmentation at the base, leaf sheath and tips of seeds of the BS217/BS370 cross. Basmati grains have been reported to have 0.03 ppm to 0.09 ppm of 2-acetyl-1-pyrroline in milled rice (Verma & Srivastav, 2022). Basmati 217 has for a long period been preferred by consumers for its stronger aroma but detested by farmers because of its susceptibility to pest and environmental stress that affects its productivity in the long run. Generally, aromatic rice cultivars are less resilient to biotic and abiotic stressors (Ndikuryayo et al., 2022). Aromatic volatiles in rice plants have been found to play the role as signaling agents in defense against pests and diseases (Hinge et al., 2016). This could also apply to Basmati 370, considering aromatic plants produce secondary metabolites that act as antioxidants, antibiotics and antifungals. These compounds help the plant defend against biotic and abiotic stresses, including diseases and pests. Basmati 217 retains more secondary metabolites, produces less metabolites for defense hence more retainance on aroma and ultimately more diseases.

In this study, hybridization attempts between BS370 (female) and BS217 (male) were unsuccessful, with no anthocyanin pigmentation and minimal seed formation. Anthocyanin pigmentation is not consistently expressed across all Basmati rice crosses, suggesting that anthocyanin pigmentation may be governed by interactions between specific alleles unique to the parental varieties. Such variation between a direct and reciprocal cross supports the hypothesis that anthocyanin expression in rice is not solely

dependent on the presence or absence of individual alleles but on how these alleles interact within specific genetic backgrounds. This complexity underscores the need to further investigate epistatic relationships and gene regulatory networks influencing anthocyanin pigmentation in Basmati rice breeding programs. Conversely, the reciprocal cross, BS217/BS370, yielded successful crosses that produced seeds. This outcome strongly suggests the influence of maternal effect. From prior research some of the potential mechanisms underlying mechanisms that could support these results include cytoplasmic incompatibility endosperm imbalance unilateral incompatibility and hormone imbalance (Tonosaki et al., 2016; Chen et al., 2016; Haghi et al., 2022). The cytoplasm of BS370 may be incompatible with the nuclear genome of BS217, resulting in disrupted embryo or endosperm development that have been previously been reported in maize and sorghum (Bogdanova, 2019). Angiosperms need a balanced endospermal maternal to paternal genome ratio, usually 2:1, for proper seed formation. Crosses involving divergent genotypes may disrupt this balance, especially if imprinting or genomic dosage effects differ between the lines (Stadler et al., 2021). Concerning hormonal imbalance, BS370 may not provide the necessary physiological environment for seed development when crossed with BS217, possibly due to differences in flowering synchrony, hormone signaling, or nutrient provisioning. The incompatibility observed could be a case of unilateral cross-incompatibility, where one direction of the cross is rejected due to stigmatic barriers, often governed by sporophytic or gametophytic factors (Zakharova et al., 2025). Recognizing and characterizing maternal influences is essential in optimizing hybrid breeding strategies and ensuring the success of new varieties.

Selection in the crossing and backcrossing generation utilized height and anthocyanin pigmentation as well as molecular markers targeting the fragrance gene enabling early and accurate selection of desirable dwarf and aromatic genotypes. From the molecular analysis of BC_1F_1 and BC_1F_2 individuals, six and three individuals displayed a clear and distinct single band at 257 bp, which corresponds to the homozygous recessive allele responsible for the fragrant phenotype. In a review by Friedt & Ordon (2022), according to Mendel's laws, when a heterozygous plant (Fgrfgr) is crossed with a homozygous recessive (fgrfgr), the expected genotypic ratio among the progeny is 1:1, producing 50%

heterozygous non-fragrant (Fgrfgr) and 50% homozygous recessive fragrant (fgrfgr) individuals. Traits governed by single recessive genes typically segregate in predictable ratios such as 1:1 or 3:1, depending on the parental genotypes (Chatterjee, 2022). The appearance of these six and three homozygous recessive plants is consistent with this expected segregation ratio**,** indicating that the fragrance trait followed monogenic recessive inheritance (Reddy, 2023). The fgrfgr individuals are phenotypically aromatic due to the loss of functional *BADH2* gene, leading to the accumulation of 2-acetyl-1-pyrroline (2AP)**,** the compound responsible for the characteristic Basmati aroma (Huang et al., 2024). The successful identification of these fragrant plants demonstrates that marker-assisted selection was effective in isolating true-breeding lines, which can now be advanced in breeding for stable aromatic rice cultivars.

