MORPHO-PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF RICE GENOTYPES SUITABLE FOR DIRECT SEEDED CULTIVATION

Thesis

Submitted to the Punjab Agricultural University
In partial fulfilment of the requirements
For the degree of

MASTER OF SCIENCE
in
PLANT BREEDING AND GENETICS
(Minor Subject: Biotechnology)

By

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(L-2020-A-106-M)

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CERTIFICATE I

This is to certify that the thesis entitled, **"Morpho-physiological and molecular characterization of rice genotypes suitable for direct seeded cultivation"** submitted for the degree of **M.Sc.** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Mr. Sutej Singh Bains (L-2020-A-106-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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CERTIFICATE II

This is to certify that the thesis entitled, **"Morpho-physiological and molecular characterization of rice genotypes suitable for direct seeded cultivation"** submitted by **Mr. Sutej Singh Bains** (**L-2020-A-106-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

Direct seeded rice (DSR) has gained importance in Punjab in view of water and labour saving potential of this technology. The varieties available to the farmers for cultivation under direct seeded conditions have been basically developed for puddled transplanted rice (PTR) conditions. The PTR adapted varieties do not possess adequate capability for seedling emergence from depth. The DSR ideal plant type thus needs to have the ability to germinate under anaerobic condition alongwith with tolerance of early submergence and good seedling vigor. The present study evaluated the potential of 48 lines with regard to important morpho-physiological as well as economic traits under DSR conditions. Thirty eight test entries used in the were advance derivatives from a multiparent cross where each parent contributed specific DSR related traits/QTLs along with disease and insect pest resistance. Another six entries represented elite aerobic rice breeding lines. Four released varieties namely PR126, PR128, PR130 & PR131 were included as checks. The morpho-physiological evaluation of this set of lines was carried out for mesocotyl and coleoptile lengths. Test entries 15, 17, 18 and 32 showed fast growth between day 5 and day 7. Nine test entries viz 15, 17, 21, 24, 26, 29, 30, 37, 38 and advance breeding line RYT-3872 had more than 80% germination under anaerobic conditions as well as better response than check PR126 and PR128. Further the test entries and checks were planted in a replicated trial at two locations viz Ludhiana and Kapurthala during 2021 crop season. Observations were recorded for days to flowering, plant height, ear bearing tillers in a meter square, grain weight, spikelet fertility and yield per plot. The dwarf plant entry at both location were entry number 18 and 37 which are similar to PR126 in height. Lines 7,16, 27, 32 and 36 show higher ears bearing tiller in a meter square at Kapurthala and Ludhiana with minor differences. Most of the genotype had good number of tillers at both the locations. Lines having superior grain weight were 2,9,17, and 22 at both the locations. Entry number 4, 13,17 and 35 were similar at both the locations for spikelet fertility. PR126 had 71% fretile spikelets at ludhiana and 76.5 % at Kapurthala. On the basis of the pooled yield and other traits EN21 ,EN15 & EN10 were found to be most suited for DSR as compared to PR126. Thirty four samples with three replicates were used for generating sequence data from RNA. This was filtered to remove low quality bases by trimming the undesired parts of sequences. After evaluating the complete dataset putative genes were identified in already mapped genomic region associated with mesocotyl elongation on chromosome 7 alongwith the position of gene, their expression, fold change and function. A total of ten candidate genes were identified. With six of them being upregulated and four being down regulated. The function related to the identified up and down regulated candidated genes were assigned as well.

Keywords: Anaerobic germination, direct seeded rice, emergence, mesocotyle, QTL

Signature of Major Advisor **Signature of the Student**

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ਝੋਨੇ ਦੀ ਸਿੱਧੀ ਬਿਜਾਈ ਪਾਣੀ ਅਤੇ ਹੋਰ ਸਰੋਤਾਂ ਨੂੰ ਬਚਾਉਣ ਵਾਲੀ ਵਿਧੀ ਹੈ ਪ੍ਰੰਤੂ ਇਸ ਲਈ ਅਨੁਕੂਲ ਕਿਸਮਾਂ ਵਿਕਸਤ ਕਰਨੀਆਂ ਜਰੂਰੀ ਹਨ। ਇਸ ਖੋਜ ਮੰਤਵ ਦੇ ਸੰਦਰਭ ਵਿੱਚ ਸਿੱਧੀ ਬਿਜਾਈ ਲਈ ਨਵੀਆਂ ਤਿਆਰ ਕੀਤੀਆਂ ਲਾਇਨਾਂ ਦੀ ਬਹੁ-ਪੱਖੀ ਪਰਖ ਕੀਤੀ ਗਈ। ਜੰਮਦੇ ਬੀਜ ਦੀ ਲੰਬੀ ਤੂਈ ਸਿੱਧੀ ਬਿਜਾਈ ਵਿੱਚ ਚੰਗੇ ਪਲਾਂਟ ਸਟੈਂਡ ਲਈ ਸਹਾਈ ਹੈ । ਪਰਖ ਅਧੀਨ ਲਾਈਨਾਂ ਵਿੱਚ ਇਹ ਲੰਬਾਈ 22.4 mm ਤੋਂ 40.9 mm ਤੱਕ ਪਾਈ ਗਈ ਜਦਕਿ ਚੈਕ ਕਿਸਮ ਪੀ ਆਰ 126 (PR 126) ਵਿੱਚ ਇਹ ਲੰਬਾਈ 36.9 mm ਸੀ। ਹਵਾ ਰਹਿਤ (anaerobic) ਹਾਲਾਤਾਂ ਵਿੱਚ ਬੀਜ ਦੇ ਜੰਮਣ ਦੀ ਦਰ 42.78 ਪ੍ਰਤੀਸ਼ਤ ਤੋਂ 91.67 ਪ੍ਰਤੀਸ਼ਤ ਤੱਕ ਰੀਕਾਰਡ ਕੀਤੀ ਗਈ। ਡੂੰਘਾਈ ਅਤੇ ਹਵਾ ਰਹਿਤ ਹਾਲਤਾਂ ਵਿੱਚੋਂ ਜੰਮਣ ਦੀ ਸਮਰੱਥਾ ਉਚੇਰੇ ਜੀਨਾਂ (QTLs) ਦੀ ਲਾਇਨਾਂ ਵਿੱਚ ਹੋਂਦ ਨਾਲ r=0.618 ਅਤੇ r=0.906 ਦੇ ਕ੍ਰਮਵਾਰ ਦਰ ਨਾਲ ਕੋਰਿਲੇਟਿੰਗ ਸੀ। ਸਿੱਧੀ ਬਿਜਾਈ ਵਾਲੀਆਂ ਹਾਲਾਤਾਂ ਵਿੱਚ 44 ਟੈਸਟ ਐਂਟਰੀਆ ਅਤੇ 4 ਚੈੱਕ ਕਿਸਮਾਂ ਨੂੰ ਲੁਧਿਆਣਾ ਅਤੇ ਕਪੂਰਥਲੇ ਵਿਖੇ ਪਰਖਿਆ ਗਿਆ। ਨਿਸਾਰੇ ਲਈ ਦਿਨ, ਪੌਦੇ ਦਾ ਕੱਦ, ਪ੍ਰਤੀ ਵਰਗ ਮੀਟਰ ਵਿੱਚ ਸਿੱਟਾ ਯੁਕਤ ਸ਼ਾਖਾ, ਫੀਸਦੀ ਭਰੇ ਦਾਣੇ ਅਤੇ ਜਾੜ ਰੀਕਾਰਡ ਕੀਤਾ ਗਿਆ । ਅੰਕੜਿਆ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਤੋਂ ਜਗ੍ਹਾ ਅਤੇ ਜੀਨੋਟਾਇਪ ਦਾ ਅਸਰ ਮੁੱਖ ਪਾਇਆ ਗਿਆ । ਝਾੜ ਪੱਖੋਂ ਜੀਨੋਟਾਇਪ 15, 21 ਅਤੇ ID ਦਾ ਪੱਧਰ ਚੈੱਕ ਕਿਸਮ ਪੀ ਆਰ 126 ਨਾਲੋਂ ਬੇਹਤਰ ਰਿਹਾ। ਜੀਨੋਟਾਇਪ 10 ਨੇ ਨਿਸਾਰੇ ਲਈ 81 ਦਿਨ ਲਏ ਜੋ ਕਿ ਘੱਟ ਸਮਾਂ ਲੈਣ ਵਾਲੀ ਕਿਸਮ ਪੀ ਆਰ 126 ਨਾਲੋਂ ਵੀ ਘੱਟ ਹਨ। R 146 (IRGC 38606-1) ਨਾਮੀ ਜਨੈਟਕ ਸਟਾਕ, ਜੋ ਮੀਸੋਕੋਟਾਇਲ ਦੀ ਵੱਧ ਲੰਬਾਈ ਲਈ ਜਾਣਿਆ ਜਾਂਦਾ ਹੈ, ਨੂੰ ਐੱਮ ਆਰ ਐੱਨ ਏ (mRNA) ਅਤੇ ਜੀਨ ਐਕਸਪ੍ਰੈਸਨ ਪੱਖੋਂ ਘੋਖਿਆ ਗਿਆ। ਢੁੱਕਵੇਂ ਸਮੇਂ ਅਤੇ ਟਿਸ਼ੂ ਤੋਂ ਲਏ ਸੈਂਪਲਾਂ ਦੇ ਅਧਿਐਨ ਤੋਂ ਲੰਬੀ ਮੀਸੋਕੋਟਾਇਲ ਲਈ ਕਾਰਗਰ ਜੀਨਸ ਦੀ ਨਿਸ਼ਾਨਦੇਹੀ ਕੀਤੀ ਗਈ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਝੋਨੇ ਦੀ ਸਿੱਧੀ ਬਿਜਾਈ, ਮੀਸੋਕੋਟਾਇਲ ਲੰਬਾਈ, ਵੰਸ਼-ਕਣਾਂ ਦਾ ਪ੍ਰਗਟਾਵਾ

