MARKER AIDED SELECTION FOR DWARFNESS IN F_2 POPULATION OF RED RICE VARIETY HPR-2795

THESIS

By

AMAN VERMA

(A-2019-30-036)

Submitted to

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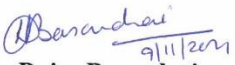
CERTIFICATE-I

This is to certify that the thesis entitled, **"Marker aided selection for dwarfness in F_2 population of red rice variety HPR-2795"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of Genetics and Plant Breeding of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mr. Aman Verma (Admission No. A-2019-30-036)** son of **Mr. Santosh Kumar Verma**, under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Palampur, H.P
Date: 9 November,2021



Dr. Daisy Basandrai
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CERTIFICATE- II

This is to certify that the thesis entitled, **"Marker aided selection for dwarfness in F_2population of red rice variety HPR-2795"**, submitted by **Mr. AmanVerma (Admission No. A-2019-30-036)**son of **Mr. Santosh Kumar Verma**, to CSK Himachal Pradesh KrishiVishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of Genetics and Plant Breeding has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Needless to say, all omissions and errors are mine.

Place: Palampur

Date: 9th November, 2021

(AMAN)

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LIST OF ABBREVIATIONS USED

IRRI	**:**	**International Rice Research Institute**
SES	**:**	**Standard Evaluation System**
G	**:**	**Gram**
Mm	**:**	**Millimeter**
CD	**:**	**Critical Difference**
Df	**:**	**Degree of freedom**
PCV	**:**	**Phenotypic Coefficient of Variation**
GCV	**:**	**Genotypic Coefficient of Variation**
*****	**:**	**Significant**
NS	**:**	**Non-significant**
RBD	**:**	**Randomized Block Design**
%	**:**	**Percent**
SE	**:**	**Standard Error**
SC	**:**	**Standard Check**
q/ha	**:**	**Quintal per hectare**
h^2_{ns}	**:**	**Heritability (narrow-sense)**
σ^2A	**:**	**Additive genetic variance**
σ^2D	**:**	**Dominant genetic variance**
σ^2E	**:**	**Environmental variance**
CV	**:**	**Coefficient of Variance**
GA	**:**	**Genetic Advance**
ANOVA	**:**	**Analysis Of Variance**

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ABSTRACT

The present study was carried out keeping in view, the lodging problem due to tall height of the otherwise popular red rice variety HPR-2795 which necessitates to incorporate a semi dwarfness gene in this variety. The morphological and foreground selection for semi dwarfness in segregating progenies of crosses between HPR-2795 and semi-dwarf basmati variety PB-3 using SSR marker was carried out. Besides this, the phenotypic study of 165 F_2 plants along with the parents and HPR-2880 was carried out during *Kharif* 2020 at the experimental farm of the Department of Genetics and Plant Breeding, CSKHPKV, Palampur. Fifty five randomly selected F_2 plants (out of total 165 F_2 plants) along with their parents were screened for the presence of *sd1* gene using SSR marker. Banding pattern revealed that the parent PB-3 showed band at 300 bp while parent HPR-2795 showed presence of bands at 280 bp indicating the presence and absence of *sd1* gene respectively. Molecular analysis implies that 44 F_2 plants were homozygous for *sd1* gene, three plants were having both the bands which represented the heterozygosity and in 8 F_2 plants *sd1* gene was absent. Out of 44 F_2 plants, twenty six F_2 plants showing presence of *sd1* gene along with red seed color were selected for further breeding program. Inheritance pattern of seed coat colour and plant height was studied in 165 F_2 plants. A segregation ratio of 9 red :3 segregating : 4 white was observed, which indicated the presence of supplementary gene action for seed coat color. For plant height, segregation ratio of 3 tall :1 dwarf was observed, which indicated that plant height is controlled by single dominant gene. Segregation for plant height was based on cut off height for tall and dwarf phenotype set at 123.5 cm, which is the mid parent value both the parents. Frequency distribution studied for agro-morphological traits indicated continuous frequency distribution and presence of few transgressive segregants indicating the quantitative nature of traits studied. The high magnitudes of PCV and GCV were recorded high for thousand grain weight and flag leaf length. Heritability in broad sense was high for plant height followed by 1000 grain weight, grain length, grain breadth, flag leaf length and L/B ratio, revealing lesser influence of environment and greater role of genetic component of variation. High genetic advance was observed for 1000 grain weight followed by flag leaf length, L/B ratio, plant height, grain length and grain breadth indicating that these characters are governed by additive genes and selection will be rewarding for improvement of such traits. Grain yield/plant was positively and significantly correlated with number of tillers/plant suggesting effective selection for these traits. The highest direct positive effect was exhibited by L/B ratio followed by number of tillers/plant, thousand grain weight, and panicle length suggesting as the best selection indices for grain yield improvement.

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INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop and staple food for 60% of the world's population (Khush, 2005). It has two cultivated and 22 wild species. It is grown in an area of 162.05 million hectares, with a production of 755.47 million tonnes and a productivity of 4.06 tonnes/ha in the world. In India, rice is grown in an area of 43.78 million hectares with production of 117.64 million tons and productivity of 4.05 tons/ha (FAOSTAT, 2019) whereas, in Himachal Pradesh, it is cultivated in an area of 71.81 thousand hectares with production and productivity of 116.50 thousand tons and 1.62 tons/ha, respectively (Directorate of Economics and Statistics, 2018-19). Drought, lodging, weeds, soil acidity/sodicity/poor fertility, diseases, insect pests etc. are the factors that contribute to low yields. India is one of the centers of origin of rice leading to enormous diversity. White rice is the main food, however many specific cultivars of rice with colour pigments i.e. black, purple, red etc. are quite popular and traditionally consumed in China, Korea, Japan and many other countries in Southeast Asia. Presently, whole grain pigmented red rice has been classified as functional food because it possesses phenolic compounds i.e. anthocyanins in the pericarp (Ryu et al., 1998; Abdel-Aal et al., 2006; Yawadio et al. 2007). The outer pericarp of red rice contains polyphenols, anthocyanin and possess antioxidant properties.

Rice has a wide genetic diversity, with thousands of varieties farmed around the world, including 6000 variants in India (Rathna et al., 2019). Until 1970, India had over 110,000 rice land races, all of which were wiped out due to the Green Revolution which emphasized on hybrid crops and monoculture. Rice types with bright colours are valued for their health benefits, the red pericarp and/or red tegmen gives its name i.e. 'red rice'. Iron and zinc are abundant in red rice cultivars. Red rice is quite prevalent and commonly cultivated in the Himalayas, Southern Tibet, Bhutan, and areas of North East and South India. It is characterized by the pigmented pericarp due to the presence of anthocyanin, pubescent light-green leaves and pubescent seeds, which distinguishes it from cultivated rice (Diarra et al., 1985). It is also taller than the majority of present day cultivars. In addition to it, zinc (Zn) and iron (Fe) content of red rice is 2-3 times higher than that of white rice (Ramaiah and Rao; 1953) and is also rich source of micronutrients *viz.* Mg, Na, K, Mn and Cu, hence it is considered as ideal health and functional food.

Red rice is an ancient treasure of Himachal Pradesh and a valuable commodity in export market. It has importance in various ceremony and local festivals. Red rice varieties have various medicinal values i.e. in hypertension, leucorrhea, abortion complications, constipation, digestive problems, etc. (Laandrault *et al.*, 2001; Shahidi, 2000; Wilson, 1999). With the increase in life style related diseases like diabetes, cancer and heart problems, scientists are looking at red rice for its antioxidant property, high nutritive, medicinal and scavenging activity due to presence of polyphenols. The red rice is used for preparing vinegar, tart, cosmetics, red kojic, and red rice yeast, which is utilized for medicinal purposes. In Himachal Pradesh, the cultivation of Jatu rice is a religious event. Red rice varieties have many special attributes i.e. resistance to abiotic stresses, aroma, resistance to prevalent rice diseases. Varieties like Hbj-Aman 3 (Assam), Br 14, Br 15 (Bihar), Taotabi (Manipur), Varappu kudaincha (Tamil Nadu) are tolerant to deep water; Jatu, Matali (Himachal Pradesh), Majehra, Jhaildu (Uttar Pradesh), Lal Nakanda 41 (Punjab) are tolerant to cold and Mtu 17, Mtu 18 (Andhra Pradesh), Kalla modan, Chennellu, Ptb 35, Ptb 28, Ptb 12, Ptb 7, Ptb 2 (Kerala) are tolerant to drought (Ahuja et al. 2007).

There is a great diversity of agro-climatic condition under which rice is grown in Himachal Pradesh, hence, there is a great variability among the landraces of rice crop as well. Some of the traditional local red rice varieties like Chhohartu (Rohru, Shimla), Sukara, Jhinjan, and Karad (Chamba), Jattoo, Deval, and Matali (Kullu) and Desi dhan, Kalizhini, Achhoo, and Begmi (Kangra) are being grown in different regions of Himachal Pradesh. However, the prevailing traditional genotypes have inherent problems of poor yield, poor organoleptic taste, aroma, lodging, poor response to nitrogen fertilizers and blast susceptibility.

Landraces of red rice played an important role in the food security and sustainable development of agriculture, and as valuable genetic resource for rice genetic improvement. The color of red rice is due to its anthocyanin content and has a nutty flavor and as it is eaten with the germ intact it has the highest nutritional value. In Himachal Pradesh, two local landraces of red rice have been released as varieties i.e. HPR-2720 and HPR-2795. HPR-2795 is a high yielding, early maturing disease resistant variety. It has thick stem and have large and long flag leaf which increases the photosynthetic efficiency and thereby ultimately the yield. However, variety HPR-2795 is very tall and is prone to lodging which may reduce its production. Hence, there is need to incorporate semi-

dwarfness and blast resistant character in red rice variety HPR-2795 to prevent losses due to lodging and diseases.

Semi-dwarf rice (*Oryza sativa* L.) was introduced in the 1960s, resulting in record productivity gains across Asia. In current rice cultivars, the primary semi dwarfing allele, *sd-1*, discovered in the Chinese variety *Dee- geo-woo-gen* (DGWG), is widely employed. Dwarfism is caused by the deficiency of plant growth hormones gibberellin (GA) in *Sd-1*. The semi-dwarfing gene (*sd-1*) produces a shorter culm with increased lodging resistance and a better harvest index, allowing for more nitrogen fertilizer use due to its recessive nature. It was crossed with Peta (tall) to produce the semi-dwarf cultivar IR8, and served as the foundation for the development of other high-yielding semi-dwarf plant varieties (Tomita and Ishii, 2019). Marker assisted selection of the recessive *sd-1* allele was based on the gene-derived markers formed from the sequence information of cloned *sd1* and were widely used in breeding populations (Ellis and Spielemeyer 2002; Rajpurohit et al. 2011).

Keeping in view of the findings, the present studies were designed to perform marker-assisted selection for dwarfness in the F_2 population with the following objectives:

1.) Morphological and foreground selection for semi dwarfness in segregating progenies of crosses between HPR-2795 and semi-dwarf rice variety PB-3 using SSR marker and

2.) Phenotypic study of F_2 population for important agronomic traits like seed color, flag leaf size and panicle length.

2. REVIEW OF LITERATURE

Rice is the world's second most extensively grown cereal crop and is the staple food of more than half of the world's population. Rice production has been hampered by a various biotic and abiotic stresses. Many traditional rice varieties have the problem of lodging because of tall height which leads to reduction in yield. In contrast to the conventional breeding programs, molecular analysis and Marker Assisted Selection (MAS) has emerged as a powerful tool in identifying the genes responsible for the improvement of these characteristics and paved a way for efficient breeding programs. Incorporation of semi dwarfness in traditional rice cultivars can eliminate the problem of lodging and MAS can be used as an efficient method for quick and precise identification of the genes responsible for it. It can be used to track the presence or absence of genes in breeding populations and can be used in combination with traditional breeding methods. MAS can improve plant breeding efficiency by allowing accurate transfer of genomic regions of interest and faster recovery of the recurrent parent genome (Wijerathna 2015).

Knowledge of variability parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, and genetic advance aid in the identification of superior genotypes with greater yield and nutrient quality potential. It is critical for effective breeding programmes to determine the relationship between yield and its component traits, as well as their direct and indirect effects on yield contributing components. By identifying promising parent genotypes, diversity can be harnessed by an appropriate breeding programme for increasing qualitative and quantitative traits of rice cultivars.

Relevant literature has been reviewed on the morphological and molecular traits linked to rice crop under the following headings:

2.1 Semi-dwarfness in rice

2.2 Marker Aided Selection

2.3 Morphological traits and pericarp color in rice

2.4 Genetic variability, heritability and genetic advance

2.5 Correlation coefficient and path analysis

2.1 Semi-dwarfness in rice:

The discovery of sd1 mutants in rice contributed in a semi-dwarf phenotype that breeders used to improve yields. In the 1960s, research on sd1 mutants prompted the "green revolution" for rice, which averted famine. The smaller plant height imparted by the sd1 allele confers lodging resistance even with high nitrogen fertiliser levels. Guang-chang-ai-carrying sd1 was the first high-yielding rice variety to take advantage of the semi-dwarf trait, with the goal of significantly increasing rice yield in China. The International Rice Research Institute bred IR8, known as Miracle Rice, in 1966 using the dwarf source Dee-geo-woo-gen and the Indonesian high-quality tall rice variety Peta. Ever since, a large number of semi-dwarf and high-yielding rice varieties containing the semi-dwarf gene sd1 have increased rice yield by 20% (Peng et al, 2021).

Foster and Rutger (1978) studied the inheritance pattern in ten parent diallel cross of diverse rice cultivar. Parents included eight semi-dwarf lines and two japonica lines. Data from F_1, F_2 and F_3 generations indicated that variation in height was due to 3 major genes with additive loci effect. Varieties D51, 72/2234-11 and G33 (derived from Dee-geo-woo-gen) possessed an allelic, partially recessive semi dwarfing gene (*sd1*). Additional semi dwarfing gene were detected in D66 (sd_2, fully recessive) and in CI 9858 (sd_3, partially to fully recessive). It was concluded that relative magnitude of the additive effects was $sd_1 > sd_2 > sd_3$ and variation between parent and F_1 and parent and F_2 was due to additive and dominance effect. Epistasis was not detected.

Jiachang et al (1997) crossed dwarf rice varieties Guangchong and Xiaolitetepu, reciprocally and test crossed with cvs. Aizizhan (*sd1*), Dijiaowujian (*sd1*), Xuheaizao sd s(t), and 80-7010 dg(t). The plant height of the F_1 populations outgrew their parents, with some being 19%-107% higher than the higher parents. In F_2 populations, of crosses between Guangchong and the varieties with the gene *sd1* gene or dg (t) developed three types of plants, i.e. high, dwarf, and special dwarf, with in a segregating ratio of 9:6:1. However, In F_2 populations from crosses between Xiaolitetepu and various dwarf varieties segregated into high, dwarf, and special dwarf plants but the proportion was not consistent and were widely dispersed. The dwarf genes in varieties Guangchong and Xiaolitetepu were non allelic, and also they were not allelic to the *sd 1, sd 2*s(t), and dg(t) genes.

Monna et al. (2002) studied numerous PCR-based marker technologies viz. cleaved amplified polymorphic sequence, derived-CAPS and single nucleotide polymorphisms. In all, 3477 segregants were analysed, revealing 1 ORF in a 6-kb candidate interval. Normal-type rice cultivars had the same sequence in this region, having three exons i.e. 558, 318 and 291 bp and two introns 105 and 1471 bp. Dee-Geo-Woo-Gen-type sd-1 mutants had a 383-bp deletion from the genome (278-bp deletion from the expressed sequence), from the centre of exon 1 to upstream of exon 2, with a 105-bp intron, resulting in a frame-shift that results in a termination codon after the deletion site. Calrose 76, a radiation-induced sd-1 mutant, features a 1-bp alteration in exon 2 which resulted in an amino acid substitution. There was at least one more locus of gibberellin 20-oxidase which suggested the existence of at least one more locus which might have prevented severe dwarfism in sd-1 mutants.

Asano et al. (2007) reported that the short stature of IR8 was due to loss-of-function of the *sd1*gene which encoded a *GA20 oxidase-2* (*GA20ox-2*) that catalyzed late steps of gibberellin biosynthesis. Sequence analysis of the *sd1* locus of 57 semi-dwarf varieties revealed at least 7 *sd1* alleles used in the breeding of semi-dwarf rice varieties in China, USA and Japan. Hence, use of high number of different alleles controlling the same target trait, highlighted that mutations in *GA20ox-2* induced an agronomically beneficial changes in rice.

Neerja et al. (2009) tried to identify alternative sources of the DGWG allele of *sd1* gene among 29 induced and 3 spontaneous dwarf accessions using markers for the DGWG allele of *sd1* gene and exogenous gibberellic acid application (GA3). Examination of DGWG allele of the *sd1* gene and the GA3 response revealed dwarf accessions with the DGWG allele and without the DGWG allele of the *sd1* allele, with variable response to GA3. 22 of 32 dwarf accessions with variable responses to GA3 showed absence of DGWG allele of *sd1* gene which could be used as an excellent alternate source for DGWG allele of *sd1* gene and could be utilised to enhance the genetic base for plant height, reducing the possibility of genetic susceptibility.

Tomita (2009) fixed an allele of gene *sd1* by eight recurrent backcrosses. It resulted in the production of a semidwarf variant of Koshihikari named ‘Hikarishinseiki' which was about 20 cm shorter than Koshihikari and had 99.8% of the Koshihikari genome. It was highly resistant to lodging and was registered as the first semidwarf form of Koshihikari.

Vikram et al. (2016) studied the genetic diversity in some Indian rice cultivars using molecular markers. “Tall” allele of the *sd1* gene was identified in well-known drought-tolerant landraces or traditional types, indicating the tight linkage, pleiotropy, or both linked with this gene.

Tomita and Ishimoto (2019) compared gene effects on the yield performance among promising semi dwarf genes viz. novel gene d60, representative gene *sd1* with two sources IR8 and Jukkoku and double dwarf combinations of d60 with each *sd1* allele, in a Koshihikari background. Hence, semi-dwarfing gene d60 was as effective as gene *sd1* and was the best possible choices in practical breeding.

Srivastava et al. (2019) itrogressed semi-dwarfing gene (*sd1*) from CSR10 using marker assisted breeding in variety Kalanamak Improved semi-dwarf Kalanamak lines were analyzed for the *sd1* gene and presence of aroma, sensory analysis test and amplification with betaine aldehyde dehydrogenase 2 (*badh*2) derived primer was done.

