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INTROGRESSING sd-1 GENE INTO BASMATI 370 RICE

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ABSTRACT

Rice (*Oryza sativa*) is depended upon by over half of the world's population as a primary staple food. Basmati 370 rice (BS 370) is popularly grown in many parts of the world for its good aroma and long, slender grains with excellent cooking and eating quality. However, its tallness nature and weak stem structure render it susceptible to lodging, particularly when 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 BS 370 to a short stature plant was needed to address this challenge. This research initiated a breeding program to develop a semi-dwarf rice line of Basmati 370 origin while being keen to retain the aromatic qualities. To realize this, Basmati 370 being the female parent, was crossed with a semi-dwarf variety IR64, the male parent using the emasculation and dusting method. This was followed by using unique anthocyanin pigmentation for hybrid identification to distinguish successful crosses from non-successful cross breeds. Molecular marker-assisted selection was performed to confirm whether the distinctive aroma associated with Basmati rice was present. The F_1s were advanced to F_2s. The F_2 segregation was analyzed for performance against the parental lines. The findings demonstrate the successful development of a semi-dwarf BS 370 rice line with reduced height, shortened culm length, fairly shorter leaf lengths and, anthocyanin pigmentation on the leaves, stems and tips of seeds which are a good indicator of a semi-dwarf line that will ultimately address the lodging issue once stabilized in advanced filial generations. This breakthrough offers promising prospects for reduced grain loss, and improved efficiency in mechanized harvesting. This will ultimately benefit Kenyan Basmati rice farmers and the rice industry as a whole.

Key words: Aroma**,** Basmati, lodging, *Oryza sativa*, Semi-dwarfing gene, rice, breeding

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INTRODUCTION

Over half of the global population relies on rice (*Oryza sativa*) as their primary staple food[1]. The main consumers of rice are in Asia America, and Europe. However, in Africa, rice consumption is increasing more than production as food preferences change [2]. With the increasing global population that currently stands at over 8.1 billion, rice demand will continue to increase, which results in importing between 10% and 90% of rice needs, costing Africa over USD 5.5 billion annually [3,4].

One of the main contributors to rice yield improvement is the Green Revolution of 1966, which resulted in the development and release of high-yielding varieties derived from a gene in the semi-dwarf variety Dee-gee-won [5]. The novel dwarf rice variety, IR8, resulted in high yields throughout Asia ranging from 6 t/ha to 10 t/ha in tropical irrigated lowlands. However, IR8 had several defects and high susceptibility to diseases and pests, which decreased the yield and led to poor grain quality [6]. The weaknesses of IR8 led to the introduction of a new, improved variety, IR36, in early 1980s, which had disease and pest resistance, early maturity, and relatively high yields. In 1985, another variety by the name, IR64 was introduced to farmers by the International Rice Research Institute (IRRI), Philippines, as an improvement to IR36, which further pushed to 8t/ha [7].

The semi-dwarfing gene is recessive and located in chromosome 1 [8]. It is widely used to produce varieties with an erect, shortened culm, dark green leaves, and high tolerance to lodging. Semi-dwarf stature increases tolerance to high doses of nitrogen fertilizers, leading to increased yield per hectare [9]. Nitrogen in rice facilitates swift plant growth and improves grain yield by boosting tillering capacity, leaf area expansion, grain formation, and grain filling [10]. Semi-dwarfism is a result of a deficiency of the bioactive gibberellin acid (GA) hormone that is due to a defective GA 20-oxidase gene found in the *sd-1* locus [11,12]. The semi-dwarfing gene contains gene mutations that lead to changes in GA content. This mutation blocks the GA biosynthetic pathway [13]. Gibberellic Acid-responsive dwarf mutants have been discovered in numerous species, including rice [14]. This defective GA 20-oxidase is responsible for shorter internodes and overall plant dwarfism [15]. Combining the dwarf gene, hybridization and good husbandry practices has increased rice yield from about 1.7 tonnes/hectare before the green revolution to about 17.1 tonnes/hectare [16].