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is cultivated on more than 44 million hectares in India and forms the staple diet of about 70% of the population (Kumar and Harikesh 2018). During the green revolution period of late 1960s and early 1970s, rice-wheat became the predominant cropping system of Punjab. Over the last 50 years Punjab has been the top rice contributor to the national food grain pool with an average share of about 25% in the last one decade (Anonymous 2021). In fact, surplus production beyond national food security needs has made India the number one exporter of rice in the world.. Rice acreage in Punjab has continued to increase in the post green revolution decades. It occupied an area of 31.33 lakh hectares during 2021-22 (Anonymous 2022). As a non-traditional crop with high irrigation requirement its impact on ground water resources is a major issue for Punjab agriculture. Average fall in ground water level over last one decade in the state has been estimated at 84.4 cm per year by Ground Water Cell of Punjab Department of Agriculture and Farmers' Welfare. Crop diversification efforts as a way of defusing the unsustainability issue have not been able to take off. In view of the urgency to conserve groundwater resources on one hand and maintain food security as well as farmer's livelihood on the other, technologies aimed at saving water in rice cultivation hold significance. Direct Seeded Rice (DSR) is one such promising technology. Traditional system of rice cultivation involves raising of nursery and transplanting of 25 to 35 day old seedlings into a puddled field with impounded, standing water. Puddling prevents seepage of water beyond the crop root zone and promotes evaporation losses. The standing water requirement aimed at suppressing weed growth further increases water use to unsustainable levels particularly in areas where rain is insufficient and ground water has to be pumped out. Further, manual transplanting adds to labor costs. Direct seeded rice seeks to reduce these requirements by sowing of seeds directly into an unpuddled field and a diversity of DSR techniques suitable for different agroecological situations and regions have been developed. Presently, direct seeded rice (DSR) is becoming more prevalent in South Asia, South East Asia and to some extent, in West Africa. Apart from water and labor saving, DSR offers advantage of early crop maturity. DSR ameliorates the problem of shallow hard pan formation due to repeated puddling which leads to water logging and hindrance to root growth in other crops in the rotation and also has lower emission of methane gas (Dhillon and Mangat 2018).The cultivation of DSR is being practiced with several modifications of tillage or land preparation and crop establishment with a site-specific package, but has not gained the required popularity because of few unresolved constraints such as high infestation of weeds and weedy rice, micronutrient deficiencies, increased nematodes population, poor crop establishment, lodging, floret sterility, incidence of blast,

brown leaf spot, impact on milling traits, etc. (Sandhu *et al* 2015). Comparative yields in DSR can be obtained by adopting various cultural practices *viz*., selection of suitable cultivars, proper sowing time, optimum seed rate, proper weed and water management.

Punjab Agricultural University recommended direct seeding of rice in 2010. After an initiation of adoption in the state, the area under DSR became restricted to water logged soils of South Western districts. A refined methodology (*Tar-Wattar* DSR) was recommended in 2021 (Bhullar and Gill) and its promise became evident during the COVID 19 induced labor shortage. The varieties recommended for DSR however have been selected from the varieties used under conventional transplanting conditions. Rice cultivars, by virtue of continuous selection for performance as puddled transplanted rice (PTR) lack the traits required for doing well under aerobic DSR conditions. Over the past few years, several researchers have reported the importance of traits like anaerobic germination (Angaji *et al* 2010), early uniform seedling emergence and vegetative vigor (Dixit *et al* 2015, Quilloy *et al* 2021). Several QTLs for grain yield have also been reported in lowland and upland rice (Sandhu *et al* 2015) and a large number of studies have been reviewed by Quilloy *et al* (2021) and Sagare *et al* (2020).

International Rice Research Institute (IRRI), Philippines, National Rice Research Institute, Cuttack, India and Directorate of Rice Research, Hyderabad, India have released varieties such as Sahbhagidhan, DRR 42, DRR 44, CR dhan 201, CR dhan 203, and Swarna Shreya (Sandhu and Kumar 2017) which have been developed for DSR conditions. Genomics-assisted breeding has contributed to development of DSR adapted CR dhan 801 variety (Sandhu *et al* 2021). The milling rice recovery and head rice recovery are generally lower in direct seeded rice as compared to PTR (Chen *et al* 2020) and breeding efforts would also be required to bridge this gap.

Short duration non-Basmati rice varieties such as PR 126, PR 128, PR 129 and *Basmati* varieties such as Pusa Basmati 1509, Pusa Basmati 1121 and Punjab Basmati 7 have been recommended for DSR conditions in Punjab. However, these varieties being used for DSR have been basically developed for transplanted puddled rice (TPR) and lack several of the attributes required for better adaptation to direct seeding. Poor emergence and inadequate seedling establishment can lead to yield losses. Under Punjab conditions, the problems of anaerobic germination and emergence from depth are encountered if there is incidence of rain within 4-5 days of sowing, causing anaerobic environment for germinating seeds/seedlings or resulting increase in depth of sowing with soil flowing into the seeded furrow with rain water. An ideal plant type for DSR should have the ability to germinate under anaerobic condition coupled with tolerance of early submergence and good seedling vigor. Mesocotyl and coleoptile lengths are directly related with seedling emergence in deep seeding. A longer mesocotyl will minimize sensitivity to seeding depth in drill seeding and improve seedling establishment. The highly dry and desiccating conditions in June necessitate that seeds as

placed at some depth in the soil to ensure moisture availability. The modern semi dwarf cultivars, adapted to transplanting mode, have a short mesocotyl which does not allow crop establishment especially when seeds are drilled deeper in the soil and land levelling is not precise. To breed rice accessions capable of germinating from depth under direct-seeding, there is need to explore the QTLs or candidate genes for mesocotyl length that can be further used in molecular breeding by marker assisted selection. Additionally, transcriptomic analysis based on RNA sequencing, which measures gene expression in a genome-wide manner can be used to reveal genes related to various traits whose expression can influence seedling emergence from variable depths.

The rice breeding programme at Punjab Agricultural University, Ludhiana has taken initiative to identify and characterize potential donors of DSR adaptive traits and start backcross breeding to transfer and consolidate these traits with the help of screening in appropriate conditions as well as use of molecular markers linked to these traits. The overall aim of this programme is to develop rice varieties specifically adapted to direct seeded conditions. The present study aimed to contribute to this work by evaluating a set of advanced breeding lines from the ongoing aerobic rice breeding programme, generated from complex crosses involving parents possessing suitable DSR traits and well adapted popular rice varieties possessing productivity and quality traits. Attempts were also made to elucidate the transcriptome dynamics in the two cultivars which exhibit differential germination from depthso as to identify the genes involved and investigate their differential expression further facilitating the breeding of rice cultivars suitable for direct seeded conditions.