Samal (2019) investigated the introgression of *pi 9* gene and *sd 1* gene from Basmati donor PB1 to develop semi dwarf and blast resistant fixed derivatives of traditional Ranbir Basmati. Twenty-three plants were homozygous for both the genes.

Angira et al. (2019) reported that in US rice breeding projects the *sd1* semi dwarf genes was a desirable choice for marker-assisted selection. Seven haplotypes were observed using the IRRI SNP-Seek database to describe the haplotype diversity present at the *SD1* gene across a group of rice accessions. These SNPs were evaluated among 359 elite US genotypes using Kompetitive allele specific polymerase chain reaction (KASP) tests and two haplotypes, including the semi dwarf deletion allele, were observed in US germplasm and among the US medium-grain germplasm, a third haplotype was discovered, which was a semi dwarf allele resulting from the ‘Calrose76' induced mutation.

Bhuvneshwari et al. (2020) reported a new SNP in the *SD1* gene of the rice genotype Pusa 1652. The semi-dwarfism in Pusa 1652 was monogenic and recessive, although it does not possess the *sd1*-d allele. The reaction to gibberellic acid (GA3) treatment and subsequent bulked segregant and linkage studies revealed that the *SD1* gene lowered plant height in Pusa 1652 and it was sequenced. It revealed a new transition in exon 3 (T/A), resulting in a nonsense mutation at the 300th codon. The stop codon caused premature termination, leading to a shortened OsGA20ox2 protein that obstructed the GA3

production pathway. This unique recessive allele, '*sd1*-bm' was derived from Bindli Mutant 34 (BM34), α -ray induced mutant of the Bindli (short-grain aromatic landrace). The semi-dwarfing allele, *sd1*-bm, was confirmed by creating the AKS-*sd1* derived cleaved amplified polymorphic sequence (dCAPS) marker. This gene is an alternative to the most often used *sd1*-d allele in rice improvement and the functional dCAPS marker will allow for marker-assisted introgression of the semi-dwarf trait into tall genotypes.

2.2 Marker Aided Selection

Rajpurohit *et al*. (2011) had pyramided semi-dwarfing gene (*sd1*) from PR106-P2 in Type 3 Basmati using marker-assisted selection and selection for *sd1* gene were done based on linked molecular markers of this gene.

Bhatia et al. (2011) developed a dwarf and bacterial blight resistant variety from the two traditional basmati rice varieties Basmati 370 and Basmati 386 using marker assisted selection for *sd1* and BB resistance gene with stringent phenotypic selection during back crossing.

Luo et al. (2014) developed an improved version of Indonesian traditional tall rice variety 'Siputeh' by introgressing *sd-1, Wxb, xa-4* and *Xa-21* genes from the donor line WH421. The linked markers were used to select the gene positive plants in different backcross generations eventually resulting in the selection of a semi-dwarf line TS4 with genes *sd-1, Wxb, Xa-4* and *Xa-21* genes. TS4 was semi-dwarf, short duration high yielding cultivar with reduced growth duration, good grain quality and broad-spectrum resistance to *Xoo* strains.

Zhang et al. (2015) reported genetic analysis of flag leaf size and the gene determining major QTL for flag leaf width. The core recombinant inbred lines of Liang-You-Pei-Jiu (LYP9) revealed a positive correlation between flag leaf width and yield per plant. Based on the high-resolution linkage map, 43 QTLs were detected for flag leaf size and shape traits and yield per plant, among which 31 QTLs were new and QTLs were common in two environments. A new major QTL 'qFLW7.2' was identified for flag leaf width which was fine mapped within 27.1 kb region on chromosome 7. Two candidate genes were selected based on sequence variation and expression difference between two parents, which facilitated further QTL cloning and molecular breeding in super rice.

Wu et al. (2017) reported an intragenic recombination between two parental non-functional *sd-1* alleles which resulted in the generation of a functional SD1 allele in a tall line RI92 (160 cm height) which was obtained from the cross between two semi-dwarf *indica* cultivars Zhenshan 97 and Minghui 63. Liu et al. (2018) reported a new lodging resistance gene, *Shortened Basal Internodes (SBI)*, which encoded gibberellin 20- oxidase which was also encoded by *sd-1* gene in semi dwarf rice varieties and reported to control the elongation of culm basal internodes through deactivating GA activity. *SBI* was predominantly expressed in culm basal internodes and found to show partial dominance in affecting plant height and lodging resistance.

Borale et al (2021) used a simple sequence repeat (SSR) marker to assess the segregating population (F_2) of breeding material for quality parameters. Twenty-four SSR markers were used to assess this segregating population using bulk segregant analysis, among marker RM 147 revealed polymorphism between long and short slender parents and respective bulks. There was a relationship of RM147 with grain length gene in the segregating population.

2.3 Morphological traits and pericarp color in rice

Lee et al. (1987) revealed that the grain yield was highly correlated with the number of panicle of red rice. Dewande et al., (1991) reported a positive correlation between panicle length and yield. Alvarado and Pedreros (1991) reported variation in morphological characters, awns and node color.

Kwon et al. (1992) reported that growth and developmental differences were stronger between red rice (strawhull) and ‘Lemont' and ‘Newbonnet' rice. Red rice was taller, generated more culms per m^2 and aboveground dry weight, and had higher leaf area indices and larger flag leaf area. It showed lower leaf to stem ratios late in the season, higher crop growth rate early in the season but a lower crop growth rate late in the season and a lower grain weight than rice.

Yu et al. (2002) investigated the genetic origins of heading date and plant height at both single-locus and two-locus levels in a population of 240 F2:3 families from a cross between two elite rice lines. The qualities were measured over the course of two years in replicated field trials. With 151 polymorphic marker loci, a linkage map was created and interval mapping was performed using Mapmaker/QTL. Six QTLs were discovered for

plant height and heading date. The QTLs for heading date accounted for more phenotypic variance than the QTLs for plant height.

Katoch and Kumar (2006) studied 54 genotypes comprising landraces, japonicas and new plant types along with checks China 988, RP 2421 and Nagger dhan. Genotypes, RJ-100, Chiti Zeeni and Kalizhini (land races), IR 72884-181-3-2-1 and IR 69853-70-3-1-1 (yield attributing traits), IR 71146-40-7-2-1-2-1 and IR 72870-21-2-1-2 (quality traits) in new plant types Hinohikari, desi dhan and Yunlen-18 (japonicas) were the best genotype. A total of 56 primers were subjected to PCR using 10 primers. Amplification generated 81 reproducible markers, of which 95% were polymorphic. Cluster analysis revealed presence of 8 clusters and both D^2 statistics and molecular markers were effective for diversity studies.

Ahuja et al. (2007) reviewed the history, classification, of red rices; their food and medicinal uses and their use in breeding strategies for the improvement of cultivated varieties.

Sood et al. (2008) reported that cvs. Achhoo, Baldhar, IC3131159, Kijun and Tiyun (red rice); RLC 3, Lal Nakanda-41, R-575 and Purple Baldhar (purple leaved rice) and LC99-5B, IC3131155 and Rajpur Basmati (quality rice) were promising genotypes for further exploitation. Grain yield had significant positive correlation with grains per panicle, plant height and spikelets per panicle inferring that direct selection for these characters will be effective. Based on Path coefficient analysis showed that tillers/ plant, panicle length, grains/ panicle and 1000-grain weight had high positive direct effects on yield. Tremendous variation was observed in anthocyanin pigmentation in different plant parts in all red, purple and quality rice.

Wei et al. (2010) reported that a newly detected QTL, DTH8 responsible for days to heading, was detected on chromosome 8 and it was found in almost all tissues and its protein was found in the nucleus. DTH8 could down-regulate the transcriptions of Ehd1 (for Early heading date1) and Hd3a (for Heading date3a; a rice orthologue of flowering locus T) under long-day circumstances. The photoperiodic flowering genes Ghd7 and Hd1 could inhibit Ehd1 and Hd3a (a rice orthologue of constans) and DTH8 transcription was independent of Ghd7 and Hd1. A spontaneous mutation in this gene resulted in reduced photoperiod sensitivity and lower plant height. It was suggested that DTH8

functions as a new suppressor in the photoperiodic blooming signal network in the regulation of plant height and yield potential.

Sharma et al. (2012) reported that among six land races viz. Karad, Matali, Begmi, BhriguDhan, Sukara and Chohartu of district Chamba, the highest content of Fe was observed in Matali (0.063 mg/100g) and minimum in Chohartu (0.029 mg/100g). Zinc was maximum in Matali (0.031mg/100g) and minimum in HPR 2143 (0.020 mg/100g) whereas, magnesium content 0.925-1.386 mg/100g and Mn content were highest in Bhrigudhan (0.053 meq. /l)

Gealy et al. (2012) reported that red rice plants are taller than white rice cultivars and the majority of biotypes are either awnless with straw-colored hulls or have long awns with black-colored hulls (blackhull). Outcrossing between rice and red rice seldom takes place, leading in a diverse range of plant forms. SSR markers revealed that short-stature awnless (LhtsA) and awned (LhtsA+) types accounting for about 5% each out of 460-accessions, were genetically related to their normal-sized counterparts but not to cultivated rice. A short-awned, medium high type, 'Sawn,' ~ 4% of the accessions, was genetically separate from all other types.

Patil (2014) studied genetic variability, correlation and path analysis in red rice genotypes and reported that yield components like number of productive tillers/plant, filled grains/ panicle, 1000 grain weight, grain length and harvest index were important in selection programme to improve grain yield per plant of red rice. These characters also had significant positive correlation at genotypic and phenotypic levels and high positive direct effects on grain yield.

Yogita (2016) reported that among three CMS lines viz., IR79156A, CRMS 31A and CRMS32A and eight testers CRMS 32A was identified as good general combiner for grain yield per plant due to its positive GCA effects for grain yield per plant.

Puren (2017) studied thirty genotypes of red and brown rice, which include three checks grown in RBD in three replications, with the goal of studying the diversity and comparing the performance of red rice genotypes, elite cultivars, and their derivatives, and identifying promising genotypes for yield, quality, and disease resistance. Analysis of variance showed that the mean sum of squares due to genotypes were highly significant for all the traits studied. Grain yield per plant, number of panicles per plant, spikelets per

panicle, grains per panicle, total anthocyanin, total phenol, and total flavonoid all had high PCV, GCV, genetic advance, and heritability. On the basis of mean performance, red rice genotypes, Karad, Brighu dhan, Bongal dhan, HPR 2800, Jattoo dhan, HPR 2946, ACC 19186 and Naggar dhan proved to be promising genotypes for yield and yield contributing traits and could be used in future rice improvement programme.

Waghmode et al. (2017) studied inheritance of red kernel and hull color in eight crosses between white kernel/red kernel rice varieties *viz.*, Karjat 4/Patni 6, Panvel 2/MO 17, Palghar 1/MO 8, Ratnagiri 5/TKM 9, Karjat 184/Munga, Karjat 6/Bela, Ratnagiri 24/Valai and Karjat 8/MO 8. Segregation generations reveled that red kernel in rice was governed by one dominant gene in six crosses, two dominant genes in two crosses.

Islam et al. (2018) evaluated 50 red rice germplasm from the Bangladesh morphologically and genetically using fifty simple sequence repeat (SSR) markers, and identified 162 alleles with 2-7 alleles per marker. Markers RM282 and RM304 had the highest and the lowest polymorphic information content (PIC) indices of 0.75 and 0.04, respectively. The genetic diversity was moderate, ranging from 0.05 to 0.78. (average: 0.35).

Lingaiah et al. (2018) reported that in F_2 population, PCV > GCV for all the characters and there was little difference between GCV and PCV for physical quality characters indicating less influence of environment on these characters. High heritability was observed for traits i.e. plant height, grains/panicle, spikelet fertility (%), Kernel length, breadth and L/B ratio, whereas, high genetic advance (% of mean) was observed for plant height, productive tillers, number of grains/ panicle, head rice recovery and kernel L/B ratio.

Kristamtini et al. (2019) studied inheritance of pericarp pigment in a cross between black and white rice. Purple pigment was controlled by two mutually complementary dominant genes with recessive epistasis (9:3:4) which followed the model of additive × additive and dominant × dominant interaction. Action of black/purple pigment gene of rice was perfect dominance which was directed to the parent with purple pericarp (black rice).

Mustikarini et al. (2019) assessed genetic parameters i.e. variability, heritability and genetic progress in F_2 lines from mutant red rice crossing MR1512 × Inpago 8, MR1512 × Banyuasin, Inpago 8 × Balok, Balok × Banyuasin and Balok × Inpago 8, local red rice accession and some upland rice varieties using single plant design The phenotypic

variability for full grains and grain weight was high whereas, the genotypic variability for plant height and productive tiller numbers was low. The plant height, flowering time and harvest time had high heritability, it was suggested that plant height, productive tiller numbers, flowering time and harvest time were essential features for use in rice lodging tolerance breeding programmes.

2.5 Genetic variability, heritability and genetic advance

Padmaja et al. (2008) studied eleven features in 150 genotypes including five check varieties. Analysis of variance showed extremely significant variations for all the parameters except leaf width and 100-seed weight. All the traits, except days to 50% blooming and panicle length, showed high genotypic and phenotypic coefficients of variation (GCV and PCV).

Bisne et al. (2009) studied 13 yield and yield-related traits in four CMS lines, eight testers and thirty-two hybrids. The genotypic and phenotypic coefficients of variation were low, moderate and high. Harvest index, total number of filled spikelets/panicle, 100-grain weight and % spikelet fertility had high genotypic and phenotypic coefficients of variation. Harvest index, total number of chaffy spikelets/panicle, grain yield per plant, total number of filled spikelets/panicle and % spikelet fertility showed strong heritability and genetic progress, suggesting that selection may be effective for these features.

Veerabadhiran et al. (2009) studied the degree of diversity and genetic characteristics in 15 rice hybrids for 17 qualitative qualities and grain yield. The difference between PCV and GCV was very small, indicating less environmental effect. High GCV and PCV (>20%) were recorded for gelatinization temperature and gel consistency. All of the traits had significant heritability estimates (>61%) except breadthwise expansion.

Anbanandan et al. (2009) studied diversity, heritability and genetic advance in four crosses in F_2, F_3 and F_4 generations. Cross 1 performed better than the other crosses for all the economic test traits both in F_3 and F_4 generations. This cross showed improved performance in F_3 to F_4 generations showing improved performance with the advancement of generation. In both the, High phenotypic and genotypic coefficients of variation were detected for grain production per plant in F_3 and F_4 generations for cross 1, followed by cross 2. Cross 1 and cross 2 had high heritability and genetic progress for the number of productive tillers per plant, 1000-grain weight and grain yield per plant in all F3 and F_4 generations

Anjaneyulu et al. (2010) reported that in 50 rice germplasm lines number of grains/panicle, fertility percentage and grain yield/plant had the highest PCV and GCV. The number of grains/panicle, plant height and fertility percentage showed high heritability along with high genetic advance, indicating the suitability of these traits for selection.

Singh et al. (2011) studied 81 genotypes for thirteen quantitative variables. The variations between the 81 genotypes were significant for all of the traits except flag leaf width. Number of spikelets/panicle had the highest estimates of GCV and PCV, followed by harvest index, grain yield/ hill and number of panicles/ hill. Biological yield/ hill had the highest broad sense heritability, implying that this attribute will respond to selection.

Lal et al. (2011) reported that plant height, stem length, seeds/panicle and L/B ratio had high GCV, whereas, 1000 seed weight, flag leaf length, leaf breadth, days to flowering, seed/plant and days to maturity had moderate GCV. Panicle length had low GCV. The heritability of all of the traits was very high.

Sumanth et al. (2017) reported that among 23 rice genotypes genotypes LSD-1, TP29654 and TP29737 showed high seed yield/ per plant and had the highest GCV and PCV, followed by flag leaf length, number of spikelets/ panicle, biological yield/ plant and panicles/plant, indicating that these characters were useful for crop improvement selection. Plant height, flag leaf length, biological yield/plant, spikelets/panicle and panicles/plant all had high heritability values whereas, spikelets/panicle and plant height showed high genetic advance indicating the preponderance of additive gene effects and the possibility of efficient selection for the development of these traits.

Abebe et al. (2017) reported that in 36 rice genotypes plant height, culm length, number of unfilled grains/panicle, biomass yield and grain yield had higher PCV and GCV inferring that these traits may be improved by selection. Culm length had the highest heritability, followed by plant height, biomass production and panicle length. Plant height, biomass yield, grain yield and number of unfilled grains/panicle showed moderate to medium heritability, high GCV and high genetic progress as a percentage of means.

Srujana et al. (2017) determined genetic diversity and heritability in 29 rice genotypes for 13 quantitative traits. There was little difference between PCV and GCV implying that the environment had minimal impact on the expression of the test traits. Grain yield/hill,

harvest index, spikelets/panicle, tillers/hill, flag leaf length and panicles/hill had high to moderate GCV and PCV estimates. Spikelets/panicle, days to maturity, biological yield, grain production/hill, panicles/hill and tillers/hill all had high heritability estimates. Spikelets/panicle, seed output/plant, tillers/plant, panicles/plant and biological yield/hill had high estimates of heritability and moderate to low estimates of genetic progress.

Prasad et al. (2017) determined variability, heritability and genetic advance in yield and yield contributing features in 50 boro rice genotypes. Filled grains/ panicle, unfilled grains/ panicle and grain production/ plant had high GCV and PCV values. Plant height, number of tillers/plant, number of productive tillers/plant, number of filled grains/panicle, number of unfilled grains/ panicle, 1000-grain weight and grain yield/plant had high heritability and genetic advance as a percent of mean, indicating that these traits were controlled by additive gene action.

Adhikari et al. (2018) evaluated 26 advance genotypes of lowland irrigated rice to study genotypic and phenotypic variability, heritability, genetic advance and correlation of yield and yield-related parameters. Days to flowering (0.88), maturity (0.79), 1000 grain weight (0.48) and plant height (0.43) showed high heritability, indicating that these variables showed strong genetic influence. Comparing PCV and GCV revealed that the expression of traits was influenced by the environment. Grain yield (11.98) and days to flowering (10.32) exhibited moderate genetic advance as a % of the mean. These qualities were governed by non-additive genes with high to low heritability and moderate to low genotypic progress as a percent of mean, indicating direct selection was not advantageous.