Many high-yielding rice varieties lack good cooking qualities when compared to Basmati [17]. However, many consumers prefer Basmati due to its favorable cooking and eating attributes. Basmati fetches premium prices between Kes.140 to 200 (USD 1.2 to 1.8) per kilogram compared to other non-aromatic rice varieties imported from Pakistan that are sold at KES.100–120 (USD 0.9 to USD 1.1) per kilogram [18].

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Consumer preference for Basmati makes farmers in areas like India, Pakistan and Thailand produce Basmati rice to meet this demand. Basmati growing originated in India. The major Basmati rice varieties grown in the Indian subcontinent include Basmati 370, Basmati 580, Pusa Basmati and Jasmine. Efforts to improve their yield include population improvement, hybrid development, and the production of dwarf rice varieties, which have not been very successful [19]. In Kenya, locally known as *Pishori*, Basmati rice is the most preferred rice by consumers because of its aroma and good cooking qualities. The National Irrigation Authority introduced it in the late 1990s [20]. Basmati is mainly grown in Mwea irrigation scheme. The main Basmati types available and grown in Kenya are Basmati 370, Basmati 217 and, to some extent, Pusa 1120 and Pusa 1121. Basmati 370 is mainly grown because it has a slightly higher yield compared to other Basmati types. The yield of Basmati in Kenya is about 3.6 t/ha which is relatively low compared to dwarf lines like IR8, which have recorded yields up to 8 t/ha [21, 22].

Kenya's annual rice consumption is approximately 22,000 metric tonnes, while domestic production only amounts to 1,162,000 metric tonnes. This substantial gap between consumption and production creates a significant deficit, which the country addresses by importing rice to meet the demand [23]. Kenyan consumers have continued to demand better, fragrant rice, leading to a more extensive acreage of land being put under Basmati by rice farmers. However, Basmati rice continues to register low yield due to some inherent breeding weaknesses, such as a weak stem, which makes it lodge in cases of high nitrogenous fertilizer application and windy weather [24]. The tall nature of Basmati rice contributes to its susceptibility to lodging during the heading stage, resulting in pre-harvest grain loss of up to 20% in the field [25]. Continuous grain losses due to lodging exacerbating low yield in Basmati contributes to the deficit of rice consumed in the country hence high consumer prices and, ultimately, food insecurity. The low Basmati yield in Kenya brings a need for improvement to reduce the gap between demand and supply. This study sought the impact of incorporating *sd-1* genes into Basmati 370 through introgression. It is a promising indicator of the successful development of dwarf basmati breeder seeds and it is a major step in the plant breeding process.

MATERIALS AND METHODS

Study site description

The experiment was set up and crosses were conducted at Kenya Agricultural and Livestock Research Organization (KALRO)-Mwea, located in Kirinyaga County, Kenya. The site is located at 0°39' S, 37°22' E and an elevation of 1,159m above sea level [26]. The area receives about 500 to 850 mm of rainfall annually with long rains (March-June) and short rains (October-December). The site has vertisols with a pH of 5.97. The study was conducted for three growing seasons: August to

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December,2021, January to May, 2022 and August to December, 2022. Temperatures ranged from 15.6ºC to 28.6°C with a mean of 22°C.

Plant materials

Rice cultivar IR64 was used as the *sd-1* gene donor and was sourced from IRRI through KALRO –Mwea and Basmati 370 was provided by the National Irrigation Authority. Characteristic descriptors of the two test lines (Basmati 370 and IR64) are outlined in Table 1[27,28].

Experimental design and layout

Basmati 370 and IR64 seeds were soaked in 30% hydrogen peroxide for 24 hours and the seeds were placed in an oven at 35°C for 48 hours to initiate the breaking of seed dormancy. The pre-germinated seeds were sowed in plastic trays with biochar as the seedling media and given 21 days to grow before transplanting. Transplanting was done in 10-litre polythene pots with two seedlings, each with a spacing of 15cm. Soils used to fill the pots were of vertisols type. There were four planting staggers, each spaced apart by a seven-day difference. Each experimental stagger comprised 20 polythene 10-liter pots for Basmati 370 and IR64 varieties.