The analysis of agronomic traits among the three genotypes; BS370, IR64, and BC_1F_2 highlights the complex interplay of genetic inheritance and trait expression in rice breeding. The significant genotypic differences observed in traits such as plant height, culm length, tillering ability, anthocyanin pigmentation, and awn presence highlight the genetic divergence among the parental lines and their hybrid. According to IBPGR standards, mean plant height of 114 cm classifies the BC_1F_2 plants as tall. In contrast, the BC_1F_2 generation exhibited a noticeable reduction in height, with most individuals falling within the semi-dwarf range. The study confirms achievement of a near semi-dwarf line. The tall stature and longer culms expressed in BS370 reflect its traditional landrace background, which is often associated with lower yield but preferred grain and aromatic quality traits (Wanatabe et al., 2021). In contrast, IR64's high tillering capacity and compact plant architecture are indicative of its high-yielding, semi-dwarf genetic lineage, a trait commonly exploited in modern rice breeding for enhanced productivity (Mackill & Khush, 2018). The low tillering capacity in the BC_1F_2 line can be further harnessed under different nitrogen levels and closer spacing to explore its yield potential per acre since the number of panicles per tiller and 1000 grain weight fairly competes with the parents. The strong expression of anthocyanin pigmentation in BC_1F_2, absent in both parents, points toward complementary gene interaction or epistasis, possibly activated by recombination during hybridization. This trait can serve as a useful morphological marker

in Basmati breeding, but also reflects the underlying genetic complexity in apiculus pigment expression. Anthocyanin accumulation in plants serves multiple roles, including protection against UV radiation, involvement in hormone regulation, and responses to both biotic and abiotic stresses (Naing & Kim, 2021). Additionally, the presence of awns in BC_1F_2 and BS370, but absence in IR64, supports the dominance or partial dominance of the awned trait, with possible modulation in the hybrid background. The uniformity in leaf width across genotypes despite clear differences in leaf length suggests that some traits are more conserved and less influenced by hybridization. The relatively high number of panicles in BS370 and BC_1F_2, compared to IR64, may signal a reproductive advantage or adaptation linked to panicle architecture, which could influence grain yield under specific environments (Parida et al., 2022). Collectively, these findings emphasize the value of strategic parent selection and careful evaluation of trait segregation in early-generation populations for Basmati rice improvement.

The observed differences in grain and kernel traits among BS370, IR64, and the BC_1F_2 hybrid reflect the influence of both genetic background and hybridization effects on grain morphology and weight (Gao et al., 2024). These are key parameters in rice breeding for quality and market preference. Grain length and kernel length are important determinants of consumer appeal, especially for Basmati rice, which is prized for its long, slender grains. While BS370 maintained its characteristic long grain, the slightly shorter grain size in the BC_1F_2 suggests a reduction, potentially due to introgression from IR64, a high-yielding but shorter-grained variety. The minimal reduction in kernel length in the hybrid, despite visible variation, points to the preservation of certain endosperm traits post-hulling, governed by additive gene effects. The lack of significant variation in grain and kernel width across genotypes suggests a level of stability in these traits, which could be attributed to shared alleles or reduced sensitivity of width traits to hybridization. In contrast, the observed differences in 1000 grain weight highlight a positive heterotic effect in the BC_1F_2 generation. The slightly higher grain weight recorded in the hybrids compared to the parents implies enhanced endosperm development or better grain filling, which are often targeted traits in yield improvement (Gao et al., 2024). These findings collectively indicate that while some grain quality traits such as length may be partially

compromised in the hybrid, compensatory gains in grain weight may provide a yield advantage, supporting the strategic use of IR64 in hybridization to balance grain quality and productivity in Basmati breeding programs.