2.6 Correlation coefficient and path analysis

Kiani et al. (2012) studied F_2 populations of fifty-four rice genotypes and reported that grain yield was positively associated with panicles/ plant (r = 0.751) and filled grains/panicle (r = 0.458), whereas grain yield was inversely associated with non-filled grains/panicle (-0.297). Grain yield was linked to panicles/plant and full grains/panicle, with direct effects of 0.691 and 0.568, respectively, according to path coefficient analysis whereas, filled grains/panicle, panicle length (0.301) had the largest indirect effect. Hence, panicles/plant and filled grains/panicle may be utilized as selection criteria for improving grain yield in segregating rice populations.

Babu et al. (2012) investigated the correlation and path analysis in 21 rice hybrids and found that number of productive tillers/plant has a substantial positive relationship with grain production/plant indicating that selecting these traits can increase yield. The analysis of path coefficients revealed that panicle length and the number of productive tillers/plant had a positive direct effect on yield.

Sarker et al. (2014) studied the yield and yield contributing characters of 32 exotic early maturing rice lines using correlation and path coefficient analyses. Path analysis revealed that days to maturity (0.87), number of tillers (0.25), effective number of tillers (0.48), panicle length (0.68) and number of filled grain panicles (1.29) showed positive direct effects on yield. Hence, these were the credible criteria for enhancing yield of early maturing rice.

Lakshmi et al. (2014) studied the nature and extent of correlation between yield and yield attributing characters in 70 genotypes of rice Days to maturity, number of productive tillers/plant, plant height and kernel length were significantly and positively correlated with grain yield/ per plant, inferring that these traits may be useful criteria in yield improvement programmes.

Kishore et al. (2015) reported that among 73 rice cultivars number of filled grain/panicle and the number of panicle bearing tillers were positively correlated with grain yield and have significant direct effects on grain yield/plant. Features like panicle bearing tillers per hill had high indirect effects, highlighting the trait's usefulness as a selection criterion in breeding programmes.

Ratna et al. (2015) reported that in six advanced Basmati rice lines and one check variety, BRRI Dhan 29 genotypic correlation coefficients were greater than phenotypic correlation coefficients and a substantial positive association between plant height and panicle length. There was substantial positive correlation between the number of filled spikelets/panicle and yield and a significant negative correlation between plant height and yield.

Devi et al. (2017) reported that in 27 rice genotypes grain yield/plant was significantly and positively correlated with filled seeds/panicle, plant height, flag leaf length, effective tillers, flag leaf width and panicle length, indicating that these characters are important

for yield improvement. Path analysis revealed that test weight (3.48), effective tillers (1.57) and filled grain/panicle (1.41) had a favorable direct effect on grain yield/plant.

Bhujel et al. (2018) investigated the correlations and path coefficients for seven morphological traits in 24 rice genotypes. Grain yield was positively correlated with 1000-grain weight, reproductive rate and panicle length. Path coefficient analysis revealed that 1000-grain weight had the greatest positive direct effect on grain yield, followed by panicle length and % fertility.

Sivasankar et al. (2018) evaluated 35 rice genotypes, including one check. Days to 50% flowering, plant height, tillers/hill, panicles/plant, flag leaf width, flag leaf length, spikelets/panicle, days to maturity, biological yield and harvest index, and test weight had a positive and significant correlation with grain yield at both the genotypic and phenotypic levels. Path analysis revealed that panicle length, flag leaf width, spikelet count/panicle, days to maturity, biological yield and harvest index and test weight had a significant positive direct effect on grain yield/hill at both the genotypic and phenotypic levels.

Hossain et al. (2018) reported that in 33 rice genotypes, a highly significant positive association with a positive direct influence in the number of grains/panicle, number of panicles/hill, unfilled grains/panicle, primary branches and the number of secondary branches were observed.

Bhargava et al. (2021) reported that in segregating F_2 population in an aerobic restorer AR 9-18 YPK 198 analyzed for eight yield and yield contributing traits. The PCV and GCV of productive tiller number, grain number/ panicle, and plant yield were high. Plant yield was significantly correlated with plant height, productive tiller number, panicle length and grain number/panicle. Productive tiller number, grain number/panicle and plant height had a significant positive direct effect on plant yield.

3. MATERIALS AND METHODS

The present investigation entitled "Marker aided selection for dwarfness in F_2 population of red rice variety HPR-2795" was carried out during *Kharif*, 2020 at the Experimental Farm and the molecular analysis was carried out at MCTL Lab, Department of Genetics and Plant Breeding, CSKHPKV, Palampur. Geographically, the farm is located at an elevation of about 1290 m above mean sea level, latitude 36°6'N and 76°3'E longitude representing the mid hill zone (Zone-II) of Himachal Pradesh, characterized by humid sub-temperate climate with high rainfall (~2,500 mm per annum). The details of the materials used and methods employed in the present investigation are described in this chapter under the following headings:

3.1 Materials and layout of the design

The experimental material comprised 165 F_2 single plant from the cross HPR-2795 xPB-3. The F_2 plant population along with parents was evaluated for different agro-morphological characters and molecular markers. The material was planted following row to row and plant to plant spacing of 20 and 15 cm, respectively. In all, 165 plants were tested for agro-morphological characters out of which, 55 randomly selected plants were screened for the presence of gene *sd1*. The brief description of varieties is as follows:
Punjab Basmati 3 (PB-3): It is an improved version of variety Basmati 386 (Bhatia et al 2011, Singh et al 2014). It is a photoperiod sensitive, semi dwarf, lodging tolerant variety about 105-110 cm tall and matures in about 139 days after seedling. It is the very first Basmati variety, resistant to bacterial blight (BB). It's average grain yield is 16q per acre.

HPR-2795 (Him Palam Lal Dhan 1): It is improved land race Sukana from Chamba. It has red decorticated grains and developed by CSKHPKV, Palampur during 2017. It is

recommended for cultivation in rainfed uplands of low and mid hills (up to 1500 m amsl) of H.P, Meghalaya and Manipur. It attains harvestable maturity in 120-125 days after transplanting. Its mean yield ranges between 2.7 and 3.5 q/ha.

3.2 Observations recorded

Field data were recorded for all the 165 F_2 plants for following quantitatively measured traits during the cropping season. The data from the entire one sixty-five plants was pooled to calculate the average.

Table 3.1 : List of traits recorded in the study

S. No.	Trait	Observation recorded
1.	**Days to 50% flowering**	Recorded as number of days from planting to the day on which 50 per cent of the plants in a row of genotype flowered.
2.	**Days to 75% maturity**	Number of days from planting to the day on which the plants were ready for harvesting was counted.
3.	**Plant height(cm)**	Measured in centimeters from the base to the tip of the main shoot.
4.	**No. of tillers/plant**	The total no. of tillers/plant at maturity.
5.	**Panicle length (cm)**	The length of the main panicle measured from base of main rachis to the tip of the top most grain of panicle.
6.	**Flag leaf length (cm)**	The length of upper most leaf below the panicle was measured using scale in centimeters.
7.	**Grain length (cm)**	Length of dehusked grains of each plant was recorded in millimeters using digital vernier calliper.
8.	**Grain breadth (cm)**	Using digital Vernier calliper dehusked grains of each plant whose length was earlier recorded were used for recording grain breadth in millimetre.
9.	**L/B ratio**	L:B ratio was calculated by dividing grain length by its breadth.
10.	**1000-seed weight(g)**	From single plant harvest, 1000seeds were taken at random and weighed in grams.
11.	**Seed yield per plant(g)**	Panicles harvested from each plant were dried, thrashed, cleaned and weighed in grams.

3.3 Molecular analysis

Simple Sequence Repeats (SSR) assay

Fifty-five randomly selected F_2 plants from the cross of HPR-2795 x PB-3 including parents were subjected to SSR assay as per the following procedure:

3.3.1 Primer used

Table 2.2: Details of marker used in this study

Gene (trait)	Linkage group	Marker	Sequence	Type of marker	Linkage phase	Source
sd-1 (Semi dwarfing)	1	*SD-1*	F: 5'CACGCACGGGTTCTTCCAGGTG 3' R: 5'AGGAGAATAGGAGATGGTTTACC 3'	Gene Derived Indel	Co-dominant	Eliss and Spielmeyer (2002)

3.3.2 Extraction of plant genomic DNA

Genomic DNA was isolated from young leaf tissue (0.5-1g) of each plant using CTAB method (Murray and Thompson, 1980). The leaf tissues were rinsed in deionized water, dried on tissue paper discs and ground to fine powder in liquid nitrogen in autoclaved pre-cooled pestles and mortars. The ground tissue was transferred to a separate 2 ml eppendorf tubes containing 800 µl of extraction buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, 1.4 mM NaCl and 1% PVP, pH 8.0) maintained at 60^0C in water bath and mixed vigorously. The mixture was incubated at 60^0C for 1 hour with occasional mixing. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the tubes followed by its gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4^0C. The aqueous phase was then transferred to a fresh tube, followed by addition of 500 µl of pre-chilled isopropanol. The contents of the tubes were gently mixed and the mixture was incubated at-20^0C for 1 hour. DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes using centrifuge. The supernatant was drained and the resultant pellet was washed twice with 1ml of 70% chilled ethanol. The pellet was dried in a stream of sterile air in a laminar air flow cabinet for 3-4 h. Dried DNA pellet was dissolved in 1 ml TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The dissolved DNA was treated with 1 µl of RNase (10 mg/ml).

3.3.3 Purification of DNA

For purification of extracted DNA, 100 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the tubes followed by gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4^0C. The aqueous phase was transferred to fresh tube, followed by addition of 200 µl of pre-chilled isopropanol. The contents of the tubes were mixed gently and the mixture was incubated at -20^0C for 30 min. DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes using centrifuge. The supernatant was drained and the resulting pellet was washed twice with 70 µl chilled ethanol. The pellet was dried in a stream of sterile air in a laminar airflow cabinet. Dried DNA pellet was dissolved in 80 µl TAE buffer. The quantity and quality of DNA was estimated through electrophoresis using 0.8% agarose gel (HIMEDIA).

3.3.4 PCR amplification of DNA

For the amplification of genomic DNA, a reaction mixture of 12.5 µl volume was prepared using 7.15 µl of sterilized distilled water, 1.0 µl template DNA (25 ng/µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 1.0 µl $MgCl_2$ (25 mM), 1.25 µl 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.0 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (Gotaq® DNA Polymerase, Promega). PCR amplification was carried out in a thermocycler (Proflex PCR System; Applied Biosystems, Life Technologies, USA) using specific temperature profiles.

Table 3.3: PCR conditions used for amplification of gene *sd1* in parents and F_2 plants genomic DNA.

Primer Type	Steps	Temperature and time	Cycles
SSR	Initial denaturation	94^0C for 5 minutes	1
	Denaturation	94^0C for 45 seconds	
	Annealing	57^0C for 45 seconds	35
	Extension	72^0C for 1 minute	
	Final extension	72^0C for 5 minutes	
	Storage	4^0C for ∞	1

To verify the presence of *sd-1* gene, amplification was carried out with the *SD-1* marker. The conditions for PCR amplification of *SD-1* was that 35 PCR cycles were carried out at 94^{o}C for 45 seconds, 55^{o}C for 45 seconds, 72^{o}C for 1 min with a final extension at 72^{o}C for 5 min. Ten µl of each PCR product was analyzed on 2% agarose gel prepared in 1X Tris borate-EDTA (TBE) buffer (0.05 M Tris, 0.05 *M* boric acid, 1 mM EDTA, pH 8.0). The PCR amplicons for marker *SD-1* was resolved on 2% agarose gel. The gel was run at a constant voltage of 100 volts for 1 h. The gel was stained with ethidium bromide (0.5µg/ml) for 5 min after the completion of electrophoresis. The gel was de-stained for 10 min by keeping under running tap water. The resolved PCR products were visualized over an ultraviolet trans-illuminator and a photograph of the gel was stored in Gel-Doc system (BIO-RAD).

3.4 Statistical analysis

The data recorded for 165 plants for different characters were statistically analysed for working out the following values:

3.4.1 Chi square (χ^2) test for goodness of fit in the segregating population

χ^2 test was carried out at $p < 0.05$ and $p < 0.01$ probability at appropriate degrees of freedom depending on the number of classes. Where 'p' is the probability that deviation of the observed frequency from the expected frequency is due to chance alone. χ^2 is the sum of the squared difference between observed and the expected data, divided by the expected data in all possible categories. The χ^2 value for the various field observations was calculated by formula:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

Where;

O = Observed frequency and

E = Expected frequency

3.4.2 Morphological analysis of F_2 population

Estimation of parameters of variability

The genotypic and phenotypic coefficients of variation were estimated by following method of Burton and DeVane (1953) as follows:

Phenotypic variance due to progenies (Vp) $= \sigma_p^2 = \sigma_g^2 + \sigma_e^2$

Genotypic variance due to progenies (Vg) $= \sigma_g^2 = M_p - M_e$

Environmental variance due to progenies (Ve) $= me = \sigma_e^2$

Where,

Genotypic coefficient of variation (GCV %) $= \frac{\sigma g}{\bar{x}} \times 100$

Phenotypic coefficient of variation (PCV %) $= \frac{\sigma p}{\bar{x}} \times 100$

Environmental coefficient of variation (ECV %) $= \frac{\sigma e}{\bar{x}} \times 100$

Where,

σ_g = genotypic standard deviation

σ_p = phenotypic standard deviation

σ_e = environnemental standard deviation

$\bar{x}$ = grand mean

Heritability (broad-sense) (%)

Heritability in broad sense (h^2_{bs}) was calculated as per the following formula given by Burton and DeVane (1953) and Johnson et al. (1955):

$$h^2_{bs}\ (\%) = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}\ x\ 100$$

Where,

h^2_{bs} = heritability

σ^2_g = genotypic variance

σ^2_e = environmental variance

$\sigma^2_g + \sigma^2_e$ = phenotypic variance

Genetic advance (%)

The expected genetic advance (GA) resulting from the selection of 5 superior individuals was calculated as per the formula given by Burton and DeVane (1953) and Johnson et al. (1955).

GA = $k. \sigma_p. h^2_{bs}$

K = 2. 06 (selection differential at 5% selection intensity)

σp = phenotypic standard deviation

h^2_{bs} = heritability (broad-sense)

Genetic advance as percentage of mean (GAM) % = $\frac{\text{expected GA}}{\text{grand mean}} \times 100$

Limits used for categorizing the magnitude of different parameters:

PCV& GCV	>20%	High
	10- 20%	Moderate
	<10%	Low
Heritability (h^2_{bs})	>70%	High
	40- 70%	Moderate
	<40%	Low
Genetic advance	>30%	High
	20- 30%	Moderate
	<20%	Low

3.4.3 Correlation coefficients

For computing correlation coefficients, analysis of variance was carried out in all possible pairs of combination of the traits.

$$r = \frac{Cov.(x.y)}{(Vx . Vy)}$$

Where,

Cov. (x.y) = $[(\sum xy) - \{(\sum x)(\sum y)/N\}]/N-1$

Vx= S^2x = $[(\sum xy) - \{(\sum x)/N\}]/N-1$

Vy= S^2y = $[(\sum xy) - \{(\sum y)/N\}]/N-1$

The significance of coefficients of correlation was tested against ‘r’ values as given by Fisher and Yates (1963) at n-2 degree of freedom.

3.4.4 Path coefficient analysis

The correlation coefficients were used in finding out the direct and indirect contribution of traits towards yield/plant as proposed by Wright (1921). The direct and indirect paths were carried out by following Dewey and Lu (1959) as follows:

$Py_{1+}\ py_2r_{12}+py_3r_{13}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots+py_nr_{1n} = ry_1$

$Py_1r_{12}+py_2+py_3r_{23}+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots+py_nr_{2n} = ry_2$

$py_1r_{13}+py_2r_{23}+py_3+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots+py_nr_{3n} = ry_3$

:

:

$py_1r_{n1}+py_2r_{n2}+py_3r_{n3}+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots+py_n= ry_n$

Where,

py_1, py_2, py_3----------, py_n are the directs path effects of 1, 2, 3---------, n variables on the dependent variable “y”

r_{12}, r_{13}, ----------, $r_{(n-1)\ n}$, are the possible coefficient of correlation between various independent variables with dependent variables “y”

The variation in the dependent variables which remained undetermined by including the other variables was assumed to be due to the variables (s) not included in the present investigation. The degree of the determination (P^2xR) of such variables was calculated as follows:

Residual effect (P x R) = $(1-R^2)^{1/2}$

Where,

$R^2 = py_1ry_1+py_2ry_2+$----------$+py_nry_n$

and R^2 is the square multiple correlation coefficient and is the amount of variation in yield that can be accounted by the yield component trait.

4. RESULTS AND DISCUSSION

Red rice is an ancient treasure of Himachal Pradesh and a valuable commodity in export market. There is a great diversity of agro- climatic conditions under which rice is grown in Himachal Pradesh. The name of the rice is associated with the color pigment (red, black, purple) formed by deposits of anthocyanin in the pericarp layer, seed coat or aleurone layer (Chaudhary 2003). Coloured rice is potential source of anti- oxidants. However, inheritance of pigmented pericarp of red rice was conducted by Hsieh and Chang (1964) and Rahman et al. (2013) but still this is not clear.

Red rice variety HPR-2795 is an early maturing, high-yielding, disease-resistant variety released by Central Variety Release Committee for H.P, Manipur and Meghalaya is a pureline selection of traditional Sukara landrace. It has a thick stem and long flag leaf, which enhances its photosynthetic efficiency leading to higher yield. However, it is very tall and prone to lodging, which may lower its yield potential. Hence, there is a need to incorporate a semi dwarfness gene into the red rice variety HPR-2795 to prevent the lodging losses. The semi- dwarfing gene (*sd1)* identified in the Chinese variety Dee Geo Woo Gen (DGWG) is one of the most important gene utilized in modern rice breeding. The gene confers semi-dwarf stature, reduces culm length and improves lodging tolerance (Pinthus 1974), thus allowing increased use of nitrogenous fertilizers for obtaining high yield (Murray et al. 2002). The *sd1* gene has been cloned and gene derived markers were developed from the sequence information of cloned *sd1* which is being used for marker assisted selection of recessive *sd1* allele in breeding population (Ellis and spielemeyer 2002; Rajpurohit et al. 2011).