Development of F_1 hybrids

During heading stage, when BS 370 panicles had extended approximately one-third beyond the flag leaf, crossing was performed to generate the first filial generation. 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, which could lead to self-pollination, were cut off using clippers. Mature pollen from IR64 was extracted from the flower by carefully cutting off the panicle and tapping gently onto the emasculated Basmati 370. The emasculated and dusted flower was then covered with parchment paper to avoid unwanted cross-pollination. The experiment was performed during the August – December 2021 planting season.

Morphological selection of Basmati 370/IR64 F_1 hybrids

A total 9 individuals were planted in 20-litre plastic buckets with a circumference of 94.2cm filled with well-decomposed farm soil in a spacing of 15cm between plants. The phenotypic selection was done after vegetative growth using anthocyanin pigmentation, which was used as a morphological marker to confirm successful crosses. Anthocyanin pigmentation manifests at the base of the plant where Basmati has been used as one of the parents in the cross-breed [29]. Five F_1 plants were selected as successful crosses based on anthocyanin pigmentation.

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

The presence or absence of aroma genes in the F_1 population was confirmed using linked molecular markers. Genomic DNA from 21-day-old leaves was extracted

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using a modified DNA extraction protocol [30]. Amplification of DNA was done on a conventional 9800 thermocycler Polymerase Chain Reaction (PCR) machine. The PCR reaction mixture of 20 µl consisted of 1.5µl-50 ng/µl of rice genomic DNA, 1 µl-10 µM of the primers; Internal Fragrant Antisense Primer (IFAP), External Sense Primer (ESP), Internal Non-Fragrant Sense Primer (INSP) and External Sense Primer (ESP), 12 µl molecular grade water, 1 µl-5mM dNTPS, 4 µl Accuris™ Taq-buffer and 0.2 µl of 5 units Accuris™ Taq polymerase.

For PCR amplification, IFAP(5'CATAGGAGCAGCTGAAATATATACC3'R) and ESP (R3'TTGTTTGGAGCTTGCTGATG5'F) were used for fragrance gene amplification whereas INSP(F5'CTGGTAAAAAGATTATGGCTCA3'R) and ESP (R3'AGTGCTTTACAAAGTCCCGC5'F) for non-fragrant gene amplification [31].

The PCR profile was as follows: an initial denaturation stage of two 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 non-fragrant(355bp), homozygous fragrant (257bp), heterozygote non-fragrant(355bp+257bp) and heterozygote fragrant with three bands (580bp+355bp+257bp) according to Bradbury *et al*. [31].

Evaluation of F_2 hybrids

Experimental design and layout

After molecular amplification was performed, all selfed seeds were harvested for F_2 segregation trial. A sum of 240 F_2 seeds, along with an equivalent number of parental seeds, were planted in pots using a completely randomized design layout. Each experimental plot was constituted of 120 polythene ten-liter pots. Gapping was done for the seedlings that had major transplanting shock. The experiment was laid out as in the F_1 generation trial. All rice husbandry practices were observed.

Data collection

Data from each of the four plants within a pot were collected at maturity as follows: Plant height in cm (measured from the ground to the top of the longest flag leaf), number of productive tillers (total number of tillers with panicles), culm length in cm (the length between the ground surface and the panicle base) , presence of awns (presence was assigned a score of one, while absence was scored as zero), anthocyanin pigmentation (presence was assigned a score of one), while absence was scored as zero), leaf length(cm) and leaf width (cm).

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Data analysis

Quantitative data underwent to one-way analysis of variance (ANOVA) using Statistical Analysis System version 9.4. Separation of means was determined using Tukey's studentized Range (HSD) at a 95% confidence level using SAS software. The correlation between the quantitative traits was evaluated using the correlation coefficient(r) within an Excel Workbook. The segregation analysis of F_2 hybrid heights was conducted using the chi-square test for goodness of fit. This was used to determine significant differences between the observed and expected frequencies in the collected data set.

RESULTS AND DISCUSSION

Anthocyanin Selection of Basmati 370/IR64 F_1 hybrids

Anthocyanin in F_1s was observed at the base in (c-1), leaves in (c-2), and tips in (c-3). All successful F_1 crosses showed an intense anthocyanin concentration at the base of their stems, leaf sheath, and tips of seeds, unlike the parents and self-pollinated plants that had none. The unsuccessful crosses had no anthocyanin pigmentation, similar to the female and male parents.