The present study used a set of 38 lines generated from a complex cross at International Rice Research Institute, Philippines and advance breeding lines from ongoing aerobic rice hybridization programme. Further this set of lines was characterized for QTLs linked to yield under aerobic conditions, drought tolerance, seedling vigour and anaerobic germination. Competitiveness of this material with respect to currently best adapted material for direct seeded conditions such as PR 126 was also tested to understand the progress towards development of varieties specifically adapted for DSR conditions.

For molecular genetic investigations, the donor line IRGC 38606-1, with a distinct superiority for mesocotyl elongation and ability to emerge from sowing at depth of about 10 cm in pot experiments was used. Gene expression study (transcriptome analysis) of this material while undergoing elongation in response to deep sowing was employed as the strategy to pinpoint the genetic factors underlying this behaviour.

In view of this background, the study had been taken up with the following objectives:

- Morpho-physiological evaluation of advanced derivatives with DSR related traits in well adapted background

- Evaluation of advanced derivatives for yield performance and milling quality traits under direct seeded conditions
- Identification of genes with differential expression from candidates associated with mesocotyl elongation under deep sowing

CHAPTER II

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is one of the predominant cultivated cereal crop with respect to proportion of human population dependent on it as staple food. Rice is essential part of Indian agriculture and forms the staple diet of nearly 70% of the population (Kumar and Harikesh 2018). With rice-wheat as the largest cropping system in the state, rice is an integral part of Punjab agriculture, both in terms of production and trade. Rice was grown over an area of 31.33 lakh hectares in the state during 2021-22(Anonymous 2022), mainly supported by groundwater irrigation. The present set of varieties lack several of the attributes required for better adaptation to direct seeding. Serious problems inherent to use of conventional rice varieties for direct seeding, such as poor germination under anaerobic conditions and inability of seed to emerge from depth, higher incidence of brown spot, nematode infestation, iron deficiency under light soils and poor milling quality pose a challenge to wider adoption and success of DSR. These problems needs to be addressed using both conventional and molecular interventions. In the recent years scientists have been concentrating in developing suitable varieties and agronomic packages for promoting DSR. In this content, the relevant literature with respect to the performance , challenges and prospects of direct seeded rice, its key traits and breeding approaches has been summarised and reviewed under the following headings

2.1. Direct Seeded Rice

2.2. Comparative studies on direct seeded and transplanted rice

2.3. Quantitative trait loci governing performance under direct seeded conditions

2.4. Molecular genetics of mesocotyl length

2.5. Molecular genetics and physiological basis of anaerobic germination ability

2.6. Breeding approaches for DSR

2.1 Direct Seeded Rice

Rice cultivation requires technologies and practices that are based on less water and mechanization. Practices like direct seeding and alternate wetting and drying may be the best possible measures to make rice more water efficient. This will also promote mechanization, thereby decreasing the manual labor requirements and ultimately cost. Under Direct seeding, seeds are sown directly in the field instead of transplanting seedlings grown from the nursery. Kumar and Ladha (2011) on the basis of field conditions have classified DSR in three types: water seedling, wet-DSR and dry-DSR, and water seeding. As per Kumar & Ladha (2011), Dry-DSR is sown by broadcasting, drilling, or dibbling of dry seeds on normal conventionally tilled or not tilled soil which has not been puddled. For wet-DSR sowing of pre-germinated seeds is after puddling in the soil. This can also be called as aerobic and anaerobic wet-DSR,

respectively. Lastly, in the case of water seeding (WS), pre-germinated seeds are to be broadcasted in standing water either puddled (wet-WS) or unpuddled (dry-WS). Fanish in 2016 concluded that DSR can help in production of rice in water-scarce areas by making the rice cultivation water efficient. Dry direct seeding helps in foregoing puddling, and transplanting followed by maintenance of 4–5 cm of standing water throughout the season. Also, PTR fields are the biggest producers of greenhouse gases, in that particularly methane., In different areas of Punjab, DSR is reported to reduce total global warming potential by about 33%, from 2.0 to 4.6 t CO_2 eq./ ha to 1.3–2.9 t CO2 eq./ha as given by Pathak *et al* in 2013. It was found that CO_2 and methane were produced less in DSR as against PTR. DSR has labour and water saving attributes with around 30% of water (Fanish 2016) and 11.2% of labour costs. In addition, in case of DSR there is no transplanting injury and the plants attain physiological maturity in fewer days leading to early maturity. However, as of now the varieties developed specially for direct seeding are not available. The ultimate DSR adoption and success depends on breeders by giving varieties having early seedling vigor, anaerobic germination (AG) tolerance and also with ability to germinate from deep soil.

Seedling vigour is an important agronomic trait for DSR. They studied 684 accessions from the 3K-RG (3000 Rice Genomes) population. The genetic basis of rice seedling vigour was deciphered through multiple seedling traits under both, laboratory and field conditions at three planting depths. GWAS study revealed that the main QTL for mesocotyl length, percentage seedling emergence and shoot biomass were co-located on the short arm of chromosome 7. The haplotypes in the indica subgroup from this region can be used to predict the seedling vigour of 3K-RG accessions. Su and co workers reported in 2022 that long time submergence of rice seeds will lead to difficulties germination, emergence, and seedling levelling. This may also cause rotting of seeds, seedlings, affecting the growth, development, and finally the yield. Submergence as well as stress from hypoxia similarly effect the germination and growth of rice (Li *et al* 2018).

The low input cost of for labour and water favours DSR over the conventional rice (Goyal *et al* 2022). The Central Soil Salinity Research Institute (CSSRI), Karnal evaluated 44 genotypes and identified few good suited ones for DSR like CSR MAGIC-167 , CSR 58 & 49 etc (Goyal *et al* 2022). In a similar study by Singh *et al* (2022) advanced breeding lines were evaluated under direct seeded as well as transplanted conditions. The yield under DSR was comparable to TPR but the grain quality was lower as indicated by poor milling and finally low head rice recovery. However, the selected genotypes performed better for DSR related traits like seedling establishment and root traits.

Panda *et al* 2021 reviewed efficient root system architecture (RSA) which has great potential to increase resource-use efficiency and grain yield, especially under direct-seeded rice, by adapting to aerobic soil conditions. They identified that root architecture traits viz.

Number, density, length, thickness, etc are important for moisture and nutrient uptake. Candidate genes for PSTOL1 (early root growth enhancer), DRO1 (deep rooting),QTL qSOR1 (surface rooting) and many transporters have been identified and validated under different environments.

Adhikary *et al* (2022) conducted a controlled pot experiment for seed priming agents for improved emergence under Direct-seeded rice. They examined the effectiveness of sodium selenite, zinc oxide nano particles and sodium selenate and their combinations as priming agents for DSR in West Bengal, India. Priming gave early emergence and vigour by using combinations of all the test agents. While in field , use of priming agents in combinations enhanced the chlorophyll, phenol and protein contents, as well as improved the leaf area index, crop growth rate, uptake of nutrients and yield of DSR over the control.

2.2. Comparative studies on direct seeded and transplanted rice

Kato *et al* (2009) conducted the study for estimating yield potential and rooting behavior in direct seeded and transplanted method with rice cultivars Takanari, Akihikira, Lemont and IRAT 109. They concluded that the average yield under DSR conditions was similar to or even higher than that achieved with flooded conditions (8.3 t/ha in 2007 and 9.4 t/ha in 2008 in direct seeded rice and 8.2 t/ha for transplanted in 2007, 2008). Takanari, super-high-yielding cultivar achieved yields greater than 10 t/ha with no yield penalty in direct seeded rice due to the favorable agronomic characteristic of its greater sink capacity (grain number and grain weight). The highly stable productivity of the Takanari in direct seeded rice was related to its better root growth characteristics. The rooting vigor at the early growth stage in direct seeded rice resulted in greater individual root growth development and higher root-to-shoot ratio.