Conventional breeding is time-consuming and selection is based on phenotypic data, which require higher plant population whereas, marker-assisted selection (MAS) based on selection of plants using molecular markers reduce the selection cycle, are precise and robust in nature. MAS selection requires screening of a small population size, and hence reducing the time span.

Over the last 20 years, SSRs are extensively used markers for genotyping plants because they are highly informative, co dominant, experimentally repeatable and transferrable between related species (Mason, 2015). SSR markers are found throughout eukaryotic genomes and can be used as highly variable and multi-allelic PCR-based genetic markers

(Brown et al., 1996). The application of SSR techniques to fingerprint plant species was first reported by Akkaya et al. (1992).

In this context, the present investigation entitled "Marker aided selection for dwarfness in F_2 population of red rice variety HPR-2795" was carried out with an aim to fast-track development of the semi dwarf derivatives of a cross between red rice variety HPR-2795 and basmati rice variety Punjab Basmati 3 (PB-3). PB-3 has short stature and the presence of *sd1* gene has been confirmed with gene derived markers.

The biotechnological tools of marker-assisted selection were used to ensure precise selection of gene positive homozygous progenies and morphological analysis of each F_2 plant for identification of superior plants having short length and red grain color.

The results obtained in the present study are presented under the following headings:

4.1 Marker aided selection for semi-dwarfness in F_2 plants

4.2 Chi-square test in F_2 population for seed coat color and plant height

4.3 Morphological analysis of F_2 population for the important agronomical traits

4.4 Correlation coefficient analysis

4.5 Path coefficient analysis

4.1 Marker Aided Selection for semi-dwarfness in F_2 plants

Out of 165 plants of cross between HPR-2795 x PB-3, 55 plants were selected for molecular studies. Marker Assisted Selection (MAS) was conducted on parents PB-3, HPR-2795 and 55 F_2 plants of cross between HPR-2795 x PB-3. The parents and F_2 plants were screened for *sd1* gene through gene specific markers in PCR as suggested by Eliss and Spielmeyer (2002). Plant height, seed colour, panicle length, flag leaf length, seed yield per plant and presence or absence of molecular band with sd1 primer is given in table 4.1. HPR-2795 had no amplification with the *sd1* gene specific primer while PB-3 showed amplification in the PCR. Height of HPR-2795 ranged from 111cm to 125cm and PB-3 ranged from 99cm-106cm suggesting the presence of *sd1* gene in PB-3 and absence of *sd1* in HPR-2795. Out of 55 F_2 plants, 47 plants were *sd1* positive and rest of the plants were *sd1* negative. The presence of positive *sd1* allele in segregating progenies indicated the introgression of the gene from the donor variety PB-3. The amplified products with *sd1* primer from one parent were 300 bp while the other parent was about 280 bp which

could be easily resolved on 2% agarose gel. Some of the plants were having both the bands which represented the heterozygosity present in them.

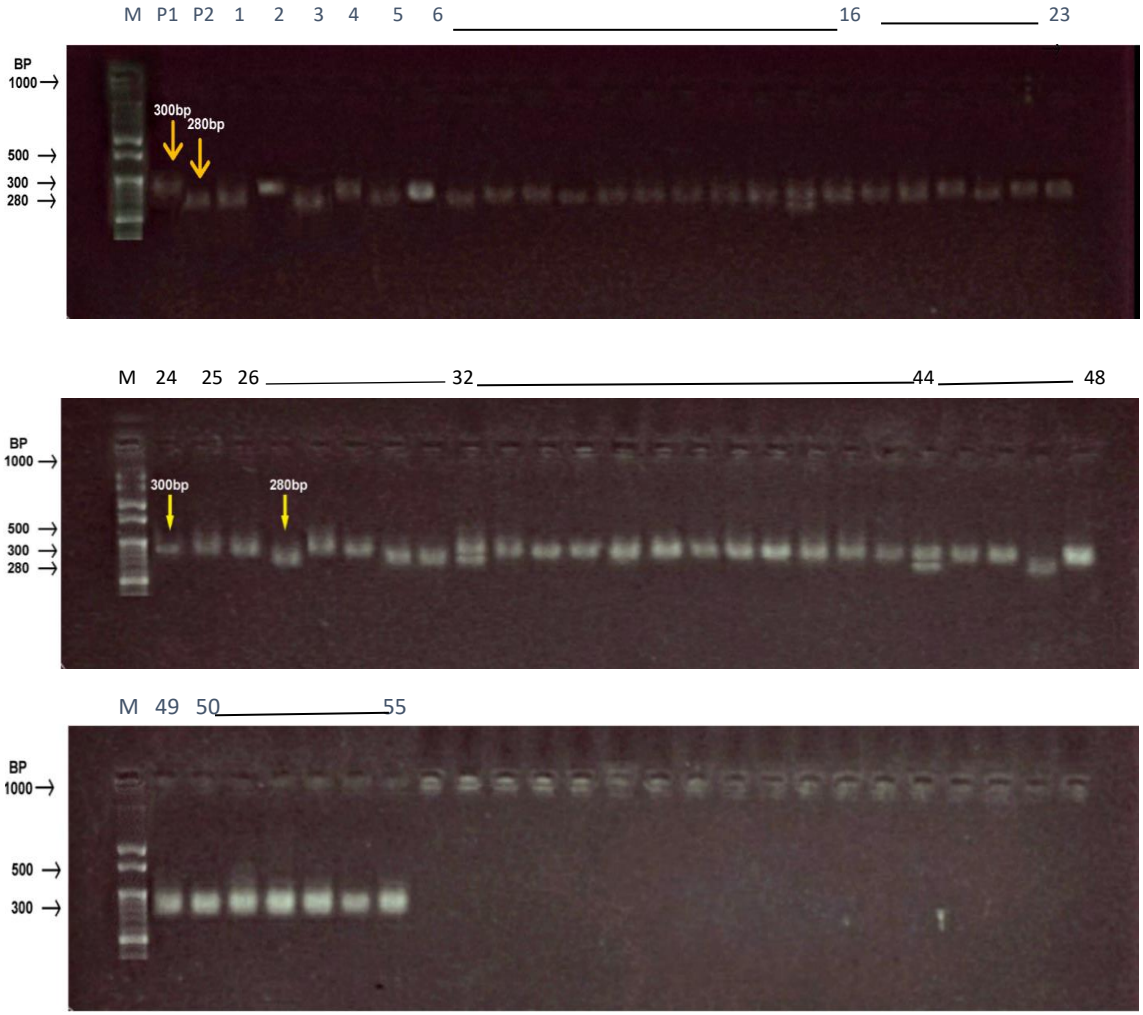

Figure 4.1: A representative gel depicting the F_2 plants of cross HPR-2795 x PB-3 with *SD1* gene-derived SSR marker sd1. P1=Punjab Basmati 3 (sd1); P2 = HPR- 2795. PCR products were resolved in 2.0 % agarose gel and stained with ethidium bromide. *Sd1* gene is present in PB-3 and absent in HPR-2795

Plant height of variety PB-3 ranged from 99cm to 106 cm with white seed colour whereas, HPR-2795 was having height in the range of 111cm to 125cm with red seed colour (Table 4.1). Out of 55 F_2 plants, in plant number 4, 6, 19 and 36 plant height ranged between 75cm to 90cm, in plant number 2, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29, 32, 33, 34, 35, 38, 39, 40, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54 and 55 plant height ranged from 91 to 110cm and in plant number 1, 3, 5, 7, 27, 30 and

31 plant height ranged between 111cm to 130cm and plant number 31 has plant height ranged between 131cm to 140cm.

Molecular analysis implied that 44 plants were homozygous for the gene *sd1*. Three plants i.e. plant number 16, 32 and 44 showed heterozygosity. Twenty-six plants sowed red seed color however, segregation in colour was also observed in plant number 32, 33 and 51 in which *sd1* gene was present (Table 4.1).

Table 4.1: Morphological and molecular data of selected F_2 plants in cross HPR-2795 x PB-3

S. No.	Bands (+/-)	Plant Height (cm)	Seed Colour	Panicle Length (cm)	Flag Leaf Length(cm)	Seed Yield/Plant (gm)
P1 - PB 3	**Present**	**99 – 106**	**White**	**28-30**	**39-43**	**21-22**
P2 - HPR 2795	**Absent**	**111 – 125**	**Red**	**28-32**	**43-51**	**15-19**
F_2population						
Plant 1	Absent	125.6	White	22.2	41.1	18.47
Plant 2	Present	103.8	Red	24.6	44.1	17.87
Plant 3	Absent	122.5	White	28.4	29.2	21.87
Plant 4	Present	79.3	White	21	45.2	20..67
Plant 5	Absent	125.3	Red	27.6	20.5	20.07
Plant 6	Present	88.9	White	29	33.2	19.87
Plant 7	Absent	129.7	White	24.3	31.1	21.07
Plant 8	Present	105.5	White	29.4	26.1	18.77
Plant 9	Present	103.9	Red	25.8	40.5	17.87
Plant 10	Present	100.2	Red	18	34.5	18.67
Plant 11	Present	99.7	White	26.5	23	18.37
Plant 12	Present	105.7	Red	26.8	39	19.17
Plant 13	Present	106.2	White	23.2	26.4	17.87
Plant 14	Present	102.1	Red	30.5	31.2	18.17
Plant 15	Present	95.7	Red	26.8	24.3	18.27
Plant 16	Present	102.4	White	27.5	35.1	17.67
Plant 17	Present	108.5	Red	26.4	36	18.47
Plant 18	Present	105.7	White	22.5	29.1	18.47
Plant 19	Present	77.2	Red	29	16.1	18.27
Plant 20	Present	101.9	Red	23.5	38.2	20.67
Plant 21	Present	103.7	White	24.2	28.1	19.47
Plant 22	Present	101.4	Red	26.4	21.8	19.37
Plant 23	Present	106.1	White	22.9	23.5	17.27

Plant 24	Present	95.9	White	22.6	25.5	20.27
Plant 25	Present	107.1	White	27.2	23.1	16.37
Plant 26	Present	99.3	Red	30.1	32.9	19.27
Plant 27	Absent	127.6	White	30.5	34.6	17.07
Plant 28	Present	104.5	Red	24	26.1	17.37
Plant 29	Present	100.2	White	23.1	33.3	18.07
Plant 30	Absent	118.9	Red	26.5	41.5	15.87
Plant 31	Absent	132.6	White	23.5	39.2	19.47
Plant 32	Present	107.4	white, red, light red	23.4	34.7	17.87
Plant 33	Present	102.9	white and light red	25.6	36.3	17.67
Plant 34	Present	105.1	Red	27.7	33.3	17.27
Plant 35	Present	100.7	White	30.4	41.1	16.37
Plant 36	Present	87.9	White	24.7	27.2	17.47
Plant 37	Present	106.7	Red	27.3	45.7	18.07
Plant 38	Present	103.4	Red	27.9	34.3	20.67
Plant 39	Present	95.4	Red	22.4	23.3	20.37
Plant 40	Present	106.1	White	22.6	41.3	19.67
Plant 41	Present	74.5	White	24.1	26.6	20.37
Plant 42	Present	108.7	Red	27.6	27.5	20.27
Plant 43	Present	105.7	Red	25.6	36	18.07
Plant 44	Present	103.2	Red	30	25	16.37
Plant 45	Present	106.3	Red	21	33.7	18.47
Plant 46	Present	102.6	Red	29.4	29.6	17.17
Plant 47	Absent	130.9	White	26.6	29.4	20.67
Plant 48	Present	103.7	White	23.6	35.5	17.07
Plant 49	Present	100.2	White	23	34.2	15.77
Plant 50	Present	105.1	Red	26.4	47.5	17.37
Plant 51	Present	105.4	white: red	25.6	38.3	18.47
Plant 52	Present	103.4	Red	26.5	43	20.27
Plant 53	Present	100.4	Red	31.5	39.8	18.67
Plant 54	Present	106.1	Red	29.8	36.2	16.77
Plant 55	Present	99.7	Red	24	30.5	17.47

Pericarp of 28 F_2 plants was red in color and these red colored and positive plants for semi-dwarf gene *sd1* were further examined for its association with agro morphological traits i.e. plant height, panicle length, flag leaf length and seed yield/plant. Plant height with positive alleles for *sd1* gene ranged from 74.5 to 108.7 cm. Five F_2 plants with plant numbers 2, 9, 37, 50 and 52 with positive allele for *sd1* and flag leaf length of >40 cm

were selected for further studies. The F_2 plants showing absence of *sd1* band were discarded and homozygous plants showing presence of *sd1* gene with red seed colour (Table 4.1) were selected for future breeding programme. Neha (2016) used MAS for screening aromatic, semi dwarf and photosensitive genes in F_2 of cross of Katrani rice with Rajendra Sweta.

Srivastava et al. (2019) also reported that variety Kalanamak's tall stature promotes lodging, which reduces its yield and other characteristics. As has been observed in the present studies, with the use of marker-assisted breeding, the semi-dwarfing gene (*sd1*) from CSR10 was introduced and the resultant semi-dwarf nature of backcross-derived plants was identified. The amplified product with SD1 primer from the donor line was 300 bp, which could be easily resolved on 2% agarose gel.

Table 4.2: Number of F_2 plants showing presence and absence of *sd1* gene band and number of plants with red seed coat and white seed coat color

Bands	**Present (less height)**		Absent (more height)	
Seed color	White	**Red**	White	Red
F_2 plants (HPR-2795 x PB-3)	Plant 4, Plant 6, Plant 8, Plant 11, Plant 13, Plant 18, Plant 23, Plant 24, Plant 25, Plant 29, Plant 35, Plant 36, Plant40, Plant 41, Plant 48, Plant 49	**Plant 2, Plant 9, Plant 10, Plant 12, Plant 14, Plant 15, Plant 17, Plant 19, Plant 20, Plant 21, Plant 22, Plant 26, Plant 28, Plant 34, Plant 37, Plant 38, Plant 39, Plant 42, Plant 43, Plant 45, Plant 46, Plant 50, Plant 52 , Plant 53, Plant 54, Plant 55**	Plant 1, Plant 3, Plant 7, Plant 27, Plant 31, Plant 47	Plant 5, Plant 30

Out of 55 F_2 plants studied, 16 plants were having white pericarp color and plant height ranged from 74.5cm to 107.1cm. 26 F_2 plants having red pericarp color and height ranged from 77.2cm to 108.7cm. 6 F_2 plants were having white pericarp and height ranged from 122.5cm to132.6cm. Only 2 plants were showing red pericarp and was taller in height (Table 4.2). Out of these 26 F_2 plants having red pericarp color and dwarf nature were selected for further studies. Three plants *viz*., plant number 16, 32 and 44 showed band heterozygosity (Figure 4.1) and plant number 32, 33 and 51 showed segregation in seed color. (Table 4.1)

Raina et al. (2019) used high yielding genotype PAU148, carrying gene *xa21, xa13* and *sd1* genes, as a donor parent to introgress these genes in Ranbir basmati which is a tall and short-duration variety with strong aroma and excellent cooking quality but was susceptible to bacterial blight (BB) and prone to lodging. A semi-dwarf (*sd1*) and BB resistance genes (*xa21* and *xa13*) were introgressed into Ranbir Basmati using marker-assisted backcross breeding (MABB) scheme.

4.2 Chi-Square test in F_2 population for seed color and plant height

Data for plant height and seed coat color was recorded in F_2 plants of cross of red rice variety HPR-2795 and white rice variety PB-3 and χ^2 test was applied.

4.2.1 Seed coat color

Chi-square test was conducted to test the goodness of fit in the F_2 population for seed color by comparing observed and expected frequency of plants in each range. The calculated and table value of chi square has been given in table 4.3. Out of 165 F_2 plants tested, the observed value of seed color was 89, 27 and 49 for red pericarp, segregating seed coat color and white pericarp color, respectively. Goodness of fit with chi-square test showed the expected segregation ratio of 9(Red): 3(Segregating): 4(White) indicating the presence of supplementary genes in the F_2 population for seed color. However, inheritance of pigmented pericarp of red rice was conducted by Hsieh and Chang (1964) and Rahman et al. (2013) but still this is not clear.

Table 4.3: Chi-square analysis for seed color in F_2 population of cross between HPR-2795 and PB-3

Class	Observed value (O)	Expected Value (E)	$\frac{(0-E)^2}{E}$	$\chi^2 = \Sigma \frac{(0-E)^2}{E}$	χ^2table (0.05,2)	χ^2table (0.01,2)
Pericarp color red	89	92.8	0.155	**2.09**	5.991	9.210

Segregants for red and white color	27	30.9	0.499			
Pericarp color white	49	41.2	1.456			
Total	165	165	**2.09**			

4.2.2 Plant height

Chi square test was applied to test the goodness of fit in the F_2 population for the plant height at maturity by comparing observed and expected frequency of plant in each range. The cut-off for tall and dwarf plant phenotype was set at 123.5 cm, which is the mid parent value of the mean of both parents. Goodness of fit with chi-square test showed the expected segregation ratio of 3:1 (at P <0.05 and P <0.0 where P is the probability that deviation of the observed frequency from the expected frequency is due to chance alone). Hence, it indicated that the plant height is controlled by a single gene. The calculated and table value of chi square has been given in table 4.4:

Table 4.4: Chi-square analysis of plant height in F_2 population of cross between HPR-2795 and PB-3

Class	Observed value (O)	Expected Value (E)	$\frac{(0-E)^2}{E}$	$\chi^2 = \Sigma \frac{(0-E)^2}{E}$	χ^2table (0.05,2)	χ^2table (0.01,2)
Semi-dwarf (plant height<123.5 cm)	130	123.75	0.315	**1.255**	3.814	6.635
Tall (plant height> 123.5 cm)	35	41.25	0.94			
Total	165	165	1.255			

4.3 Morphological analysis of F_2 population for the important agronomical traits

As the F_2 population is mortal and every plant is different so all the observations under study were taken on individual plant basis. Total of 165 plants were evaluated along with three checks *viz.*, PB-3 (check 1), HPR-2795 (check 2) and HPR-2880 (check 3) and their mean performance with respect to different traits is presented in Annexure-I. The character wise interpretation of the results is presented in table 4.5 and figures 4.2 to 4.12:

Table 4.5: Estimates of parameters of variability for various traits in F_2 genotypes

Trait	Mean ± SD	Range	Genetic coefficient of variation (%)	Phenotypic coefficient of variation (%)	Heritability (%)	Genetic Advance (as percent of mean)
DFF	88.72 ± 1.28	79.33-90.67	0.72	1.17	37.74	0.91
DSF	108.9 ± 0.97	106.56-110.56	0.05	0.81	0.38	0.01
PH	112.05 ± 14.34	73.18-146.04	12.59	12.79	96.87	25.56
NOT	7.44 ± 2	2.89-11.56	10.56	18.5	32.55	12.43
PL	26.04 ± 2.95	17.97-33.97	6.74	11.27	35.82	8.33
FLL	33.4 ± 7.34	14.29-52.36	18.87	21.66	75.86	33.9
GL	7.56 ± 1.19	4.62-9.92	12.67	13.61	86.69	24.34
GB	2.45 ± 0.39	1.84-3.08	12.54	13.87	81.75	23.39
L/B	3.14 ± 0.73	1.59-4.71	17.14	19.79	75.03	30.63
TGW	24.76 ± 6.32	20.31-38.94	25.36	25.83	96.4	51.38
GY	18.37 ± 1.5	15.07-22.27	2.97	7.8	14.56	2.34

DFF – Days to 50% flowering; DSF- Days to 75% maturity; FLL- Flag leaf length; GB- Grain breadth; GL- Grain length; GY- Grain Yield; L/B- Length/Breadth ratio; NOT- Number of tillers; PH- Plant height; PL- Panicle length; TGW- Thousand grain weight;

4.3.1 Mean Performance

a) Days to 50% flowering

The estimates of the mean values indicated that days to 50% flowering ranged from **79.33 – 90.67** days with overall mean value of **88.72**. Out of 165 Plants, six plants were found to be significantly earlier in flowering to the best check HPR-2880 (88.67 days).