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

Figure 1: Use of anthocyanin as a morphological marker for F_1 lines. (a) and (b) represent Basmati 370 and IR64 parents, respectively, while c-1, c-2 and c-3 represent the crossbreed

The observed anthocyanin pigmentation is indicator of successful crossing. This concurs with previous studies that have shown similar observations when Basmati 370 and Basmati 217 were crossed with EGMS lines and also where anthocyanin is used to identify off types from genetically pure rice varieties [32, 33]. Considering both Basmati 370 and IR64 have no traces of anthocyanin pigmentation on the base, leaf sheath, and tips of seeds, a non-allelic two-gene variation and epistasis is a possible cause of the anthocyanin pigmentation [34].

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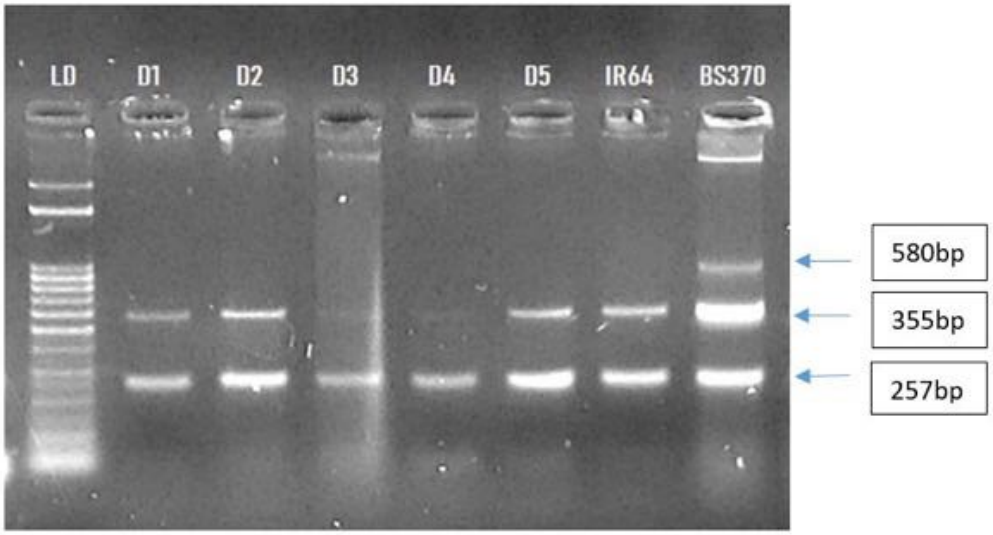
Marker selection for fragrant gene in Basmati 370/IR64 F_1 hybrids

Basmati 370 had 3 bands of 580 bp +355 bp + 257 bp, whereas IR64 had only 2 bands of 355 bp + 257 bp. D1 to D5 had 2 bands of 355bp and 257bp, which is similar to IR64 which had 2 bands of 355bp and 257bp. The hybrids exhibited non-fragrant heterozygote nature similar to IR64, while Basmati 370 with three bands was fragrant heterozygote.



LD-50bp ladder. Entries D1-D5 are F1 hybrids, IR64 and BS370 are the parental lines

Figure 2: Amplification of molecular markers linked to aroma genes

This assessment was crucial to verify the preservation of the aromatic genes in Basmati 370 as it is the characteristic most favored by consumers. The fragrance in crops is governed by a single gene, and a mutation that reduces or eliminates the function of betaine aldehyde dehydrogenase 2 (BADH2) or amino aldehyde dehydrogenase (AADH) which leads to the synthesis of 2 Acetyl Pyrolline [35]. Individuals in the F_1 population of Basmati 370 and IR64 displayed a heterozygous non-fragrant nature, similar to the male parent (IR64).