Yoichiro and Midoni (2010) characterized the root growth in flooded and aerobic culture. Total root biomass was significantly lower in DSR than in flooded rice. But the ratio of deep root biomass to shoot biomass was higher in DSR. They concluded that by vigorous root growth in soil water uptake was better and it maintained higher transpiration in DSR. Ginigaddara and Ranamukhaarachchi (2011) evaluated various morpho-physiological traits involved in direct seeded rice and transplanted rice. The days to 50% flowering showed significant difference between direct-seeded rice (DSR) and puddled transplanted rice (PTR) and were less in direct seeded rice. Plant height, ear bearing tillers, thousand grain weight was less in direct seeded rice than transplanted rice but it had more spikelet fertility percentage. Root length was more in direct seeded rice than transplanted rice. The direct seeded rice had significantly deeper and longer root system with greater dry mass, thus it had higher root: shoot ratio than transplanted rice.

Early seedling vigour (ESV) determines rapid, uniform emergence and the development of seedlings under a wide range of field conditions and it has been considered as

one of the important characteristics that determines successful crop establishment (Zhang *et al* 2005a, b) under direct seeded rice. Mahender *et al* 2015 studied yield along with seedling vigour which revealed that varieties which are better yielding in DSR also showed early seedling vigor. They concluded that the varieties which had early good seedling vigour with uniform germination yielded higher direct seeded rice. Pradhan *et al* (2016) reported lowering of SPAD values as chlorophyll content was affected under DSR as compared to transplanted which leads to reduction in yield and this might had been occurred due to less uptake of nutrients (Nitrogen and Iron).

Kaur and Singh (2017) compared direct seeded rice and puddled transplanted rice establishment methods with respect to grain yield, irrigation water applied, green house gas emissions and labor use. Direct seeded rice was found to be cost and labour-saving technology yielding at par or even higher than PTR under good management practices. They reported significantly higher yield in direct seeded rice (7.84 t/ha) than transplanted rice (7.46 t/ha) in India. The grain yield was higher in direct seeded rice is due to more number of effective tillers ($361/m^2$ in direct seeded rice and $336/m^2$ in PTR), higher thousand grain weight (26.8 g in direct seeded rice and 26.2 g in PTR), lower sterility percentage and higher panicle number than transplanted rice. Xu *et al* (2019) conducted meta analysis between indica and japonica varieties and concluded that on overall basis yield of direct seeded rice was 12% lower than that of transplanted rice due to fewer spikelets per panicle. The panicle number per m^2 was more in direct seeded rice than PTR but spikelet number per panicle was less in direct seeded rice, moreover the grain weight was less in direct seeded rice than PTR. However, yield loss was variable from 2% to 42% depending on management practices, soil type and climate conditions. The yield penalty could be narrowed down to 4% only in the better managed condition as compared to 14% in low yielding condition.

2.3. Quantitative trait loci governing performance under direct seeded conditions

Abiotic stress such as drought is one of the major factor which effects the yield of direct seeded rice. Bernier *et al* (2009) studied the effect of *qtl12.1* for grain yield under drought conditions in 21 field trial in a rice mapping population derived from Vandana and Way Rarem. The effect of QTL on grain yield was found to be increased with increasing intensity of drought stress and the effect increased to 40% more in most severe drought stress environment. The grain yield was more in drought stress (severe and moderate) but there was no effect under well watered aerobic conditions or under transplanted conditions in nine out of ten direct-seeded trials over wide range of environment. The *qtl12.1*has a large and consistent effect on grain yield under drought stress conditions.

Sandhu *et al* (2013) reported 35 QTL associated with 14 traits from a biparental MASARB-25 × Pusa Basmati-1460 population. In parallel mapping study, they identified 14 QTL for 9 traits from HKR47 × MAS26 BIP population. These included QTLs for enhancing

yield under aerobic conditions and several root-related traits. Further four QTLs viz. *qGY8.1*, *qGY2.1*, *qGY2.2* and *qRL8.2*, were identified in both populations. Later, in 2015, Sandhu *et al* (2015) conducted an experiment on backcross derived lines (BC_2F_4) from backcross populations viz. Aus276/3*MTU1010 & Aus276/3*IR64 for testing seedling emergence, early vegetative vigor, nutrient uptake, nodal root number, and root hair length and density and their genetic control. A total of 46 QTLs associated with 36 traits were mapped both the populations. Aus276 was concluded to have contributed most of the QTLs in both the populations. The grain yield QTLs *qGY1.1, qGY6.1* and *qGY10.1* were identified to be stable in both populations. The increase in grain yield was attributed by donor parent Aus276. A number of QTLs were also detected for traits hypothesized to be beneficial for direct-seeded conditions. Several of the QTLs identified by Sandhu *et al* 2015 for grain were also previously reported to be consistent across different mapping populations and under different drought severities (Dixit *et al* 2012; Ghimire *et al* 2012; Vikram *et al* 2012).

Long shoot length plays an important role in establishment of rice seedlings because short shoots face the problem of emergence from paddy water surface which leads to mortality of seedlings. The study by Fukuda and Terao (2015) reported QTLs affecting the chlorophyll content and shoot length of seedlings using 91 inbred lines which were derived from BC_1F_4 & BC_1F_5 generation of cross Habataki x Arroz da terra which are *indica* and a *japonica* rice cultivar. The QTLs *qSL1* for increased shoot length & *qCL1 for* culm length were identified from the Arroz da Terra. These were detected in the same region of chromosome 1, where the earlier reported *gibberellin 20 oxidase-2* gene almost identical to *Semi Dwarf1*: *SD1*, was located.

Anandan *et al* (2016) screened 629 rice (*Oryza sativa*. L) genotypes comprising of 25 tropical *japonica*, 57 *indica* landraces, 127 breeding lines, and 427 ARC (Assam Rice Collection) for early seedling vigor. Early seedling vigour is an essential trait for direct seeded rice to dominate and smother the weed growth. Singh *et al* (2017) evaluated the BC_3F_4 population (253 lines) Swarna x Moroberekan cross. They carried phenotyping for early vigor (EV) and 8 related and identified 14 related QTLs.

Direct seeded rice faces many problems like weed infestation, deficiency of micronutrients, susceptibility to root knot nematode, lodging susceptibility and higher seed rate. QTLs/Genes have been identified for these traits viz. *qAG1.2, qAG3.1, qAG7.2 qAG9.1* and *qAG9.2* for anaerobic germination (Angaji *et al* 2010); *qNR4.1, qNR5.1*for nodal root; *qN5.1, qP5.2, qFe5.2*for nutrient uptake; *qGY1.1, qGY6.1, qGY10.1*for grain yield (Sandhu *et al* 2015*)* and *qLDG4.1* for lodging resistance (Dixit *et al* 2015).

Nutrient deficiency (e.g iron and zinc) leads to decrease in yield in direct seeded rice (Mahajan *et al* 2012) due to several effects of physical and chemical properties of soil. In direct seeded rice availability of iron, zinc and rate of photosynthesis is affected. In very small

amounts, these elements serve as critical cofactors for several enzymes and main structural motifs required by the transcriptional regulatory proteins. Zhang *et al* (2017) studied a total of 222 *indica* rice accessions which were introduced from IRRI, from which 211 accessions were selected and a total of 29 and 31 putative QTLs affecting shoot ion concentrations (SFe or SZn), shoot fresh weight (SFW), shoot dry weight (SDW), shoot height (SH), root length (RL), root dry weight (RDW) and shoot water content (SWC) were identified at seedling stage for Fe and Zn content, respectively.

Photosynthesis is the basic productivity conferring process. The various physiological traits and agronomic parameters which lead to increase in yield indicate moderate no of tillers (10-12), higher number of panicles per plant, number of spikelets per panicle, specific leaf weight and number of functional leaves (Katsura *et al* 2007). The photosynthates assimilated in leaves are exported to stem during reproductive and late vegetative phase which leads to grain yield by grain filling (Dingkuhn*et al* 1991). Anandan*et al* (2018) observed 17% decrease in yield in direct seeded rice due to lack of normal development of root structure and due to increase in concentration of proline (20%), hydrogen peroxide (24.6%) and lower concentration of total soluble protein (20%). Therefore, the QTLs identified under direct seeded rice would be more appropriate to utilize in rice breeding for direct seeded rice than the QTLs identified form TPR condition. Introgression of such QTLs would definitely be beneficial and improves better adaptation to direct seeded rice condition with high yield potential. Several QTLs has been identified such as *qGY1.1, qGY2.1, qGY2.2, qGY8.1* and *qGY10.1* for increasing grain yield under aerobic conditions by Sandhu *et al* (2013; 2015) using HKR47 × MAS26 and MASARB25 × Pusa Basmati 1460 $F_{2:3}$ mapping populations.