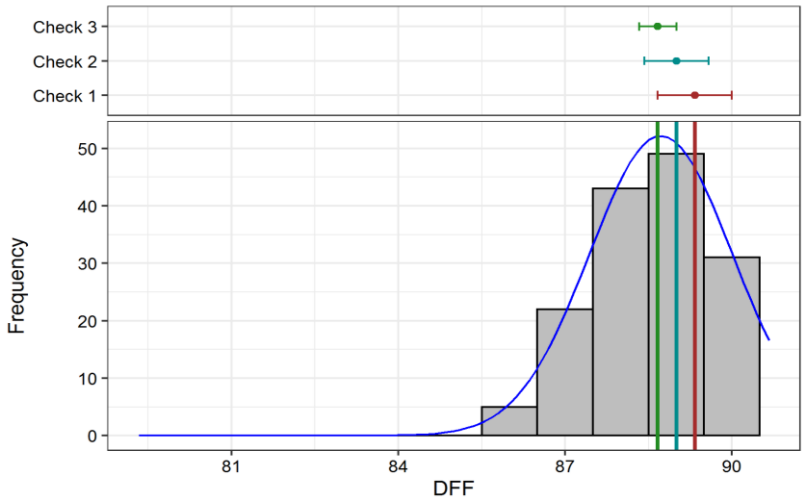

Figure 4.2: Frequency distribution curve of days to 50% flowering PB-3 (check 1); HPR-2795 (check 2) and HPR-2880 (check 3)

b) Days to 75% maturity

The range for days to 75% maturity varied from **106.56 – 110.56**days with a mean value of **108.9**. Among 165 plants, only one plant was significantly early in maturity to the best check HPR-2880 (108.67 days) whereas, 164 genotypes were statistically at par with the best check.

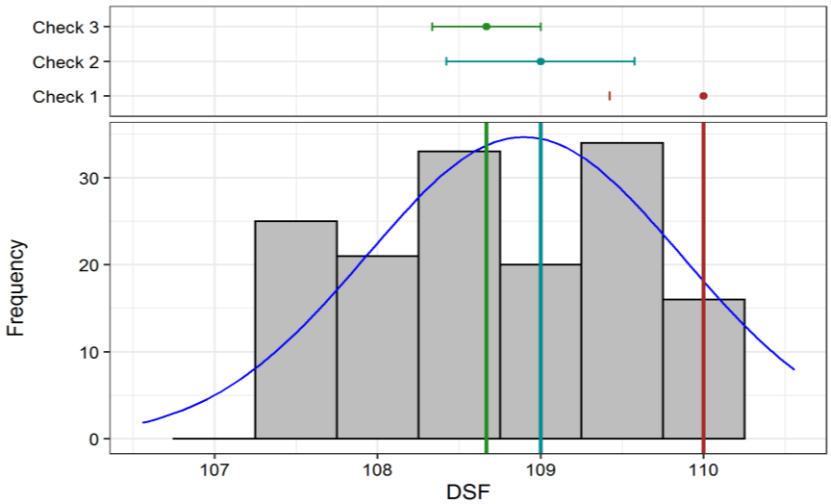

Figure 4.3: Frequency distribution curve of days to 75% maturity PB-3 (check 1); HPR-2795 (check 2) and HPR-2880 (check 3)

c) Plant height

The overall mean value for plant height is **112.05** cm and F_2 population varied from **73.18 – 146.04cm.** In 165 plants, 23 plants were significantly dwarf as compared to the best check PB-3 (105.4 cm) whereas, 63 plants were statistically at par with the best check. Average Plant height of parent variety HPR-2795 and PB 3 was 141.77 and 105.4cm, respectively. The values for F_1 plants were intermediate (Om Prakash, 2019) however, F_2 population showed segregation.

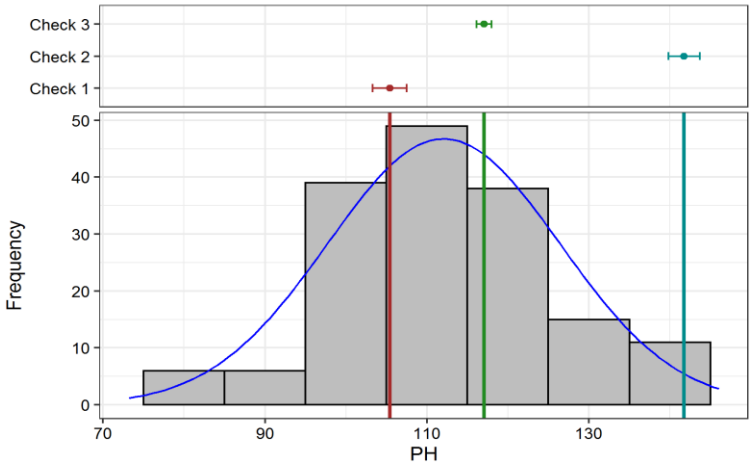

Figure 4.4: Frequency distribution curve of plant height (cm)
PB-3 (check 1); HPR-2795 (check 2) and HPR-2880 (check 3)

d) Number of tillers per plant

The overall mean value for this trait was **7.44** with a range of **2.89-11.56**. Only 3 plants were found to be superior as compared to the best check HPR-2880 and 131 plants were statistically at par with the best check.

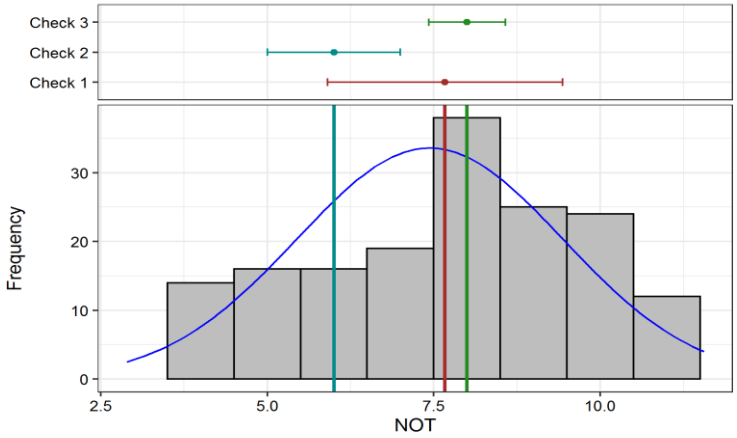

Figure 4.5: Frequency distribution curve of number of tillers per plant
PB-3 (Check 1); HPR-2795 (Check 2) and HPR-2880 (Check 3)

e) Panicle length

The mean value for this trait was **26.04 cm**, with a range of **17.97-33.97 cm**. None of the plants were significantly superior to the best check HPR-2880 (29.13) whereas, 92 plants were statistically at par with the best check.

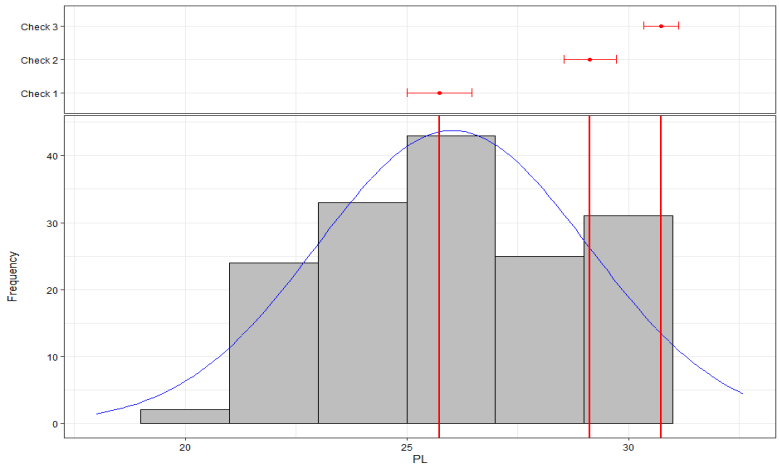

Figure 4.6: Frequency distribution curve of panicle length (cm)
PB-3 (Check 1); HPR-2795 (Check 2) and HPR-2880 (Check 3)

f) Flag leaf length

Flag leaf length varied from **14.29-52.36 cm** with a mean value of **33.4 cm.** None of the plants were significantly superior to the best check, HPR-2795 (46.4 cm) whereas, 42 genotypes were statistically at par with the best check.

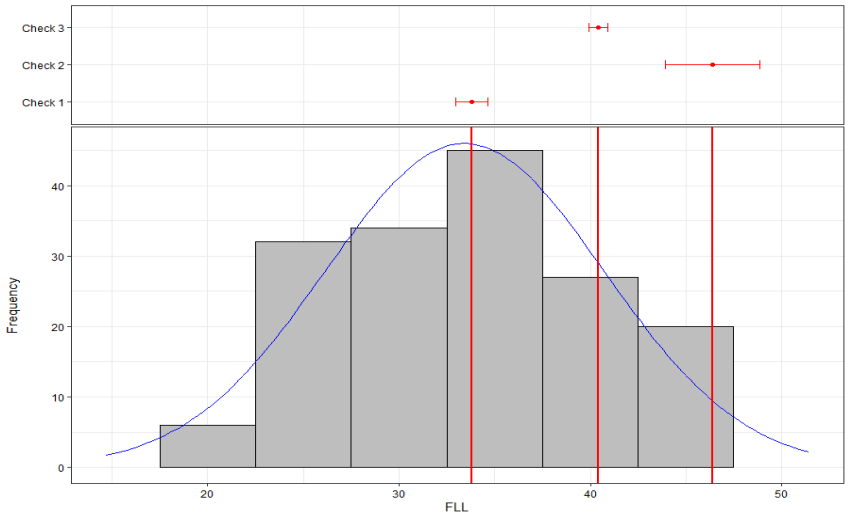

Figure 4.7: Frequency distribution curve of flag leaf length (cm)
PB-3 (check 1); HPR-2795 (check 2) and HPR-2880 (check 3)

g) Grain length

The grain length varied from **4.62-9.92 mm** with a mean of **7.56 mm**. Fifteen plants showed significantly more length compared to the best check HPR-2880 (8.1 mm) whereas, 90 plants were statistically at par with the best check.

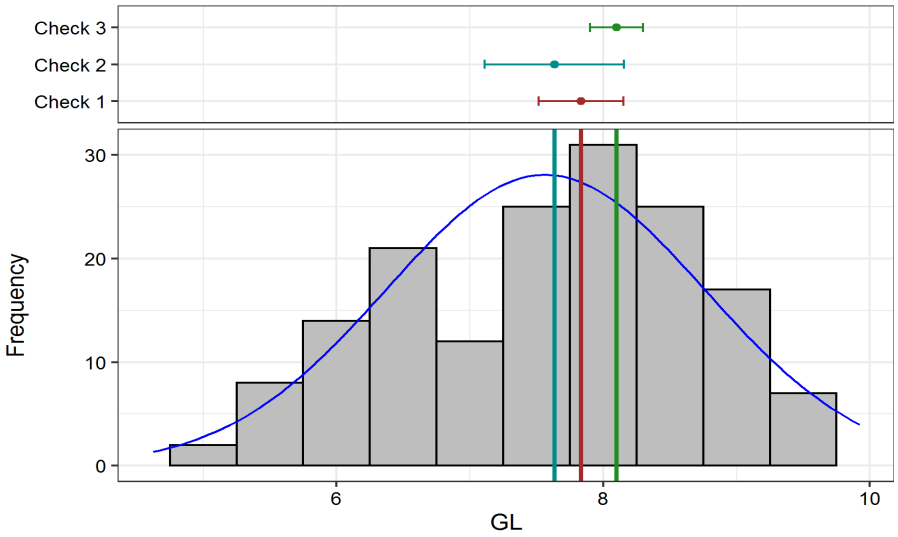

Figure 4.8: Frequency distribution curve of grain length (mm)
PB-3 (Check 1), HPR-2795 (Check 2) and HPR-2880 (Check 3)

h) Grain breadth

The average mean value was **2.45 mm**, with a range of **1.84-3.08 mm**. Nine plants exhibited significantly superior grain breadth over the best check, HPR-2795 (2.2 mm) whereas, 77 plants were found to be statistically at par with the best check.

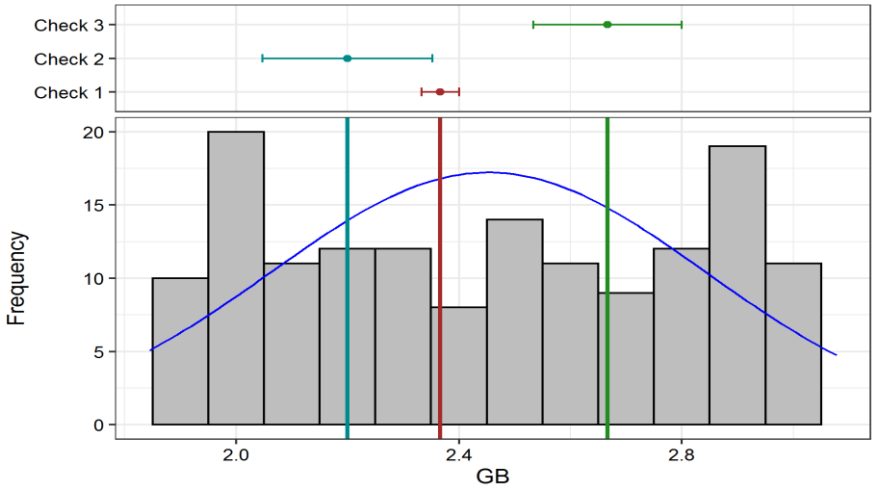

Figure 4.9: Frequency distribution curve of grain breadth (mm)
PB-3 (Check 1), HPR-2795 (Check 2) and HPR-2880 (Check 3)

i) L/B ratio

Mean value for this trait was **3.14** with a range of **1.59-4.71**. Out of 165 plants, 15 plants when compared to the best check, HPR-2795 (3.52) were found to be superior whereas, 83 plants were found statistically at par with the best check.

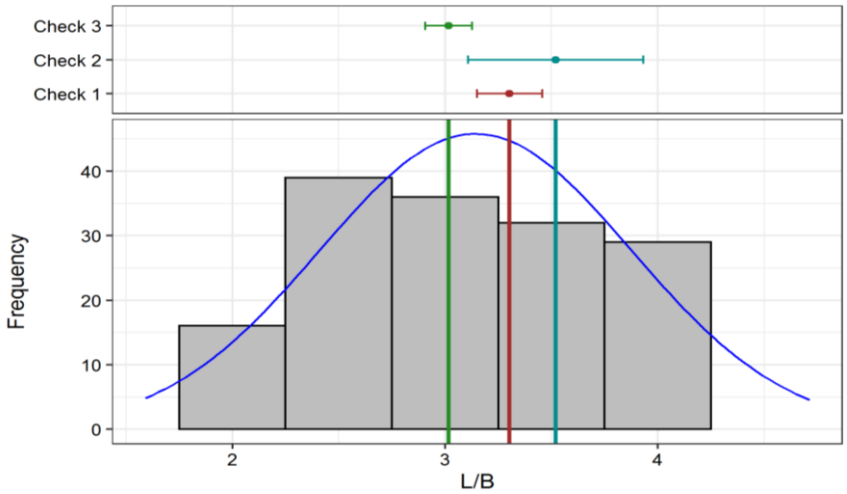

Figure 4.10: Frequency distribution curve of L/B ratio
PB-3 (check 1), HPR-2795 (check 2) and HPR-2880 (check 3)

j) 1000-grain weight

Mean value for 1000 grain weight was **24.76**gm, with a range of **20.31 - 38.94** gm. Out of 165 plants, 2 plants were superior to the best check, PB-3 (28.07g) whereas, 42 plants were statistically at par.

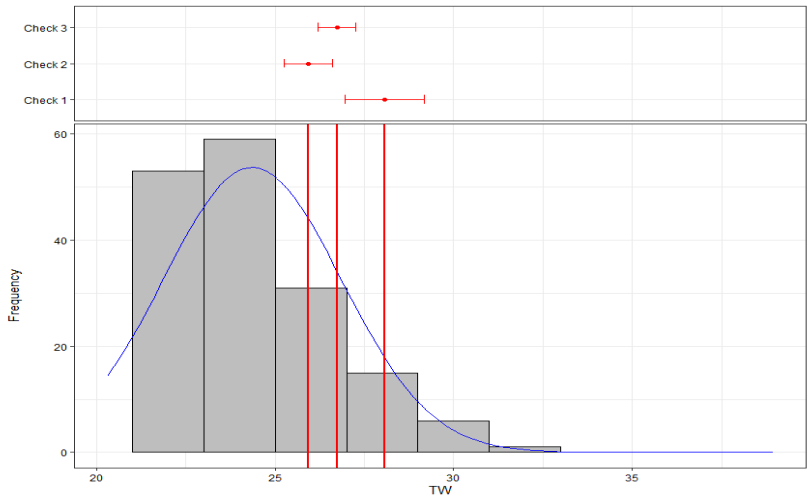

Figure 4.11: Frequency distribution curve of 1000 grain weight (gm)
PB-3 (Check 1), HPR-2795 (Check 2) and HPR-2880 (Check 3)

k) Grain yield per plant

The mean value for this trait was **18.37** g, with a range of **15.07-22.27**g. Out of 165 plants, none of the plants outyielded the best check, PB-3 (21.53 g) whereas, 66 plants were statistically at par with best check.