Morpho-agronomic characterization of Basmati 370/IR64 F_2 progenies

Positive correlation was observed between plant height and awnness (r = 0.846299), plant height and anthocyanin pigmentation (r = 0.115305), plant height and culm length (r = 0.879908), plant height and leaf length (r = 0.655931), culm length and apical pigmentation (r = 0.335853), culm length and awnness (r = 0.669972) and culm length and leaf length (r = 0.463267) and awnness and leaf length (r = 0.665009). Negative correlations were observed between plant height and productive tillers (r = -0.42844), and anthocyanin pigmentation correlated negatively to leaf length (r = -0.30046). Consequently, productive tillers correlated negatively to

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anthocyanin pigmentation (r = -0.48647), culm length (r =-0.53535), and awnness (r = -0.29062).

The significant difference recorded in culm length as well as the positive correlation between plant height and culm length resonates with earlier findings that reduced plant height and leaf length conferred in plants is as a result of reduced cell elongation in the culm and leafs [36]. In this study, reduced cell elongation was observed more in the leaves with a mean reduction of 27 cm compared to a reduction of culm by only 7 cm. Basmati 370 naturally has long awns and IR64 has no awns while the hybrids registered short, long 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 [37]. Reduced tillering ability was also observed as an effect of introgression of *sd-1* genes into Basmati 370. The negative correlation between tillering and plant height, culm length, anthocyanin 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 fewer the tillers, the fewer the panicles. On the other hand, increased tillering may cause tiller death and reduced productive tillers because of higher nutrition competition, incomplete grain filling, and diminished yield. Among the parameters, the F_2 plant height registered a significantly shorter mean length of 115.487cm compared to the female tall parent, which registered a mean of 147.662cm for Basmati 370. In contrast, the semi-dwarf male parent, IR64, registered a mean of 64.845cm. Significant difference was observed in productive tillers, with notable decrease in tillers from the F_2 hybrids which registered 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, 86.171cm, was notably reduced compared to that of the female tall parent which registered a mean culm length of 90.104cm, and was longer than that registered by the male shorter parent, IR64, with a mean of 37.895cm. A significant difference was observed in leaf length as seen in hybrids where the F_2 hybrids and IR64 had almost similar mean recordings of 29.155cm and 28.368 cm, respectively while BS370 registered mean leaf length of 56.285cm. A significant difference was also observed in awnness with Basmati 370 bearing long awns compared to the F_2 hybrids, which had some plants with awns and others with absent awns and IR64 registering no awns. Anthocyanin pigmentation was observed in the F_2 hybrids but was absent in the parental lines. There was no significant difference in leaf width among the hybrids and parents.

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Characterization of plant heights of BS370/IR64 F_2s and parents

In Figure 3, most of the samples in IR64 had plant heights ranging from 50cm to 80 cm, whereas Basmati 370 ranged from 100cm to 160cm. The F_2 segregating population ranged from 80cm to 160 cm. There was an intersection between Basmati 370, and a segregating population was observed between 80cm and 160cm. The intersection between IR64 and the segregating population is between 80cm and 90cm. A small percentage of the F_2 population and an average height of 80cm were observed in IR64. Dwarf rice plants are less prone to lodging under heavy grain loads, strong winds and rainfall hence improving harvest efficiency and reducing crop losses [38]. It also leads to increased grain yield per unit area due to improved resource allocation and better utilization of nutrients [39]. Dwarf rice varieties have a higher adaptability for high-density planting, optimizing land use and increasing overall productivity. They were recorded to be shorter than Basmati and taller than IR64 as seen phenotypically in Figure 4.