The study was conducted by Zhao *et al* (2019) for genetic analysis of roots and shoots in rice seedling by association mapping on 273 rice varieties (154 *indica* and 119 *japonica)* and reported 65 QTLs for root and 43 QTLs for shoot. Several genes have been reported which result in deep root penetration in soil for water absorbance. One such gene is *DRO1* which is related with deep rooting trait to avoid the drought conditions. The *qRL6.1, qRL7, qRL9.1, qRL9.2, qRT2.3, qRT4.1, qNR4.1, qNR5.1 qRHD1.1* and *qRHD5.1*were some of QTLs for roots. Therefore, the breeding program for direct seeded rice should aim to increase the yield and adaptability by introgression of such QTLs for that area which are struggling for water and labour.

2.4. Molecular genetics of mesocotyl length

In order to address the problem related to uniform emergence and germination from depth varieties with optimum mesocotyl length are preferred and these also have reduced lodging and increased tolerance to abiotic stresses. Molecular and genetic characterization of mesocotyl elongation and improved germination from deep sowing under depth under DSR has not been studied extensively and the available studies are discussed below.

Wu *et al* (2015) examined 270 rice accessions for mesocotyl lengths of seedlings cultivated in water in the dark or in 5 cm sand culture. There were eleven accessions with the longest mesocotyl lengths, including seven upland landraces or varieties. A whole genome SNP array (Rice SNP50) was used to genotype selected accessions and 13 loci were found for mesocotyl length that confirmed the previously reported co-location of two QTLs across populations and experiments. Associated SNPs hit 36 annotated genes including function-matching candidates like cullin and GRF.

Quantitative trait loci (QTLs) for mesocotyl and coleoptile length were detected by Lee *et al* (2017) using 98 backcross inbred lines from a cross between Kasalath and Nipponbare. Three QTLs qMel-1, qMel-3, and qMel-6 for mesocotyl length were identified on chromosomes 1, 3, and 6, respectively. Seeds of 54 chromosome segment substitution lines (CSSLs) from the cross between Kasalath and Nipponbare sown at 5 cm soil depth showed a significant positive correlation between seedling emergence and mesocotyl elongation but not with coleoptile elongation ($r = 0.05$, $P = 0.7$).

In a review paper Zhan *et al* (2020) concluded that (1) Mesocotyl elongation is responsive to abiotic stresses, such as deep sowing drought, submergence, chilling, and salinity. (2) Humus soil culture with a burial depth of 6 cm and at the temperature of 30 °C could be the optimum method for mesocotyl length phenotyping, (3)Sixty-seven QTL controlling mesocotyl length were reported, which are distributed on all the 12 chromosomes. Twelve chromosomal regions were repeatedly found to have QTL using various mapping populations and methods. Two genes with very different molecular functions have been cloned, highlighting the genetic complexity of mesocotyl elongation

A total of 26 SNP loci were detected for coleoptile length (CL) and coleoptile diameter (CD) in a set of 209 rice lines (Su *et al* 2021). Four reliable candidate genes were identified: Os01g0911700 (OsVP1), Os05g0560900 (OsGA2ox8), Os05g0562200 (OsDi19–1) and Os06g0548200. Then qRT-PCR and LC-MS/ MS targeting metabolite detection technology were used to further verify that the up-regulated expression of these four candidate genes was closely related to anaerobic germination. Under oxygen-deficiency conditions, the four novel candidate genes were linked to gibberellin (GA) and abscisic acid (ABA) regulation, as well as cell wall metabolism, and promoted coleoptile elongation while avoiding negative consequences, allowing the coleoptile to obtain oxygen, escape the low-oxygen environment, and germinate quickly.

2.5. Molecular genetics and physiological basis of germination under anaerobic ability

When direct seeding is done followed by flooding, this negatively affects germination and plant survival in most rice genotypes (Ismail *et al* 2012). Though, this flooding helps in reducing weeds and lowers the cost of manual weeding and/or application of chemical herbicide. Germination under anaerobic conditions is defined as the ability of the seed to

sprout, elongated, grow, and survive under poor or low oxygen referred to as hypoxia or anoxia when there is very little to no oxygen. This trait is the most important or the key trait for DSR systems. Owing to this the varieties have potential to mitigate crop failure at the initial stages and can be also help in weed control. The present set of the rice high-yielding varieties do not show any underwater germination. Nevertheless, some landraces have evolved in various parts of rice-growing areas that show this AG trait viz. Kalarata, Khao Hlan On, Nanhi, Ma Zhan (red) and Khaiyan.

Rice plants under extreme submergence, experience anoxia and the plant then shift to alcoholic fermentation (AF) pathway rather than respiration for energy, in the form of adenosine triphosphate (ATP). During AF, only two ATPs are being produced vis-à-vis 38 ATPs are produced in aerobic respiration (Magneschi and Perata 2009), making it nineteen times less efficient in ATP production under anaerobic conditions. The three physiological responses that can allow rice seedlings to survive under anaerobic conditions are: (1) longer coleoptile, (2) greater water imbibition, and (3) higher starch reserves. Kennedy *et al* 1980, reported that rice coleoptiles is the only plant organ which can frow under anoxia. Faster elongation in coleoptile helps the plant gain access to aeration, which will than enable the germination of the embryo (Ismail *et al* 2012). Therefore, AG tolerance can be indirectly measured using coleoptile length reported by Hsu and Tung (2015) as the "anaerobic response index.. Alpi and Beevers (1983) had also demonstrated earlier that seedlings can develop coleoptiles at a higher speed when subjected to low environmental O_2. However, despite being longer in length, coleoptile is thin and fragile with less fresh weight. This phenomenon can be referred to as the snorkel effect, whereby anoxia induces the development of a hollow coleoptile to access a better-aerated environment (Kordan 1974).m Adachi *et al* (2015) when compared the germination of intolerant (IR42) with tolerant (IR06F459) lines of rice, revealed that the coleoptiles of IR06F459 had signifcantly longer length than those of IR42. IR06F459 is an AG-tolerant line developed from backcrossing IR64 to Khao Hlan On.

Besides the efficient imbibitions of water, the content of carbohydrate has the capacity to assist the growth of plant under the condition of stress. It was suggested by Ella and Setter (1999) that the adverse effects of lowered ATP supply during the AF is relieved when the availability of the carbohydrate for breakdown is adequate. Seeds having a high content of starch are presumed to be more tolerant of AG as compared to the seeds with higher content of fat (Magneschi and Perata 2009). Under the condition of anoxia, the survival of plant was found to have a strong correlation with starch content ($r = 0.73–0.88$) (Ella and Setter 1999). However, the starch cannot be used as it is; therefore , there are some hypothesis which indicate the e role of α-amylases. These are responsible for breaking down of starch into the soluble form of sugar (El-Hendawy *et al* 2011). Amongst the cereal crops which are under anoxia, rice is the only crop which expresses the α-amylase mRNA (Perata *et*

al 1992, 1993). The whole set of enzymes which are required for breaking down of starch into the soluble forms is expressed by the a rice seed which grows under anoxia (Ismail *et al* 2009). The degradation of starch into glucose is regulated by amylases which is required in the fermentative metabolism of plant. This is activated under the condition of anoxia for the production of ATP. It was reported by Adachi *et al* (2015) that the activity of α-amylase in their tolerant line (IR06F459) was significantly more as compared to the one observed in the intolerant line (IR42). This was in line with the observations of Illangakoon *et al* (2016). The survival rates of their genotypes had a positive correlation with the activity of α-amylase activity and the contents of soluble sugars . It was documented by Ismail *et al* (2009) that the concentration of the soluble sugar was observed to be low for both tolerant and intolerant genotypes. This was true until the tolerant genotypes had a high amount of soluble sugar which was at 4–8 days after sowing. It was observed that the tolerant genotypes could breakdown the reserves of carbohydrate in the seed. They could also mobilize the resultant monosaccharide. This could lead to germination and growth under hypoxia. In 2015, Kretzschmar *et al* found that OsTPP7 was expressed in the germinating tissue of NIL-AG1 (tolerant) lines while its expression was found to be absent in IR64 (susceptible). OsTPP genes (i.e., OsTPP1 and OsTPP2) in rice convert the trehalose-6- phosphate (T6P) into trehalose, this mechanism can also be true for OsTPP7. It was also documented that the trehalose and sucrose were 2.3-fold and 2.0-fold higher in NIL-AG1 as compared to that in the IR64. It indicates that OsTPP7 affects the conversion of T6P to trehalose. This signals the low availability of sugar. Hence, the plant enables the mobilization of starch from the endosperm reserve (source) to the coleoptile (sink).