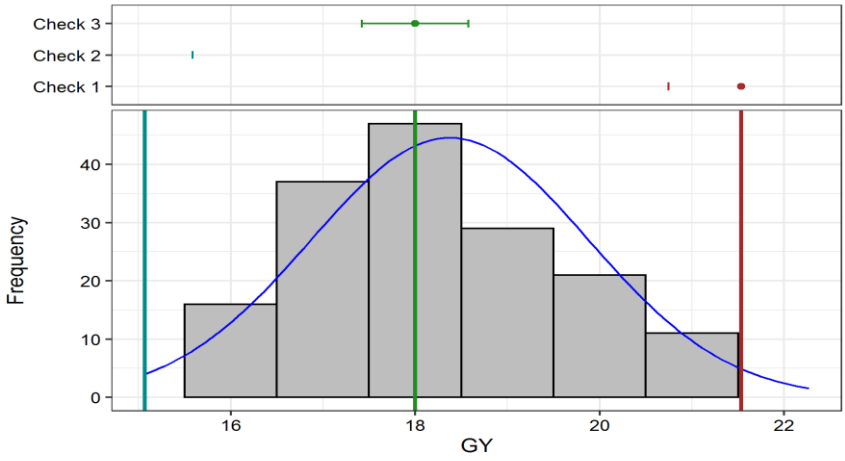

Figure 4.12: Frequency distribution curve of grain yield per plant (gm) PB-3 (Check 1), HPR-2795 (Check 2) and HPR-2880 (Check 3)

Frequency distribution studies were performed for the test traits i.e. days to 50% flowering, days to 75% maturity, plant height, panicle length, flag leaf length, number of tillers per plant, grain length, grain breadth, L/B ratio, thousand seed weight and grain yield in F_2 plant population. A continuous frequency distribution and presence of few transgressive segregants for all the phenotypic traits were observed in F_2 population except for red colour. This indicated the quantitative nature of the traits studied. The major reason for transgressive segregants could be complementary gene action for QTL's with opposite effects of parents (Thomson et al. 2003).

Neha K (2016), also studied frequency distribution for plant height, days to first flowering, sensory test for leaf and grain aroma, grain breadth and L/B ratio in F_2 and BC_1F_1 population of cross between Katrani and Rajendra sweta. She also observed continuous frequency distribution and presence of few transgressive segregants for all phenotypic traits in F_2 and BC_1F_1.

4.3.2 Estimates of variability parameters

The existence of genetic heterogeneity within a population determines the effectiveness of any selection programme. Variability at the phenotypic level develops as a result of genotypic and environmental impacts on phenotypic development. For various qualities, the estimations of mean, range, and variability factors such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense (h^2bs), and genetic advance (GA) expressed as a percentage of mean are presented in Table 4.5 and described below:

4.3.2.1 Coefficients of variation

The ability to anticipate the amount of variation in a germplasm using phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) helps in the formulation of an effective breeding programme.

The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV), in all the traits indicating that the apparent variance was not just due to genotypes but also to the significant influence of environment on genotype performance.

High PCV (>20%) values were observed for flag leaf length (21.66) and thousand grain weight (25.83). Moderate PCV (10-20%) was observed for plant height (12.79), number of tillers (18.5), panicle length (11.27), grain length (13.61), grain breadth (13.87) and length/breadth ratio (19.79) while, low PCV (<10%) was observed for days to 50% flowering (1.17), days to 75% maturity (0.81) and grain yield (7.8) (Table 4.5 and Fig 4.13).

The genotypic coefficient of variation (GCV) estimations indicated the extent of genotypic variability transferred from parents to offsprings. High GCV (>20%) values were observed for thousand grain weight (25.36). Moderate GCV (10-20%) was observed for plant height (12.59), number of tillers (10.56), flag leaf length (18.87), grain length (12.67), grain breadth (12.54) and length/breadth ratio (17.14). Low GCV (<10%) was observed for days to 50% flowering (0.72), days to 75% maturity (0.05), panicle length (6.74) and grain yield (2.97) (Table 4.5 and Fig 4.13).

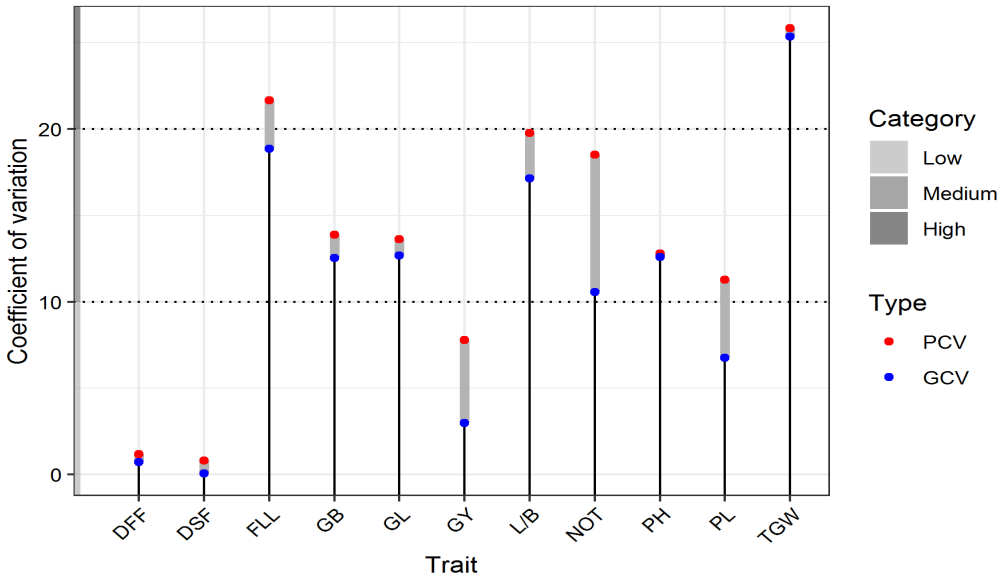

Fig 4.13: Graphical representation of Coefficient of variation for different traits

DFF – Days to 50% flowering; DSF- Days to 75% maturity; FLL- Flag leaf length; GB- Grain breadth; GL- Grain length; GY- Grain Yield; L/B- Length/Breadth ratio; NOT- Number of tillers; PH- Plant height; PL- Panicle length; TGW- Thousand grain weight; PCV- Phenotypic coefficient of variation; GCV- Genotypic coefficient of variation

The present results were corroborated by Bhat et al. (2018) in F_2 segregating population, developed from the crosses between the parental lines K343 and DHMAS. They reported the highest GCV values for spikelet density (7.24) and yield per plant (7.02), whereas it was the lowest for days to maturity (2.61). The low GCV and PCV values indicated that environmental influences on trait expression were minimal.

Present studies showed results that were in conformity with the findings of Shet et al. (2012) and Kalaiselvan et al. (2019) in which PCV as well as GCV was moderate for plant height and L/B ratio and both were low for days to 50% flowering.

4.3.2.2 Heritability in broad sense (h^2bs)

Heritable variation can be examined in terms of the degree to which it is passed down to the progeny, a concept known as heritability, when it accounts for a large amount of variability. Burton and DeVane (1953) has suggested that a genetic coefficient of variability paired with estimates of heritability would provide a more realistic picture of the predicted genetic gain through selection. The quantity of heritability refers to how reliably a genotype can be identified by its phenotypic appearance, which is crucial for breeders (Lush, 1940). Heritability estimations are useful for understanding quantitative

trait inheritance and developing breeding programmes with the needed amount of genetic development.

The present study revealed that heritability in broad sense was high (>70%) for most of the traits under study like plant height (98.87) followed by thousand grain weight (96.4), grain length (86.69), grain breadth (81.75), flag leaf length (75.86) and L/B ratio (75.03) revealing lesser influence of environment and greater role of genetic component of variation. Thus, selection for these traits on the basis of phenotypic expression would be more effective and can be relied upon. In F_2 each plant is genetically different. So heritability estimation does not give clear information. However, low heritability (<40%) was observed for days to 50% flowering (37.74), days to 75% maturity (0.38), number of tillers (32.55), panicle length (35.82) and grain yield (14.56) indicating that this trait was highly influenced by the environmental factors (Table 4.5 and Fig:4.14).

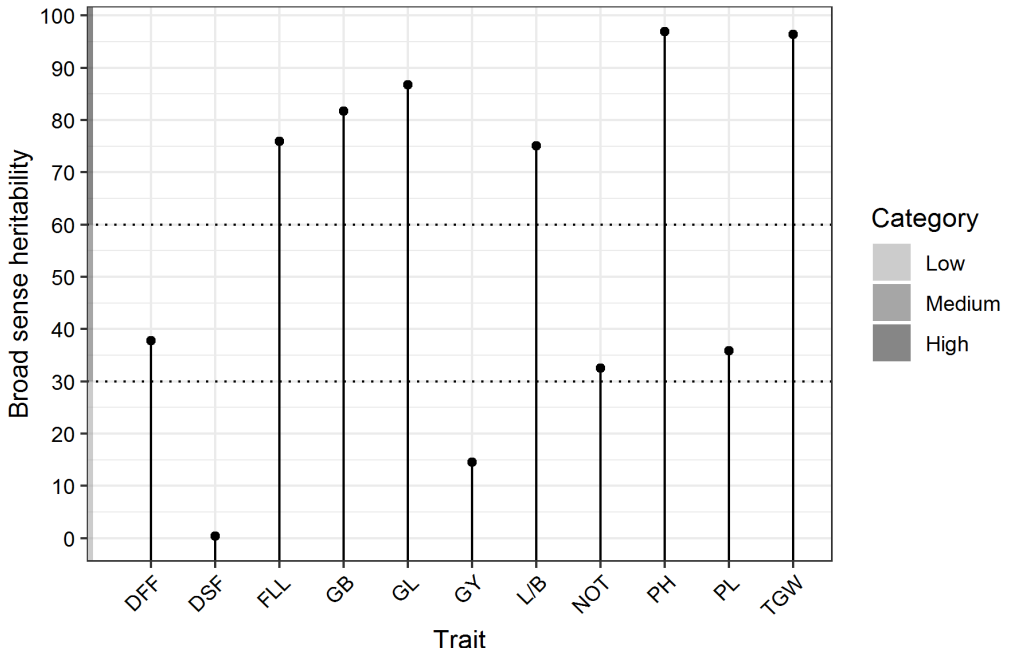

Fig 4.14: Graphical representation of broad sense heritability for different traits

DFF – Days to 50% flowering; DSF- Days to 75% maturity; FLL- Flag leaf length; GB- Grain breadth; GL- Grain length; GY- Grain Yield; L/B- Length/Breadth ratio; NOT- Number of tillers; PH- Plant height; PL- Panicle length; TGW- Thousand grain weight;

The present results were corroborated by the studies of Ogunbayo et al. (2014) for days to flowering, days to maturity, plant height at maturity, number of tiller per metre square and panicle length had high heritability estimates, indicating that the traits were primarily genetically controlled. The present results were also confirmed by Bharath et al. (2018) who reported high heritability for grain L/B ratio. Similarly, Yadav et al. (2010) and

Kalaiselvan et al. (2019) who studied that the flag leaf width and grain length breadth ratio possessed high heritability accompanied with high genetic advance which indicated the preponderance of additive gene action.

4.3.2.3 Genetic advance (GA)

The heritability estimates alone are insufficient to predict the response to selection however, when combined with estimates of genetic progress; these estimates appear to be more valuable than alone (Johnson et al., 1955). Thus, genetic advance has an added edge over heritability as a guiding factor to the breeders in various selections in breeding programmes.

Genetic advance expressed as percentage of mean was the highest (>20%) for thousand grain weight (51.38) followed by flag leaf length (33.9), L/B ratio (30.63), plant height (25.56), grain length (24.34), grain breadth (23.39) indicating that these characters were governed by additive genes and selection will be rewarding for improvement of such traits. Moderate genetic advance (10-20%) was observed for number of tillers (12.43) while, low genetic advance (<10%) was observed for days to 50% flowering (0.91), days to 75% maturity (0.01) and grain yield (2.34) indicating that these characters were governed by non- additive genes (Table 4.5 and Figure 4.15).

Heritability should be evaluated in conjunction with genetic progress while forecasting realistic estimates of additive and non-additive effects (Burton, 1953; Johnson et al., 1955). On this consideration, high heritability (>70%) coupled with high genetic advance (>20%) was observed for plant height, flag leaf length, grain length, grain breadth, L/B ratio and thousand grain weight indicating the presence of high additive gene effects providing scope for the improvement of these traits through selection.

Low heritability (<40%) coupled with low genetic advance (<10%) was observed for days to 50% flowering, days to 75% maturity, panicle length and grain yield which indicated that character was influenced by environment and selection for these traits would be ineffective.

The results of present study indicated presence of additive and non-additive gene action in the inheritance of these traits, providing scope for improvement of these traits through hybridization and selection.

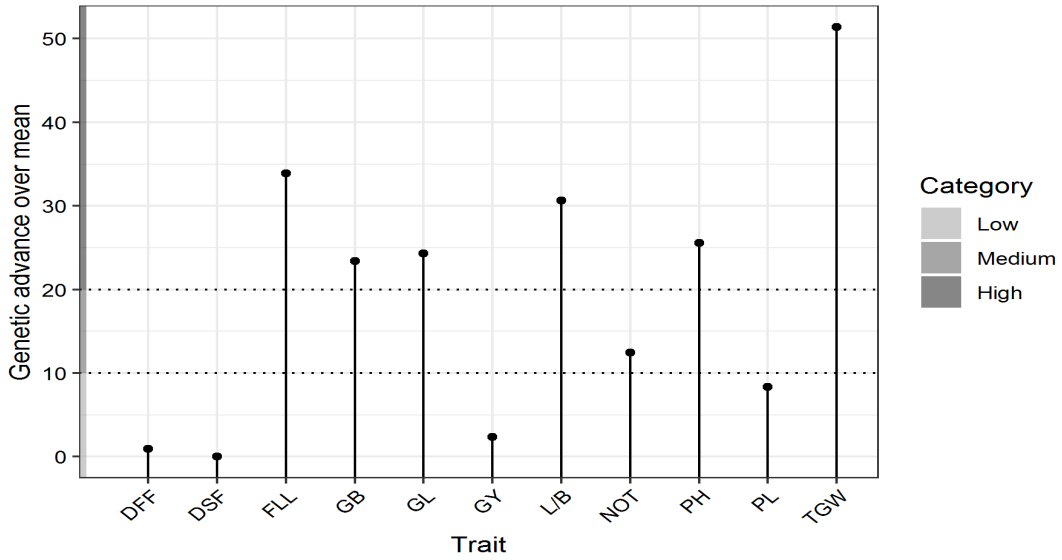

Fig 4.15: Graphical representation of Genetic advance over mean for different traits
DFF – Days to 50% flowering; DSF- Days to 75% maturity; FLL- Flag leaf length; GB- Grain breadth; GL- Grain length; GY- Grain Yield; L/B- Length/Breadth ratio; NOT- Number of tillers; PH- Plant height; PL- Panicle length; TGW- Thousand grain weight;

4.4 Correlation coefficient analysis

The study of the relationships between numerous yield-attributing variables is necessary for aggregating the best contribution of these traits to yield. Seed yield is highly influenced by environmental factors, has a complex inheritance pattern, and low heritability (Bocanski et al., 2009). However, most yield components are less complex, hence selecting the best progenies should be more accurate if another trait is substantially correlated with seed yield and has a greater heritability. (Vasic et al., 2001, Bekavac et al., 2007). The study of yield components and their inter-relationship along with yield and their direct and indirect contributions towards yield is of immense importance.

The phenotype of a plant is the result of interaction of a large number of factors. Hence, the final yield is the sum total of effects of several component factors. Therefore, it is important to know the extent and nature of inter-relationship between seed yield and its contributing traits and also among themselves, to identify traits for increasing the efficiency of both direct and indirect selection for yield improvement. Correlation analysis determines the component traits, based on which qualities can be selected to enhance grain yield genetically, and offers information about the nature and degree of relationship between various traits.

In the present study, the estimates of correlation coefficient were computed for 11 different morphological, yield and its component traits to determine the traits on which selection can be emphasized (Table 4.6) and the results obtained are discussed as under:

Seed grain yield showed significant positive correlation with grain breadth (0.19) and number of tillers (0.42) indicating that selection through these traits would be effective. However, it showed significant negative correlation with days to 75% maturity (-0.20), flag leaf length (-0.13) and L/B ratio (-0.15) and positive on significant correlation with panicle length (0.027) and thousand seed weight (0.11) hence it can be considered as an important component for realizing good yields

Days to 50% flowering showed significant positive correlation with grain length (0.39), L/B ratio (0.37) and number of tillers (0.43) indicating that selection through these traits would be effective and showed significant negative correlation with grain breadth (-0.15).

Days to 75% maturity showed negative correlation with grain breadth (-0.21), grain length (-0.23) and number of tillers (-0.36).

Flag leaf length showed significantly positive correlation with grain length (0.14), plant height (0.15) and panicle length (0.13).

Grain breadth showed significantly negative correlation with L/B ratio. However, it showed positive correlation with grain yield (0.19). Grain length showed significant positive correlation with L/B ratio (0.72) and number of tillers (0.32). L/B ratio showed significant positive correlation with number of tillers (0.20). Plant height showed positive correlation with panicle length (0.12) and thousand grain weight (0.088).

Present results were in agreement with Kiani et al. (2012) reporting that there was a highly significant correlation between grain yield with number of tillers per plant whereas, Ratna et al. (2015) reported that plant height was correlated negatively with number of tillers/plant and grain yield. Similarly, Bhargava et al. (2021) reported that number of tillers and length of panicle showed a significantly positive correlation with plant yield which indicated that improvement in length of panicle would improve grain number per panicle and plant yield. Abhilash et al. (2018) and Seneega et al. (2019) reported similar positive association between number of tillers and plant yield.