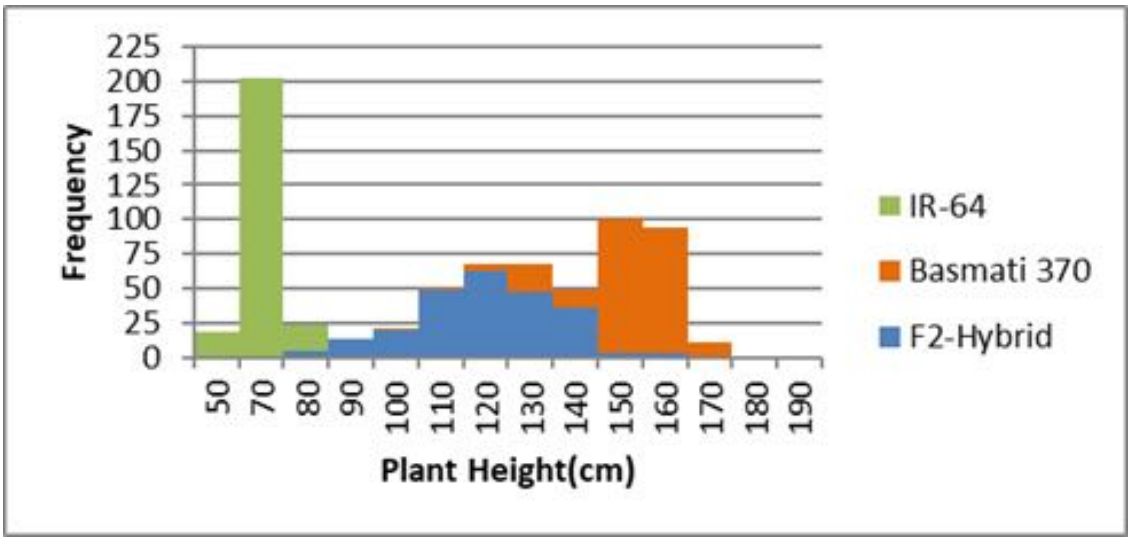

Figure 3: Frequency distribution of heights IR64, BS370 and F_2 populations

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Figure 4: Phenotypic observation of IR64, F_2 hybrid and Basmati 370 respectively, from the left

Considering semi-dwarfism in rice is primarily controlled by a single gene with a dominant allele for semi-dwarfism and a recessive allele for tallness, these findings are at par with the findings of Gaur [40]. The semi-dwarfing gene in rice is among the most crucial genes employed in rice breeding initiatives to substantially boost rice yield [41]. It is positioned at 38.38 Mb on chromosome 1's long arm, it encodes GA20 oxidase 2 (OsGA20ox2) and is recessive. Its expression 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.

Chi-Square height analysis of the F_2 BS370/IR64 progenies

A chi- square analysis (table 4) for a single gene segregation of the F_2 plants for the trait plant height, classification of the plants was applied as follows; >110 cm were classified as tall and semi-dwarf plants were those of heights <110 cm. Parental heights averaged 147.66 and 64.84 cm, categorizing the parents as tall and semi-dwarf. The distribution of plant height in the 240 F_2 plants was distinctly bimodal, with 176 tall and 64 semi-dwarf plants (Table 4). Plant height segregation exhibited a strong fit, demonstrating a 3:1 ratio (χ 2 value of 0.3555) between tall and semi-dwarf categories, with a p-value of 0.550985. This indicates that plant height inheritance in Basmati rice is governed by a single gene. According to the Chi-square analysis, it is evident that the p-value exceeds that of the null hypothesis. The null

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hypothesis is therefore rejected, meaning that introgression of *sd-1* genes into Basmati 370 causes semi-dwarfism in Basmati 370. This means tallness in Basmati is controlled by a dominant gene. Its dominance completely covers the effect of its recessive allele preventing the recessive allele from phenotypic display.

CONCLUSION AND RECOMMENDATION FOR DEVELOPMENT

Basmati tallness is a prevalent precursor for causing lodging in Basmati plants. Introgression of *sd-1* gene into Basmati 370 leads to a shorter plant stature, and reduced culm and leaf length. The segregation patterns of semi-dwarfing genes observed in the F_2 generation, particularly for plant height, support a monogenic inheritance model, providing valuable insights for rice breeding strategies to improve yield and agronomic performance. Selected semi-dwarf BS370 lines can be further tested to investigate the relationship between semi-dwarfism and the degree of lodging under different nitrogen application levels.