5.2 CONCLUSION

This study offers insightful information about Basmati rice's genetic advancement through targeted introgression and pyramiding strategies. The successful incorporation of the *sd-1* gene into Basmati 370 resulted in a significantly decreased plant height, a crucial agronomic characteristic that promotes greater production potential and boosts lodging resistance. The null hypothesis that states that introgression of *sd-1* genes into Basmati 370 causes semi-dwarfism in Basmati 370 is therefore rejected. This introgression was also connected to the expression of anthocyanin coloring on the grain's tips, base, and leaf sheath. The study also highlights the challenge of aroma retention when Basmati 370 is crossed with the non-aromatic variety, IR64. On the other hand, crossing of BS217/BS370 is a positive signal of pyramiding *fgr* genes from Basmati 217 into Basmati 370 proved successful. This demonstrates the feasibility of enhancing aroma expression through strategic gene stacking within genetically related aromatic lines and hence we reject the second null hypothesis tof this study.

Finally, hybrid lines that combined both the dwarfing (*sd-1*) and aroma (*fgr*) traits exhibited some superior performance compared to the original Basmati 370. These hybrids not only showed the desired reduction in plant height but also recorded improvements in grain traits such as seed size and 1000 grain weight. This leads us to reject the third null hypothesis of this study since there were significant differences (($p < 0.0001$) observed in plant height, culm length, leaf length among other agro-morphological traits in BC_1F_2 plants. These results affirm the potential of integrating molecular and phenotypic selection to improve traditional aromatic Basmati while retaining or enhancing their market-preferred traits. Overall, the findings of this study contribute significantly to the advancement of high-quality, high-performing aromatic rice lines, and offer a clear roadmap for future breeding efforts targeting the integration of elite agronomic traits with sensory quality in Basmati improvement programs.

5.3 RECOMMENDATIONS

1. The Basmati rice backcrosses from this study can serve as parents in a breeding programme.

2. Further selection for individual plant families can be done to confirm whether there are individual plants that perform better than BS370 and can be registered as a new cultivar.

3. Research on the semi-dwarf aromatic Basmati line on increased nitrogen levels and different weather conditions can be further studied to especially the stability and yield improvement.

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APPENDICES

Appendix 1. Modified Mahuku DNA extraction protocol (Mahuku, 2004).

1. Transfer DNA young leaves of 150 mg to a mortar and pestle and add acid washed sand. Macerate the leaves for 2 minutes and transfer them into a 1.5-mL Eppendorf (micro-centrifuge) tube. Add 500µL of TES extraction buffer (0.2 M Tris-HCl [pH 8], 10mM EDTA [pH 8], 0.5 M NaCl, 1% SDS).
2. Vortex the samples for 30 seconds to thoroughly mix and place the tubes in a water bath at 65 °C for thirty minutes.
3. Add one-half the volume (250µL) of 7.5M Ammonium acetate.
4. Mix and incubate the samples at -0.5 °C in a refrigerator for 10 minutes.
5. Centrifuge the samples at 15,000rpm for 15 minutes.
6. Transfer the supernatant to a new micro-centrifuge tube and add an equal volume (500µL) of ice-cold isopropanol.
7. Incubate the samples for 1-2 hours at -20 °C.
8. Centrifuge the samples at 15,000rpm for 10 minutes to pellet the DNA. Decant the supernatant and wash the DNA pellet with 800µL of cold 70% ethanol.
9. Turn the tubes upside-down on a clean sterile paper towels for 10-15 minutes to air-dry the DNA.
10. Elute the DNA from the pellet by adding 250µL 1xTE buffer (10mM Tris-HCl [pH 8], 1mM EDTA) centrifuging each time for 5 minutes at 15,000rpm to avoid collecting pelleted polysaccharides.
11. Transfer the DNA solution to a 1.5-mL micro-centrifuge tube and add 2µL of Ranse (10mg/mL) and incubate at 37~ for 60 minutes.
12. Recover the DNA and air-dry as described above. Elute the DNA in 50µL and store at -20°C.