Table 4.6: Estimation of correlation coefficient among various yield per plant and agro-morphological traits in F_2 population

Traits	Days to 75% maturity	Flag leaf length	Grain breadth	Grain length	L: B ratio	Number of tillers	Plant height	Panicle length	1000- seed weight	Grain yield
Days to 50% flowering	-0.10	-0.0019	-0.15*	0.39**	0.37**	0.43**	0.02	0.088	0.026	-0.074
Days to 75% maturity		-0.018	-0.21**	-0.23**	-0.013	-0.36**	0.074	0.098	0.078	-0.20**
Flag leaf length			-0.044	0.14*	0.12	-0.091	0.15*	0.13*	-0.025	-0.13*
Grain breadth				-0.073	-0.74**	0.021	-0.0082	-0.0096	-0.054	0.19*
Grain length					0.72**	0.32**	0.12	0.059	-0.099	-0.03
L: B ratio						0.20**	0.085	-0.052	-0.039	-0.15*
Number of tillers							-0.041	-0.0009	0.10	0.42**
Plant height								0.12	0.088	-0.12
Panicle length									0.0073	0.027
1000- seed weight										0.11

*Significant at p<0.05 **Significant at p<0.01

4.5 Path Analysis

The correlation coefficients are very useful in determining the components of complex characters like seed yield, but such studies do not provide an exact picture of the relative importance of direct and indirect influence of each component characteristic, as these estimates only provide the nature and magnitude of the effect. In such cases, the path coefficient is critical for partitioning the correlations into direct and indirect effects of a single causal factor (Wright, 1921; Dewey and Lu, 1959). When a dependent trait, such as yield, is to be improved and is governed by many independent traits via direct or indirect effects of other traits, even a trait with a significant correlation with yield may not be considered for improvement because its correlation with yield may be due to the indirect effects of this trait via other traits. As a result, path coefficient analysis is always more appropriate for splitting the correlation value into direct and indirect effects. By resolving the correlation coefficient of yield and its components into direct and indirect impacts, path coefficient analysis provides a better means of selection.

The present investigation was therefore, aimed to estimate the direct and indirect effects of different traits on seed yield per plant and the values are presented in Table 4.7.

a) Estimation of direct effects

L/B ratio (1.268) had the highest positive effect on grain yield/plant followed by grain breadth (1.009), number of tillers (0.525), thousand grain weight (0.084) and panicle length (0.078). However, grain length (-0.920), days to 50 % flowering (-0.266), plant height (-0.103), flag leaf length (-0.059), days to 75 % maturity (-0.033) had negative direct effect on grain yield/plant. The present results were in close agreement with findings of Karad and Pol (2008) and Minnie et al. (2013), who studied the characters like number of tillers per plant and panicle length which showed positive direct effects on yield.

b) Estimation of indirect effects

4.5. b.1 Days to 50 % flowering

Days to 50 % flowering had positive indirect effect *via* days to 75 % maturity (0.003), L/B ratio (0.469), number of tillers (0.226), panicle length (0.007) and thousand grain weight (0.002). However, it showed negative indirect effect on grain yield/plant via grain breadth (-0.151), grain length (-0.359) and plant height (-0.002).

4.5. b.2 Days to 75% maturity

Days to 75 % maturity had negative indirect effect on grain yield/plant via grain breadth (-0.212), L/B ratio (-0.013), number of tillers (-0.189) and plant height (-0.007). However, it showed positive indirect effect via days to 50 % flowering (0.027), flag leaf length (0.001) grain length (0.212), panicle length (0.008) and thousand grain weight (0.007).

4.5. b.3 Flag leaf length

In a similar trend flag leaf length had positive indirect effect on grain yield/plant via days to 75% maturity (0.001), grain L/B ratio (0.152) and panicle length (0.010) while it had negative indirect effect on grain yield/plant via grain breadth (-0.040), grain length (-0.129), No. of tillers/plant (-0.047), plant height (-0.016) and thousand grain weight (-0.003).

4.5. b.4 Grain breadth

Grain breadth showed positive indirect effect on grain yield/plant via days to 50% flowering (0.040), days to 75% maturity (0.007), flag leaf length (0.002), grain length (0.064) and number of tillers (0.011). However, this trait showed negative indirect effect via L/B ratio (-0.938), panicle length (-0.001) and thousand grain weight (-0.004).

4.5. b.5 Grain length

Grain length showed positive indirect effect on grain yield/plant via days to 75 % maturity (0.007), L/B ratio (0.913), number of tillers/plant (0.168) and panicle length (0.005) Whereas this trait showed negative indirect effect via days to 50% flowering (-0.103), flag leaf length (0.008), grain breadth (-0.070), plant height (-0.012), thousand grain weight (-0.008).

4.5. b.6 Grain L: B ratio

Grain L/B ratio had positive indirect effect on grain yield/plant via number of tillers (0.105) and panicle length (0.003). while this trait showed negative indirect effect via days to 50% flowering (-0.098), flag leaf length (-0.007), grain breadth (-0.747), grain length (-0.662), plant height (-0.009) and thousand grain weight (-0.003).

4.5. b.7 Number of tillers per plant

Number of tillers per plant showed positive indirect effect via days to 75% maturity (0.012), flag leaf length (0.005), grain breadth (0.020), grain L/B ratio (0.254), plant height (0.004) and thousand grain weight (0.008). However, this trait showed

negative indirect effect on grain yield/plant via days to 50% flowering (-0.114) and grain length (-0.294).

4.5. b.8 Plant height

Plant height had positive indirect effect via grain L/B ratio (0.114), panicle length (0.009) and thousand grain weight (0.008). However, this trait showed negative indirect effect on grain yield/plant via days to 50% flowering (-0.002), days to 75 % maturity (-0.005), flag leaf length (-0.009), grain length (-0.110) and number of tillers (-0.021).

4.5. b.9 Panicle length

Panicle length had positive indirect effect on grain yield/plant via L/B ratio (0.063), thousand grain weight (0.001) whereas, negative indirect effect via days to 50% flowering (-0.024), days to 75 % maturity (-0.003), flag leaf length (-0.008), grain breadth (-0.010), grain length (-0.055) and plant height (-0.012).

4.3.b.10 Thousand grain weight

Thousand grain weight showed positive indirect effect on grain yield/plant via flag leaf length (0.002), grain length (0.092), number of tillers (0.053) and panicle length (0.001). However, this trait showed negative indirect effect via days to 50% flowering (-0.008), days to 75 % maturity (-0.003), grain breadth (-0.051), grain L/B ratio (-0.051) and plant height (-0.009).

Kiani et al. (2012) revealed revealed that number of tillers per plant had the highest positive direct effect. However, based on F_2 population studies (Bhargava et al., 2021) reported that productive tiller number exerted maximum positive direct effect on plant yield followed by grain number per panicle but showed positive direct effect on yield via panicle length, thousand grain weight and number of tillers. Similar results of direct positive effect on plant yield were reported by Nandeshwar et al. (2010) and Kalaiselvan et al. (2019). In contrast to positive direct effect, negative direct effect on plant yield was exhibited by days to flowering (Bhargava et al. 2021). Akhtar et al. (2011) also reported the negative direct effect of plant height on yield.

The residual effect was 0.6934941, which indicated that the contribution of ten component characters on grain yield was only 30.7% in path analysis. The rest 69.3% was contributed by other factors or traits which are not studied under this analysis. This high residual effect towards yield in the present study might be due to many reasons, such as other characters, which are not included in the investigation, environmental factor, segregation in F_2 population and sampling errors.

Table 4.7: Estimation of path coefficient of grain yield per plant with different characters

Traits	DFF	DSM	FLL	GB	GL	L/ B	NOT	PH	PL	TGW	Grain yield
DFF	**-0.266**	0.003	0.000	-0.151	-0.359	0.469	0.226	-0.002	0.007	0.002	-0.074
DSM	0.027	**-0.033**	0.001	-0.212	0.212	-0.013	-0.189	-0.007	0.008	0.007	-0.200**
FLL	0.000	0.001	**-0.059**	-0.040	-0.129	0.152	-0.047	-0.016	0.010	-0.003	-0.130*
GB	0.040	0.007	0.002	**1.009**	0.064	-0.938	0.011	0.000	-0.001	-0.004	0.19*
GL	-0.103	0.007	-0.008	-0.070	**-0.920**	0.913	0.168	-0.012	0.005	-0.008	-0.030
L/ B	-0.098	0.000	-0.007	-0.747	-0.662	**1.268**	0.105	-0.009	0.003	-0.003	-0.15*
NOT	-0.114	0.012	0.005	0.020	-0.294	0.254	**0.525**	0.004	0.000	0.008	0.420**
PH	-0.005	-0.002	-0.009	0.000	-0.110	0.114	-0.021	**-0.103**	0.009	0.008	-0.120
PL	-0.024	-0.003	-0.008	-0.010	-0.055	0.063	0.000	-0.012	**0.078**	0.001	0.027
TGW	-0.008	-0.003	0.002	-0.051	0.092	-0.051	0.053	-0.009	0.001	**0.084**	0.110

Residual Effect = 0.6934941

*Significant at p<0.05 **Significant at p<0.01

Diagonal bold values denote direct effects and remaining indirect effect.

5. SUMMARY AND CONCLUSIONS

Red rice 'the red pearls of Himachal Pradesh' is considered as the "ancient treasure" of the state. Its high medicinal properties help in curing several chronic diseases due to its high nutritional value. In the present era of increased lifestyle-oriented diseases, red rice is becoming popular due to its antioxidant properties, nutritive value and scavenging activity. HPR-2795 is a high-yielding, early maturing and disease-resistant variety of red rice. It has a thick stem and long flag leaf, which enhances photosynthetic efficiency leading to higher yield. However, it is very tall and prone to lodging, which may lower its production. In this context, there is a need to incorporate a semi dwarfness gene into this red rice variety 'HPR-2795' to prevent lodging losses. Hence, the present investigation entitled "Marker aided selection for dwarfness in F_2population of red rice variety HPR-2795" was carried out during *Kharif*, 2020 at the experimental farm of the Department of Genetics and Plant Breeding, CSKHPKV, Palampur.

The experimental material for the present study comprised of 165 F_2 plants from the cross HPR-2795 x PB-3 along with parents as checks. These F_2 plants and parents were evaluated for different agro-morphological characters and subjected to molecular analysis using SSR markers. Out of 165 plants only 55 randomly selected plants were screened for the presence of *sd1* gene.

The data was recorded for yield and yield contributing traits and grain quality traits and data was analyzed as per standard statistical procedures. Samples were collected for marker aided selection to identify dwarfness gene i.e. *sd*1 gene in the F_2 population.

Marker Assisted Selection (MAS) was conducted on parents HPR-2795 and PB-3 as well as 55 F_2 selected plants of cross between HPR-2795 x PB-3 for *sd1* gene showed that the parent PB-3 showed the presence of band at 300 bp indicating the presence of *sd1* gene. However, bands of 280 bp were present in parent HPR-2795 indicating the absence of *sd1* gene. Some of the plants were having both the bands which represented the heterozygosity.

The parent PB-3 was having plant height ranging of 99 -106 cm whereas plant height of parent HPR-2795 ranged from 111– 125cm with red seed color. Out of 55 F_2 plants studied, 16 plants were having white pericarp color and plant height ranged from 74.5cm to 107.1cm. 26 F_2 plants having red pericarp color and height ranged from 77.2cm to 108.7cm.

With the absence of *sd1* gene band 6 F_2 plants were having white pericarp and height ranged from 122.5cm to132.6cm. Only 2 plants were showing red pericarp and was taller in height. Out of these 26 F_2 plants having red pericarp color and dwarf nature were selected for further studies. Three plants *viz*., plant number 16, 32 and 44 showed band heterozygosity and plant number 32, 33 and 51 showed segregation in seed color.

All the samples showing absence of gene *sd1* band were discarded and homozygous plants showing presence of gene *sd1* with red colored seed were selected for future breeding programme.

The inheritance pattern of seed coat color and plant height was studied in 165 F_2 plants of cross HPR-2795 with PB-3 and observed value of seed color was 89, 27 and 49 for red pericarp, segregating seed coat color and white pericarp color, respectively. Goodness of fit with chi-square test showed the expected segregation ratio of 9(Red): 3(Segregating): 4(White) indicating the presence of supplementary genes in the F_2 population for seed color. Goodness of fit with chi-square test showed the expected segregation ratio of 9:3:4, which indicated significant deviation from Mendelian segregating ratio. Hence, it is assumed that the supplementary gene action may be operating in the F_2 population for seed color. For plant height, goodness of fit with chi-square test showed the expected segregation ratio of 3:1. Hence, single gene is controlling plant height present in the F_2 population.

Frequency distribution studied for agro-morphological traits indicated continuous frequency distribution and presence of few transgressive segregants indicating the quantitative nature of traits studied.

For some of the traits tested, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV), indicating that the apparent variance is not just due to genotypes but also there is significant influence of environment on genotype performance.

High PCV (>20%) values were observed for flag leaf length and thousand grain weight whereas, moderate PCV was observed for plant height, number of tillers/plant, panicle length, grain length, grain breadth and length/breadth ratio while, low PCV (<10%) was observed for days to 50% flowering, days to 75% maturity and grain yield.

The genotypic coefficient of variation (GCV) estimations indicated the entire amount of genotypic variability transferred from parents to offspring. High GCV (>20%)

value was observed for thousand grain weight and moderate GCV (10-20%) was observed for plant height, number of tillers/plant, flag leaf length, grain length, grain breadth, and length/breadth ratio. Low GCV (<10%) was observed for days to 50% flowering, days to 75% maturity, panicle length and grain yield. Heritability in broad sense was high (>70%) for most of the traits under study viz. plant height, flag leaf length, grain length, grain breadth, L/B ratio and thousand grain weight revealing lesser influence of environment and greater role of genetic component of variation. Thus, selection for these traits on the basis of phenotypic expression would be more effective and can be relied upon. Low heritability (<40%) was observed for days to 50% flowering, days to 75% maturity, number of tillers, panicle length and grain yield indicating that this trait was highly influenced by the environmental factors.

Accordingly, high heritability (>70%) coupled with high genetic advance (>20%) was observed for plant height, flag leaf length, grain length, grain breadth, L/B ratio and thousand grain weight indicating the presence of high additive gene effects thereby providing scope for the improvement of these traits through selection.

Low heritability (<40%) coupled with low genetic advance (<10%) was observed for days to 50% flowering, days to 75% maturity, panicle length and grain yield which indicated that these characters were influenced by environment and selection for these traits would be ineffective.

The results of present study indicated presence of additive and non-additive gene action in the inheritance of these traits, providing scope for improvement of these traits through hybridization and selection.

Seed grain yield showed significant positive correlation with grain breadth and number of tillers indicating that selection through these traits would be effective. However, it showed significant negative correlation with days to 75% maturity, flag leaf length and L/B ratio. It showed positive correlation with panicle length and thousand seed weight hence, it can be considered as significant component for realizing good yields.

L/B ratio had the highest positive effect on grain yield/plant followed by grain breadth, number of tillers, thousand grain weight and panicle length. However, grain length, days to 50 % flowering, plant height, flag leaf length, days to 75 % maturity had negative direct effect on grain yield/plant. The highest positive indirect effects were also shown by L: B ratio for most of the studied traits.

Conclusions:

1. From the molecular analysis it was observed that 26 plants were homozygous for the *sd1* gene and 3 plants showed heterozygosity. Segregation in seed color was observed in plant number 32, 33 and 51 in which *Sd1* gene was present.
2. Based on foreground selection with functional marker of *sd1* gene plants were selected that were homozygous. This selection was further narrowed down by looking the selected plants with respect to reduced plant height and other agro-morphological traits. Homozygous plants showing presence of *sd1* gene with red seed color were selected for further breeding programme and all the plants showing absence of *sd1* band and segregating for seed color were discarded.
3. The supplementary gene action may be controlling seed coat color and plant height is assumed to be controlled by single gene in the F_2 population of cross HPR-2795 with PB-3.
4. The magnitudes of PCV and GCV were high for thousand grain weight and high PCV was observed in flag leaf length. Moderate PCV and GCV were observed for plant height, number of tillers, grain length, grain breadth, L: B ratio and moderate GCV was observed for flag leaf length.
5. High heritability was observed for the traits *viz*., days to flowering, number of tillers/plant, panicle length revealing lesser influence of environment and greater role of genetic component of variation. Thus, selection for these traits on the basis of phenotypic expression would be more effective and reliable.
6. High genetic advance was observed for number of tillers/plant indicating that this character is governed by additive genes and selection will be rewarding for improvement of such traits.
7. High heritability along with high genetic advance was observed for number of tillers which indicated that most likely the heritability was due to additive gene effects and selection may be effective.
8. Grain yield was found to be positively and significantly correlated with grain breadth and number of tillers suggesting effective selection for these traits.
9. The direct positive effect was the highest by L: B ratio followed by grain breadth, thousand grain weight, panicle length and number of tillers suggesting as the best selection indices for yield improvement.