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Author Contributions

Conceptualization, Faith Gatere, Paul Nthakanio and James Kanya; Data curation, Faith Gatere, Paul Nthakanio, James Kanya and Esther Arunga; Formal analysis, Faith Gatere, Paul Nthakanio and Esther Arunga; Funding acquisition, Paul Nthakanio and James Kanya; Investigation, Faith Gatere and Paul Nthakanio; Project administration, Paul Nthakanio; Supervision, James Kanya and Esther Arunga; Writing – original draft, Esther Arunga; Writing – review & editing, Faith Gatere, Paul Nthakanio, James Kanya and Esther Arunga.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Table 1: Descriptor of parental materials (Basmati 370 and IR64)

TRAIT	BASMATI 370	IR-64
Pedigree	Traditional Basmati variety from India	Cross between IR5657-33-2-1 and IR2061-465-1-5-5 developed by IRRI
Plant height	160cm-175cm at maturity	Approximately 100 cm at maturity
Ecological Requirements	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 about 117 days
Grain Type	Extra-long, slender, aromatic grains	Medium-long, slender, non-aromatic
Potential Yield	2.5 - 4.0 tons/ha	8.28 - 8.76 tons ha^{-1}
Rain-fed or Irrigated	Mostly irrigated	Grows well under irrigated conditions but also performs in rain-fed lowland areas
Upland/Lowland Type	Lowland, irrigated	Primarily lowland; adaptable to both irrigated and rain-fed systems
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	Tolerant to blast, bacterial leaf blight, and pests like brown and green plant hopper
Tolerance to Abiotic Stresses	Low tolerance to drought and salinity	Moderate tolerance to submergence, drought and salinity
Grain Quality	Aromatic, flavor, and fluffy texture when cooked	Non-aromatic, good grain quality and soft texture

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Table 2: **Mean performance of Agronomic characters of the F_2 population of BS370/IR64 cross breeds and parents**

Analysis of variance showing means of agronomic variables (Mean ± SE)							
Parameter	**PH (cm)**	**PT**	**CL (cm)**	**LL (cm)**	**LW (cm)**	**AP**	**AW**
F_2 Hybrids	115.487^b ± 0.573	11.604^c ± 0.294	86.171^b ± 0.464	29.155^b ± 0.439	1.452^a ±0.033	0.800^a ± 0.004	0.758^b ± 0.016
BS370	147.662^a ± 0.419	18.729b ± 0.232	90.104^a ±0.615	56.295^a ± 0.469	1.368^a ± 0.002	0.004^b ± 0.000	1.816^a ± 0.016
IR64	64.845^c ± 0.142	27.020^a ± 0.162	37.895^c ± 0.165	28.368^b ± 0.170	1.430^a ± 0.026	0.000^b ± 0.000	0.000^c ± 0.000
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.3497	<0.0001	<0.0001

(PH) Plant Height, (PT) Productive tillers, (CL) Culm Length, (LL) Leaf Length, (LW) Leaf width, (AP) Anthocyanin Pigmentation, (AW) Awnness. Means sharing the same superscript letter within a column do not show significant differences at a 5% level. SE: standard error.

Table 3: **Correlation matrix among variables**

	PH	**PT**	**AP**	**AW**	**CL**	**LW**	**LL**
PH	1						
PT	**-0.42844**	1					
AP	**0.115305**	**-0.48647**	1				
AW	**0.846299**	**-0.29062**	-0.05954	1			
CL	**0.879908**	**-0.53535**	**0.335853**	**0.669972**	1		
LW	-0.03745	-0.02084	0.049658	-0.02916	-0.02115	1	
LL	**0.655931**	-0.03216	**-0.30046**	**0.665009**	**0.463267**	-0.04153	1

Bolded values are different from 0 with a significance of p = .05. (PH)Plant Height, (PT)Productive tillers, (CL)Culm Length, (LL) Leaf Length, (LW)Leaf width, (AP)Anthocyanin Pigmentation, (AW)Awnness

Table 4: **Chi-square distribution of plant height**

	Observed genotype	**Expected genotype**	**O-E**	**$(O-E)^2$**	**$(O-E)^2/E$**	**χ^2**	**P**
Tall (>110 cm)	176	180	-4	16	4	0.3555	0.550985
Semi-dwarf (<110 cm)	64	60	4	16			
Total	240	1.0					

$\chi 2$ = Chi-Square; P = P value

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