Expression analysis, through RNA sequence (RNA seq), revealed that RAmy3D was highly expressed in tolerant genotypes under anoxia (Ismail *et al* 2009). RAmy3D is a member of the Amy3 gene subfamily, which was found to be upregulated in rice embryos under anoxia (Hwang *et al* 1999). Under the conditions of starvation, a glucose and sucrose receptor, RAmy3D, gets activated by protein kinase SnRK1A (Lu *et al* 2007).On the other hand, the SnRK1A gets stimulated by the calcineurin B-like protein kinase (CIPK15). This happens under the oxygen-limiting conditions (Kudahettige *et al* 2010). In addition to this, the interaction of calcineurin B-like proteins, like CBL4 and CBL5, with CIPK15 under the condition of anoxia is taken to be a regulating mechanism in the plant response during anoxia (Ho *et al* 2017; Sadiq *et al* 2011).

As reported by Ismail *et al* (2009, Saika *et al* 2006; Shingaki-Wells *et al* 2011), despite several rice enzymes being downregulated under anoxia for inactivation of the energy-conserving steps, the enzymes alcohol dehydrogenase (ADH), pyruvate decarboxylase (PDC), and aldehyde dehydrogenase (ALDH) are found to be active in the genotypes which are tolerant of anoxia (i.e., Khaiyan). In 2009, Ismail and co workers exhibited that the

concentration of ethylene enhanced significantly after only 72 hours of imbibitions. It was also documented that the ethylene produced affects starch hydrolysis by reducing the abscisic acid (ABA) synthesis. This also led to up regulating the synthesis of and the sensitivity to gibberellic acid (GA) of the intermodal tissues. The activity of peroxidase lowers the plant cell walls' content of lignin and the assembly of the protein (Waffenschmidt *et al* 1993). This leads to the reduction of its rigidity allowing cellular elongation. Figure 6 shows the proposed mechanism of coleoptile elongation under anoxia as interpreted by Ismail *et al* (2009). The other factors which are found to be responsible for cell expansion are constituted by a group of nonenzymatic proteins called expansins, which can affect cell wall loosening. Thus far, EXPA2, EXPA4, EXPA7, EXPB12, EXPA1, EXPB11, and EXPB17 have been documented to be induced during submergence stress (Juang *et al* 2000; Lasanthi-Kudahettige *et al* 2007; Takahashi *et al* 2011).

Prior to evaluation of the genetics behind AG, it was important to identify the lines which could germinate under a limited supply of oxygen. The largest AG screening carried out was by Angaji *et al* in 2010. A total of 8000 rice accessions from the IRRI Genetic Resources Center, were assessed. These contained collections from the gene bank accessions, elite breeding lines, IR64 mutants, and introgression lines. However, only 0.23% (19 lines) exhibited higher or equal to 70% survival. Hsu and Tung (2017) carried out the evaluation of gene expression in six genotypes. They discovered 3597 genes which were affected by water stress irrespective of the plant's genetic background. Around 5100 genes were differentially affected across genotypes and 471 genes were affected in a genotype-dependent manner. Therefore , the various genotypes are expected to exhibit different degrees of tolerance responses to anoxia.

Evaluation of another set of rice lines was carried out by Jiang *et al* in 2006. They used USSR5 and N22 as parents. Septiningsih *et al* (2013) employed 118 SSR markers for assessing the AG tolerance alleles from Ma-Zhan (red). It is a highly AG-tolerant landrace from China having 90% survival (Angaji *et al* 2010). Ma Zhan (red) when crossed with IR42 and then backcrossed exhibited six QTLs which were related to AG on chromosomes 2, 5, 6, and 7. This accounted for 5.6–30.3% of the phenotypic variation. Hsu and Tung (2015) employed both GWAS and QTL mapping for detecting genomic regions which were related to AG tolerance. A total of 144 RILs from a Nipponbare and IR64 cross were employed in the QTL mapping with 35,501 SNPs.

It was remarkable to observe that some of the identified QTLs overlapped across various studies. Few of these are (1) qAG-2 detected by Jiang *et al* (2004) and Septiningsih *et al* (2013), (2) qAG-7-2 by Angaji *et al* (2010) and qAG-7-3 from Septiningsih *et al* (2013), and (3) qAG-1-2 from Angaji *et al* (2010) and chromosome 1 QTL from Hsu and Tung (2015). Further analysis must be carried out to determine whether these QTLs are controlled

by the same gene(s). Currently , two QTLs are thought of as having prime importance for AG tolerance, AG1 (qAG-9-2) and AG2 (qAG-7-1) identifed from Khao HlanOn and Ma Zhan (red), respectively (Miro and Ismail 2013). In 2015, Kretzschmar *et al* finemapped QTL qAG-9-2 to a 50-kb region on chromosome 9. On the basis of Nipponbare as the reference genome, this region encompasses 4 genes and 1 transposable element. Contrasting this region to a de novo assembled IR64 sequence exhibited a 20.9-kb deletion of AG1 spanning across LOC_Os09g20390/Os09g0369400 (OsTPP7) and a deletion of neighbouring loci. The QTL did not reveal any structural variations against the Nipponbare besides a 4-kb deletion that was also present in IR64.

2.6. Breeding approaches for DSR

Typically, direct seeding was practised under rainfed rice cultivation in Asia under upland and to some extent under shallow lowland areas (Rao *et al* 2017). In case of DSR sowing is performed in unpuddled conditions, no standing water is maintained and aerobic conditions prevail for most of the season. DSR is now increasingly seen as an answer to shortage of human labour and depletion of water resources (Mahajan *et al* 2011, Liu *et al* 2015, Rao *et al* 2017, Chauhan *et al* 2017, Jabran *et al* 2017).

Selecting the broadly adaptable suitable aerobic rice cultivars becomes critical component of success in the aerobic rice system. As DSR adoption increased, the lack of stable yielding, dry direct seeded adapted varieties emerged as a major limitation in achieving high productivity under water and resource limited conditions. Initially and even presently, the rice varieties developed for transplanting are being used for direct seeding leading to poor adaptation and lower yields (Mahajan *et al* 2018). Consequently, unravelling key genetic factors associated with grain yield and adaptability under aerobic conditions has assumed great importance. Recent developments in genomic research have resulted in demarcation of genes conferring better performance under DSR conditions. Combining these genes in elite rice backgrounds with help of molecular markers is an opportunity that presents itself and must be availed to realise the benefits of DSR.

The National Rice Research Institute (NRRI), Cuttack, released six rice varieties for direct-seeded aerobic conditions (CR Dhan 200, CR Dhan 201, CR Dhan 202, CR Dhan 203, CR Dhan 205 and CR Dhan 206). The University of Agricultural Sciences, Bangalore (India), has released three rice varieties for direct-seeded aerobic conditions, viz. ARB 6, MAS 26 and MAS 946-1 for Karnataka state. However, these are unable to represent the outcome of a well targeted systematic breeding system which is yet in the phase of development.

There is no single methodology of DSR and each agro-ecology demands a specific set of management practices. Under Punjab conditions, emergence of direct seeded rice is likely to be affected by pre-monsoon showers. Anaerobic germination is therefore a significant element for establishing a good crop stand (Septiningsih*et al* 2013). Screening of rice

germplasm at IRRI for ability to germinate under anaerobic conditions led to identification of a japonica strain (Khao Hlan On) out of 8000 accessions and breeding lines screened (Angaji*et al* 2010). These have been exhaustively used in aerobic rice breeding programme. Traits such as early germination, higher germination rate, higher seedling vigour, mesocotyl and shoot length, etc are considered imprtant (Sandhu *et al* 2015). Subsequently underlying mechanisms were elucidated, e.g., efficient starch mobilization leading to coleoptile elongation was seen as a key trait responsible for anaerobic germination. This work paved the way for molecular tagging of quantitative trait loci associated with performance under DSR conditions. These studies included QTL mapping for starch mobilization during germination (Kretzschmar *et al* 2015), early uniform emergence (Dixit *et al* 2015, Singh *et al*2017) and early vigour (Sandhu *et al* 2015). Two QTL hot spots on chromosomes 3 and 5 were indicated in these studies.