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Appendix I: Mean value for F_2 population with respect to yield and yield contributing traits during crop season *Kharif* 2020 at Experimental farm (CSKHPKV), Palampur

Treatment	Days to 50% flowering	Days to 75% maturity	Flag leaf length (cm)	Grain breadth (cm)	Grain length (cm)	L/B ratio	Number of tillers	Plant height (cm)	Panicle length (cm)	Thousand grain weight (cm)	Grain yield
C-1 (PB3)	89.33	110	32.5	2.37	7.83	3.3	7.67	105.4	26.5	28.07	21.53
C-2 (HPR2795)	89	109	46.4	2.2	7.63	3.52	6	141.77	26.5	25.93	15.07
C-3 (HPR2880)	88.67	108.67	33.73	2.67	8.1	3.02	8	117.07	29.13	26.73	18
1	88	107.56	39.29	2.78	9.72	3.53	9.56	124.38	22.17	25.91	18.47
10	88	106.56	43.39	3.08	6.22	1.93	10.56	78.08	20.97	21.41	20.67
100	89.67	108.89	35.96	1.84	8.56	4.43	9.56	107.54	28.07	23.34	17.27
101	90.67	108.89	31.76	2.54	8.66	3.43	9.56	108.14	26.77	23.24	16.97
102	89.67	109.89	43.86	1.84	8.56	4.43	8.56	101.44	28.97	23.14	17.17
103	89.67	109.89	43.76	2.54	7.76	3.1	8.56	95.44	30.77	23.14	16.37
104	89.67	108.89	42.86	1.84	8.86	4.58	7.56	142.84	30.57	30.74	15.87
105	88.67	108.89	37.96	1.84	8.66	4.48	6.56	110.14	27.27	22.74	16.57
106	88.67	109.89	29.86	2.24	8.56	3.76	7.56	90.44	25.07	23.44	17.47
107	88.67	108.89	44.66	2.64	7.96	3.07	7.56	118.04	28.37	25.74	17.37
108	88.67	107.89	44.56	2.44	8.76	3.58	7.56	111.54	28.67	23.74	17.87
109	89.67	107.89	48.36	2.64	8.66	3.32	7.56	109.24	27.67	22.64	18.07
11	89	107.56	45.29	2.98	9.72	3.26	9.56	119.08	27.47	23.41	18.87
110	89.67	109.89	52.36	2.14	8.86	4.03	6.56	130.54	28.87	27.24	15.87
111	88.33	109.56	45.86	2.78	6.62	2.34	4.89	115.98	24.57	23.84	17.37
112	87.33	109.56	33.46	2.68	7.52	2.77	6.89	102.08	27.57	21.84	20.67
113	87.33	110.56	38.96	2.88	6.62	2.25	4.89	118.18	27.57	23.84	19.37
114	87.33	110.56	40.36	2.78	7.42	2.62	5.89	121.38	25.97	24.34	18.47
115	87.33	109.56	22.46	2.88	7.62	2.59	6.89	94.08	22.07	21.64	20.37

116	87.33	108.56	33.26	2.18	6.52	2.99	5.89	82.28	27.67	22.34	19.77
117	87.33	109.56	20.16	2.88	6.72	2.28	6.89	108.28	29.67	22.84	20.77
118	88.33	110.56	40.46	1.98	7.42	3.77	5.89	104.78	22.27	22.74	19.67
119	88.33	110.56	33.36	2.08	8.52	4.05	8.89	139.28	28.07	38.94	22.17
12	89	108.56	31.19	2.88	8.12	2.8	11.56	107.98	24.57	21.21	22.27
120	88.33	108.56	34.66	2.88	5.82	1.97	7.89	106.68	28.17	23.54	21.67
121	88.33	109.56	25.76	2.38	4.62	1.93	8.89	73.18	23.77	29.64	20.37
122	89.33	108.56	27.36	1.98	6.42	3.27	7.89	116.68	23.17	23.84	19.77
123	89.33	110.56	25.46	2.08	5.82	2.81	5.89	104.48	23.67	21.94	18.27
124	89.33	110.56	26.66	2.18	4.72	2.17	7.89	105.38	27.27	22.24	20.27
125	89.33	109.56	50.86	2.78	5.72	2.02	7.89	121.18	25.87	25.14	20.57
126	87.33	108.56	45.86	2.28	5.82	2.55	5.89	127.88	28.27	26.14	19.27
127	87.33	109.56	35.16	1.98	5.82	2.97	6.89	107.38	25.27	22.44	18.07
128	87.33	108.56	34.16	2.88	6.52	2.21	5.89	116.18	22.67	24.74	16.07
129	87.33	109.56	22.46	2.88	4.72	1.59	4.89	126.28	24.17	26.14	18.07
13	89	108.56	18.69	2.98	7.92	2.58	7.56	124.08	27.57	24.91	20.07
130	88.33	110.56	24.16	2.38	5.42	2.27	4.89	105.88	29.67	22.34	16.37
131	88.33	109.56	35.76	2.28	7.52	3.29	3.89	107.18	30.17	23.14	16.67
132	88.33	109.56	38.06	2.78	5.72	2.02	4.89	112.78	29.07	24.74	17.67
133	79.33	108.56	32.86	2.78	5.52	1.94	5.89	106.98	20.67	22.84	18.47
134	88.33	110.56	46.36	1.98	7.42	3.77	3.89	105.68	29.27	22.54	16.07
135	87.33	110.56	28.06	2.88	6.52	2.21	3.89	111.18	19.97	23.74	16.17
136	87.33	110.56	28.76	2.28	6.52	2.85	4.89	101.28	29.07	21.94	17.17
137	87.33	110.56	32.66	2.08	7.42	3.67	4.89	138.38	28.17	27.94	17.47
138	87.33	110.56	25.56	2.08	7.52	3.62	3.89	128.68	27.27	26.94	16.67
139	87.33	109.56	28.56	2.78	6.62	2.34	6.89	134.58	26.27	28.74	20.67
14	89	107.56	27.29	2.78	7.22	2.57	9.56	104.68	26.47	20.61	21.07
140	88.33	109.56	32.46	2.18	5.72	2.63	3.89	107.78	22.57	23.24	17.47

141	88.33	109.56	23.36	1.98	7.62	3.87	4.89	110.88	23.67	23.94	16.67
142	88.33	110.56	34.66	1.98	6.52	3.32	4.89	102.38	23.27	21.84	17.07
143	89.33	110.56	30.56	1.98	6.52	3.32	3.89	124.18	24.97	24.94	18.37
144	89.33	109.56	40.36	2.78	6.82	2.41	3.89	114.88	23.47	23.64	18.07
145	89.33	110.56	33.36	2.68	7.52	2.77	2.89	98.48	22.67	22.14	15.77
146	88.33	108.56	30.96	2.28	6.42	2.81	4.89	111.58	21.17	23.94	16.47
147	88.33	108.56	32.76	1.98	7.72	3.92	3.89	113.28	24.57	23.54	16.77
148	86.33	108.56	46.66	1.98	7.72	3.92	3.89	103.68	26.07	21.34	17.37
149	86.33	110.56	44.46	2.28	6.52	2.85	5.89	105.88	22.07	21.64	17.67
15	88	108.56	24.49	2.98	7.22	2.37	8.56	98.78	27.27	24.01	20.87
150	87.33	107.56	39.86	1.98	7.52	3.82	5.89	108.18	27.67	22.84	19.27
151	88.33	107.56	37.46	2.78	9.02	3.19	4.89	104.08	25.27	22.94	18.47
152	86.33	108.56	34.36	2.88	7.62	2.59	3.89	126.28	24.27	25.84	16.97
153	87.33	108.56	38.66	2.88	7.52	2.56	6.89	129.18	28.97	26.94	19.67
154	86.33	108.56	42.16	2.18	5.82	2.67	6.89	102.08	26.17	22.84	20.27
155	87.33	110.56	37.06	1.98	5.82	2.97	5.89	127.88	29.17	27.74	18.27
156	88.33	110.56	36.16	1.98	6.52	3.32	4.89	104.68	25.27	22.54	17.57
157	87.33	108.56	38.96	2.88	4.82	1.63	5.89	99.08	31.17	21.94	18.67
158	88.33	108.56	34.86	2.28	7.42	3.24	4.89	123.18	26.07	25.84	16.77
159	89.33	107.56	37.16	1.98	6.62	3.37	4.89	104.38	25.07	22.84	17.97
16	89	107.56	31.39	2.88	9.32	3.24	7.56	87.68	28.97	22.01	19.87
160	86.33	109.56	35.36	2.08	7.72	3.72	3.89	104.78	29.47	23.54	16.77
161	87.33	109.56	42.66	2.88	7.52	2.56	3.89	123.88	26.17	26.04	16.37
162	87.33	108.56	26.16	2.08	7.42	3.57	3.89	118.08	22.17	22.84	16.67
163	88.33	108.56	29.66	1.98	6.62	3.37	4.89	98.38	23.67	21.94	17.47
164	88.33	107.56	33.16	2.18	7.62	3.49	4.89	113.88	26.37	22.84	17.87
165	88.33	108.56	39.56	1.98	6.52	3.32	3.89	108.18	29.87	22.34	17.77
17	88	107.56	36.79	3.08	8.32	2.66	8.56	74.08	26.37	21.61	18.97

18	88	107.56	47.69	3.08	9.42	3.04	6.56	81.88	23.07	23.01	20.17
19	89	107.56	29.29	2.38	9.72	4.2	7.56	128.48	24.27	24.11	21.07
2	89	108.56	36.59	2.88	9.22	3.2	7.56	117.88	26.17	24.01	17.47
20	89	108.56	29.39	2.28	9.82	4.47	8.56	135.28	27.17	26.01	20.27
21	88	109.56	38.39	2.98	8.32	2.76	6.56	131.78	31.97	27.11	16.67
22	88	109.56	24.29	2.28	6.22	2.75	7.56	104.28	29.37	22.11	18.77
23	88	109.56	23.19	2.28	6.32	2.8	8.56	128.18	19.97	25.11	17.47
24	88	109.56	25.49	2.48	6.12	2.45	7.56	104.88	29.37	22.41	18.87
25	89	107.56	38.69	2.88	8.32	2.87	7.56	102.68	25.77	21.01	17.87
26	87	107.56	24.69	3.08	7.12	2.24	9.56	111.68	26.97	23.11	18.87
27	88	109.56	31.19	2.98	8.12	2.69	9.56	105.68	26.37	21.91	17.77
28	90	109.56	32.69	2.98	8.22	2.72	8.56	98.68	17.97	21.51	18.67
29	89	109.56	28.69	3.08	6.42	2	8.56	115.28	29.47	23.11	18.87
3	89	107.56	37.39	3.08	9.12	2.93	8.56	139.28	24.97	29.31	18.87
30	88	109.56	23.19	3.08	7.92	2.52	7.56	136.98	26.17	21.01	16.67
31	88	109.56	21.39	2.28	6.22	2.75	5.56	98.48	26.47	21.11	18.37
32	89	108.56	27.19	2.28	6.22	2.75	8.56	121.78	24.87	24.91	18.77
33	89	108.56	29.39	2.88	8.02	2.76	7.56	104.98	25.57	21.91	19.77
34	90	108.56	37.19	2.18	8.12	3.85	7.56	104.48	26.77	22.11	19.17
35	88	109.56	29.69	2.18	8.32	3.95	8.56	123.98	23.47	25.11	21.27
36	88	107.56	33.19	3.08	5.42	1.66	8.56	113.98	25.57	23.11	20.37
37	90	107.56	24.59	2.58	8.32	3.25	7.56	104.98	23.17	21.91	17.87
38	89	107.56	29.19	2.58	7.32	2.84	7.56	106.28	22.27	22.41	18.27
39	88	107.56	32.29	2.48	9.02	3.71	7.56	109.48	24.47	23.31	18.67
4	89	109.56	42.29	2.98	7.22	2.37	9.56	102.58	24.57	21.01	17.87
40	89	108.56	29.39	2.38	9.72	4.2	9.56	100.88	30.47	20.31	18.17
41	89	109.56	25.59	2.98	8.22	2.72	7.56	113.28	29.57	23.01	18.87
42	88	107.56	32.39	2.88	8.12	2.8	7.56	104.48	26.67	22.41	18.57

43	88	107.56	22.49	2.48	7.02	2.84	8.56	94.28	26.77	22.01	18.27
44	88	107.56	30.29	2.58	6.42	2.47	10.56	97.48	20.17	23.11	17.87
45	88	108.56	26.19	2.48	5.32	2.1	7.56	107.78	26.77	22.91	18.47
46	88	108.56	33.29	2.48	5.42	2.15	9.56	101.18	27.47	21.01	17.67
47	89	108.56	35.39	2.38	7.32	3.12	8.56	145.98	29.47	29.31	18.87
48	89	107.56	32.69	2.58	6.12	2.34	7.56	125.08	29.17	25.91	17.77
49	89	107.56	34.19	2.58	7.12	2.75	6.56	107.28	26.37	24.91	18.47
5	88	109.56	42.49	2.28	6.42	2.84	7.56	112.18	25.57	22.11	19.67
50	89	107.56	28.69	2.98	8.22	2.72	6.56	128.78	28.07	29.11	19.67
51	89	108.56	23.59	3.08	7.22	2.28	5.56	109.98	25.47	26.91	16.47
52	89	108.56	27.29	2.88	6.12	2.05	6.56	104.48	22.47	22.11	18.47
53	90	109.56	29.39	2.98	8.22	2.72	5.56	119.18	23.17	24.01	16.17
54	89	109.56	23.19	2.98	8.02	2.65	6.56	122.88	25.57	25.91	17.87
55	89	109.56	14.29	3.08	8.12	2.59	5.56	75.98	28.97	22.41	18.27
56	89.67	107.89	20.96	1.94	8.96	4.43	9.56	116.04	19.97	27.84	18.07
57	89.67	107.89	33.66	1.94	7.96	3.95	9.56	100.44	22.87	22.74	17.87
58	90.67	107.89	40.86	2.64	7.96	3.07	8.56	104.44	23.87	23.94	20.67
59	90.67	107.89	23.66	2.74	6.76	2.56	8.56	103.04	25.37	23.64	19.37
6	88	107.56	37.19	2.48	5.12	2.01	9.56	144.28	22.67	30.01	19.67
60	89.67	108.89	33.86	2.04	8.76	4.15	9.56	118.84	24.37	25.64	18.27
61	89.67	108.89	30.76	2.14	8.66	3.95	8.56	106.24	24.57	24.74	19.47
62	89.67	109.89	37.16	2.14	8.66	3.95	8.56	114.44	23.37	25.74	18.27
63	88.67	109.89	34.16	1.94	7.96	3.95	7.56	146.04	24.77	31.64	16.77
64	89.67	107.89	24.46	2.24	8.66	3.8	10.56	103.94	26.77	22.64	19.37
65	89.67	109.89	33.16	1.84	7.76	4.03	10.56	107.94	27.57	24.84	19.27
66	90.67	109.89	27.26	1.94	8.56	4.23	11.56	85.64	28.37	23.74	20.37
67	89.67	108.89	26.16	1.84	8.66	4.48	10.56	108.64	23.27	24.64	17.27
68	89.67	108.89	21.26	2.04	7.76	3.7	9.56	120.94	25.37	27.04	18.47

69	90.67	108.89	40.96	2.74	6.96	2.63	9.56	110.74	26.87	24.14	16.87
7	88	109.56	27.39	2.88	7.12	2.43	6.56	121.28	28.37	25.31	21.87
70	90.67	107.89	28.16	1.84	8.66	4.48	10.56	97.94	22.97	23.74	20.27
71	90.67	107.89	29.96	2.14	8.76	3.99	7.56	115.14	30.67	25.74	16.07
72	89.67	107.89	31.36	2.14	8.76	3.99	8.56	118.14	23.97	24.54	17.37
73	89.67	108.89	25.76	2.64	7.96	3.07	6.56	109.64	27.57	23.94	16.37
74	89.67	108.89	33.86	2.54	8.66	3.43	10.56	89.24	27.07	23.54	19.77
75	89.67	109.89	35.56	2.64	8.86	3.39	9.56	136.94	32.37	29.74	18.47
76	89.67	108.89	35.56	1.84	8.56	4.43	8.56	101.84	30.47	23.54	19.27
77	89.67	107.89	41.96	2.74	7.86	2.94	9.56	123.24	30.87	27.84	18.07
78	90.67	107.89	37.26	2.64	6.96	2.71	9.56	110.64	23.17	23.74	18.77
79	90.67	107.89	37.26	2.54	8.86	3.5	7.56	130.14	30.87	27.64	17.07
8	89	109.56	31.69	2.78	9.82	3.57	7.56	118.48	26.47	24.21	20.67
80	90.67	109.89	29.96	1.94	9.56	4.71	7.56	115.04	23.77	24.54	16.67
81	90.67	109.89	26.56	2.74	5.96	2.29	9.56	119.84	32.87	25.74	17.37
82	90.67	109.89	28.76	1.94	7.76	3.85	9.56	116.74	24.37	23.74	17.37
83	89.67	108.89	26.96	2.54	7.86	3.13	8.56	109.04	19.37	22.74	16.37
84	89.67	107.89	27.66	2.74	7.56	2.84	10.56	123.44	24.37	26.44	18.77
85	89.67	107.89	35.96	1.94	8.66	4.28	8.56	102.54	23.47	23.64	18.07
86	89.67	109.89	34.66	1.94	8.56	4.23	7.56	84.34	24.97	23.04	16.67
87	90.67	109.89	43.96	2.44	7.96	3.27	7.56	124.94	23.27	27.94	16.77
88	90.67	108.89	44.16	2.54	8.66	3.43	6.56	121.44	26.87	25.24	15.87
89	88.67	107.89	23.86	2.54	7.76	3.1	9.56	121.74	20.17	25.74	19.47
9	89	108.56	41.19	2.78	9.92	3.6	11.56	136.08	24.77	28.01	21.77
90	88.67	108.89	45.76	2.54	8.76	3.46	7.56	127.64	24.97	27.64	17.27
91	88.67	108.89	41.86	1.94	8.76	4.33	10.56	135.14	23.87	28.24	19.47
92	88.67	109.89	41.76	2.04	8.56	4.06	6.56	112.74	23.67	25.24	17.07
93	88.67	108.89	21.06	1.84	7.86	4.08	10.56	75.84	25.97	23.74	19.37

94	89.67	107.89	37.36	2.24	8.76	3.84	9.56	109.94	23.77	24.64	17.87
95	88.67	107.89	30.16	1.94	8.86	4.38	10.56	113.84	33.97	25.74	19.77
96	89.67	107.89	27.06	2.04	7.76	3.7	7.56	120.84	26.87	26.74	17.87
97	90.67	108.89	38.96	2.24	7.56	3.34	8.56	105.44	25.97	24.94	17.67
98	90.67	107.89	36.36	2.04	8.66	4.11	10.56	124.84	30.87	25.94	19.47
99	90.67	107.89	38.36	2.74	7.76	2.91	9.56	112.44	24.97	23.64	18.37

Brief Biodata of student

Name	**:**	Aman Verma
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Mother's Name	**:**	Shail Verma
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Academic Qualifications:

Examination passed	**Year**	**School/Board /University**	**Marks**	**Division**	**Major Subject**
10th	2011	Delhi Public School, Durg	89.5%	First	English, Mathematics, Social science, Science, Sanskrit, Foundation of IT
12th	2013	Oriental Public School, Ambikapur	58.6%	Second	English, Physics, Chemistry, Biology, Informatics Prac.
B.Sc. Agriculture	2019	IGKV, Raipur	79.4%	First	All agriculture and Allied subjects
M.Sc. Agriculture	2021	CSK HPKV, Palampur	82.1%	First	Major Discipline: Genetics and Plant Breeding Minor Discipline: Plant Pathology