The identification of useful QTLs with large and consistent effects set the stage for development of DSR-adapted breeding lines through targeted breeding (Wing *et al* 2018). Earlier efforts had focused on evaluating elite TPR lines under DSR but few were found to be promising under both. A complex crossing programme initiated at IRRI in 2014 had aimed at transfer of various productivity and biotic stress resistance genes from TPR rice to a line adapted to DSR conditions (Sandhu *et al* 2019b). Now with much better mapping and tagging of DSR related traits, QTL based approach for consolidating key DSR related traits in elite TPR backgrounds emerged as the chosen approach. Sandhu *et al* (2019a) carried out GWAS on a complex mapping population for 39 traits [(seedling-establishment traits (9), root and nutrient-uptake traits (14), plant morphological traits (5), lodging resistance traits and (5) yield and yield contributing traits(7)] . They documented a total of 10 significant trait-SNP associations and 25 QTLs having association with traits which are useful under DSR. In a recent paper Sagare*et al* (2020) have argued for a genomics based approach for breeding of rice cultivars suited for direct seeded conditions.

CHAPTER III

MATERIALS AND METHODS

The present study on 'Morpho-physiological and molecular characterization of rice genotypes suitable for direct seeded cultivation' comprised three experiments. The first dealt with seedling attributes related to success under DSR namely anaerobic germination and seedling length. The second experiment comprised of a field trial in which lines developed for DSR conditions were evaluated at two locations. These lines had been derived earlier from a multiparent cross, in which each parent was a donor for DSR related traits. The third experiment dealt with gene expression studies based on analysis of RNA profiles in lines differing for mesocotyl length which is a critical trait for seedling emergence under DSR conditions. The material and methods for the three experiments are described below.

3.1. Plant material for morpho-physiological trait evaluation and field trials under direct seeded conditions

Plant material for 'Morpho-physiological evaluation of advanced derivatives for DSR related traits' and 'Evaluation of advanced derivatives for yield performance and milling quality traits under direct seeded conditions' consisted of 44 test entries and 4 check varieties. Out of these 38 test entries were derived from the following complex cross:

[(IR09N538/IR93312//Tadukan/IR94225)/(Rathuhennathi/IR96322//IR 4226/Abhaya)]/[IR 91648/IRBB60//IR74371/WHD-1S]

Six entries were derived from diverse crosses from the ongoing aerobic rice hybridization programme.

Further, the parents involved in this cross (made at International Rice Research institute, Philippines) had been derived from the donors which are listed in Table 3.1 along with DSR related traits possessed by them.

Table 3.1 List of donors along with their DSR related traits and QTLs used to derive the test entries

Recipient	Donor	Trait	QTL
IR09N538	IR93312-30-101-20-13-66-6	Anaerobic germination	*qAG*$_{9.1}$
	IR74371-46-1-1	Drought tolerance	*qDTY*$_{12.1}$
	Tadukan	Blast	*Pita2*
	Rathu hennathi	Brown plant hopper	*BPH3, BPH17*
	Abhaya	Gall midge	*Gm4*
	IRBB60	Bacterial blight resistance	*Xa4+xa5+xa13+Xa21*

	IR 94225-B-82-B	Grain yield under aerobic direct seeding, nodal roots, root-hair density	*$qGY_{1.1}$*, *$qGY_{6.1}$*, *$qGY_{8.1}$*, *$qGY_{10.1}$*, *$qNR_{4.1}$*, *$qRHD_{5.1}$*, *$qRHD_{8.1}$*
	IR 94226-B-177-B	Grain yield under aerobic direct seeding, early vigor, nodal roots, root-hair density	*$qGY_{1.1}$*, *$qGY_{10.1}$*, *$qRHD_{1.1}$*, *$qEVV_{9.1}$*, *$qNR_{5.1}$*
	WHD-1S-75-1-1-127	Blast resistance	*Pi9*
	IR 96322-34-223	Drought tolerance	*$qDTY_{1.1}$*, *$qDTY_{2.1}$*, *$qDTY_{3.1}$*
	IR 91648-B-32-B	Early uniform emergence	*$qEMM_{1.1}$*, *$qEMM_{11.1}$*

Each test entry inherited a combination of genes from the above list consisting of BLB resistance genes, BPH resistance genes and DSR associated QTLS. This set of genes/QTLs includes *xa4, Xa5, Xa21, BPH3, Pita 2*, *$qAG_{9.1}$*, *Pi9*, *$qDTY_{2.1}$*, *$qDTY_{3.1}$*, *$NR_{5.1}$*, *$RHD_{1.1}$*, *$EMM_{1.1}$*, *GM4*, *$qDTY_{3.1}$*, *$qGY_{6.1}$*, *$qGY_{10.1}$*, *$qNR_{5.1}$*, *$qNR_{4.1}$*, q*$GY_{10.1}$*, q*$NR_{5.1}$*, q*$NR_{4.1}$*, and q*$RHD_{5.1}$*. The derivative lines used in the study, along with the combination of genes/QTLs they are assumed to carry on basis of previously conducted marker study are given in Table 3.2

Table 3.2 List of test entries evaluated for morpho-physiological traits and for field trials under direct seeded conditions

Geno-type	**Designation**	**Relevant QTL constitution (previously available)**
1	IR 129477-4139-439-1-1-2	*Xa4+Xa5+Xa21+Pita+AG9.1+Pi9+DTY3.1+EMM11.1*
2	IR 129477-1813-87-5-2-2	*Xa4+Pita+AG9.1+DTY3.1+GY6.1+RHD1.1+EMM11.1*
3	IR 129477-1232-81-2-1-3	*GM4+Pita+AG9.1+Pi9+DTY3.1+NR5.1+RHD1.1+EVV9.1*
4	IR 129477-3343-109-13-1-1	*Xa4+Xa5+Xa13+BPH3+Pita+AG9.1+Pi9*
5	IR 129477-1629-210-2-2-2	*Xa4+BPH3+GM4+Pita+AG9.1+DTY3.1+NR5.1+RHD1.1*
6	IR 129477-3873-201-5-1-5	*Xa13+GM4+Pita+AG9.1+Pi9+DTY1.1+NR5.1+EMM1.1*
7	IR 129477-1629-210-4-4-4	*Xa5+Xa21+BPH3+Pita+AG9.1+DTY2.1+DTY3.1+NR5.1+RHD1.1+EMM1.1*

8	IR 129477-3873-297-2-6-4	*Xa4+Xa13+BPH3+GM4+Pita+AG9.1+DTY1.1+NR5.1+RHD1.1*
9	IR 129477-991-430-1-9-4	*Xa4+BPH3+GM4+Pita+qAG$_{9.1}$+qDTY$_{3.1}$+qGY$_{6.1}$+qGY$_{10.1+qNR5.1+qNR4.1}$*
10	IR 129477-1813-191-5-7-6	*Xa4+BPH3+Pita+AG9.1+Pi9+DTY1.1+GY6.1+RHD1.1+EMM11.1*
11	IR 129477-3425-97-4-3-3	*Xa4+Xa5+BPH3+Pita+DTY1.1+GY10.1+NR5.1+RHD1.1*
12	IR 129477-4197-209-2-2-2	*Xa4+Xa5+BPH3+Pita+Pita2+AG9.1+DTY3.1+NR5.1*
13	IR 129477-4139-439-2-4-2	*Xa4+Xa5+Xa21+Pita+Pi9+DTY3.1+NR5.1*
14	IR 129477-3873-297-3-3-2	*Xa4+Xa13+BPH3+GM4+Pita+AG9.1+Pi9+DTY1.1+NR5.1+RHD1.1*
15	IR 129477-3873-297-3-3-4	*Xa4+Xa13+BPH3+GM4+Pita+AG9.1+Pi9+DTY1.1+NR5.1+RHD1.1*
16	IR 129477-1629-14-1-4-1	*Xa4+Xa5+Xa21+BPH3+Pita+AG9.1+Pi9+DTY3.1+NR5.1+RHD1.1+EMM1.1*
17	IR 129477-1629-14-1-4-2	*Xa4+Xa5+Xa21+BPH3+Pita+AG9.1+Pi9+DTY3.1+NR5.1+RHD1.1+EMM1.1*
18	IR 129477-2815-185-1-7-3	*Xa4+BPH3+Pita+qAG$_{9.1}$+qGY$_{10.1}$+qNR$_{5.1}$+qNR$_{4.1}$+qRHD$_{5.1}$*
19	IR 129477-2815-185-2-1-1	*Xa4+BPH3+Pita+qAG$_{9.1}$+qGY$_{10.1}$+qNR$_{5.1}$+qNR$_{4.1}$+qRHD$_{5.1}$*
20	IR 129477-4026-249-15-1-5	*Xa4+Xa21+BPH3+GM4+AG9.1+DTY3.1+DTY12.1+RHD1.1(H)+RHD5.1+EMM11.1*
21	IR 129477-709-375-3-5-7	*GM4(H)+Pita+AG9.1+DTY3.1+DTY12.1+GY6.1(H)+NR5.1*
22	IR 129477-2815-179-3-1-1	*Xa4+BPH3+Pita+AG9.1+GY10.1+NR5.1+NR4.1+RHD5.1(H)*
23	IR 129477-991-456-6-4-5	*Xa4+BPH3+Pita+AG9.1+DTY12.1(H)+NR5.1*
24	IR 129477-902-121-10-1-3	*Xa4+BPH3+GM4+Pita+AG9.1+DTY3.1+GY6.1(H)+GY10.1+NR5.1+NR4.1*
25	IR 129477-3343-500-36-5-1	*Xa4+Xa5+Xa13(H)+GM4+Pita+DTY3.1+AG9.1+RHD1.1+EMM11.1*
26	IR 129477-1510-100-7-5-2	*Xa4+BPH3+GM4(h)+Pita+AG9.1+DTY3.1+NR5.1+RHD1.1*
27	IR 129477-4026-249-15-1-2	*Xa4+Xa21+BPH3+GM4+AG9.1+DTY3.1+DTY12.1+RHD1.1(H)+RHD5.1+EMM11.1*
28	IR 129477-2815-179-3-1-5	*Xa4+BPH3+Pita+AG9.1+GY10.1+NR5.1+NR4.1+RHD5.1(H)*
29	IR 129477-902-121-10-1-4	*Xa4+BPH3+GM4+Pita+AG9.1+DTY3.1+GY6.1(H)+GY10.1+NR5.1+NR4.1*
30	IR 129477-4026-	*Xa4+Xa21+BPH3+GM4+AG9.1+DTY3.1+DTY12.1+RHD*

	249-15-1-7	*1.1(H)+RHD5.1+EMM11.1*
31	IR 129477-1629-14-1-1-5	*Xa4+Xa5+Xa21+BPH3+Pita+AG9.1+Pi9+DTY2.1+DTY3.1+NR5.1+RHD1.1+EMM1.1*
32	IR 129477-1510-100-7-5-6	*Xa4+BPH3+Pita+AG9.1+DTY3.1+NR5.1+RHD1.1(h)+EMM11.1*
33	IR 129477-991-456-6-4-4	*Xa4+BPH3+Pita+AG9.1+DTY12.1(H)+NR5.1*
34	IR 129477-1510-100-7-5-4	*Xa4+BPH3+GM4(h)+Pita+AG9.1+DTY3.1+RHD1.1*
35	IR 129477-709-375-3-5-6	*GM4(H)+Pita+AG9.1+DTY3.1+DTY12.1+GY6.1+NR5.1*
36	IR 129477-4026-249-15-1-4	*Xa4+Xa21+BPH3+GM4+AG9.1+DTY3.1+DTY12.1+RHD1.1(H)+RHD5.1+EMM11.1*
37	IR 129477-3343-500-36-5-4	*Xa4+Xa5+Xa13(H)+GM4+Pita+DTY3.1+AG9.1+RHD1.1+EMM11.1*
38	IR 129477-902-121-10-1-1	*Xa4+BPH3+GM4+Pita+AG9.1+DTY3.1+GY6.1(H)+GY10.1+NR5.1+NR4.1*
39	RYT-3872	
40	RYT-4008	
41	RYT-4009	
42	DRR Dhan 44	
43	Sahbhagi Dhan	
44	MTU 1010	
45	PR 126	
46	PR 128	
47	PAU 6260-3-1-6 (PR 130)	
48	PAU 6707-10-2-2-1 (PR 131)	

Two of the advanced lines (PR 130 and PR 131) got released as varieties after the initiation of this study as indicated in the above table.

3.2. Methodology for conducting the morpho-physiological evaluation of advanced derivatives with DSR related traits

The set of test entries given in the Table 3.2 represent the 'advanced derivatives with DSR related traits'. This experiment focused on the analysis of shoot/mesocotyl length as a factor for seedling emergence from different depths in this material as this is a crucial morpho-physiological trait for success of DSR. The following methodology was followed:

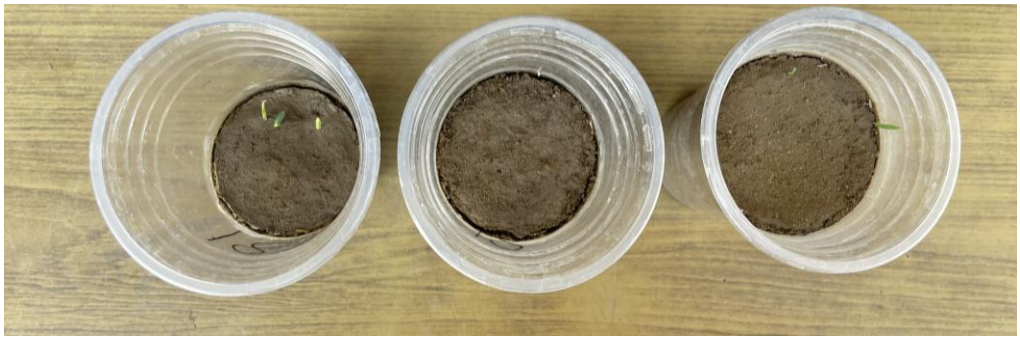
- The test entries along with standard checks were sown in cups at 3 different soil depths (3 cm, 6 cm & 9 cm)
- Six seeds were sown per cup and six cups per treatment were prepared with two

(a)

(b)

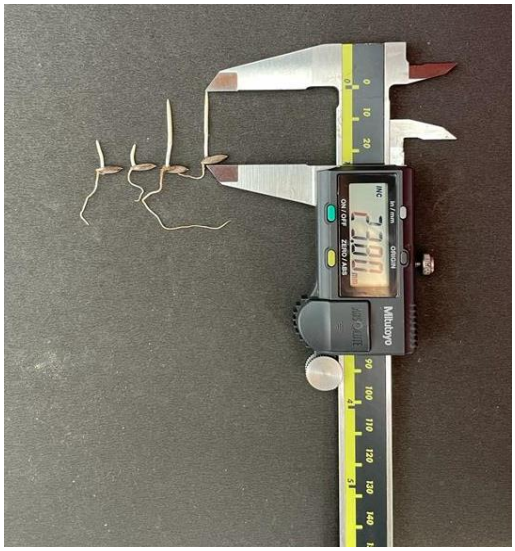
Plate 1 : View of field trial conducted at (a) Ludhiana and (b) Kapurthala during kharif 2021 crop season

(a)



(b)

(c)



(d)

Plate 2: (a) (b) (c) and (d) Evaluation of genotypes for shoot length and germination under partial anaerobic conditions using vernier calliper

replications for each depth

- These were maintained in green house till seedling stage under DSR related stress conditions
- Observations were recorded at 5th and 7th day after sowing
- The mesocotyl and shoot length was measured in mm using vernier caliper

3.3. Methodology for 'Evaluation of advanced derivatives for yield performance and milling quality traits under direct seeded conditions'

The test entries were evaluated at two locations, viz., (i) Rice Experimental Area (B-Block) Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, and (ii) PAU Regional Research Station, Kapurthala.

The plant material evaluated in this experiment has already been described in Section 3.1. The test entries along with checks were sown in alpha lattice design with two replications under direct seeded conditions at two locations during *Kharif* 2021.

The field was laser levelled and prepared well for sowing the trial. The trial was sown on 15th June at Ludhiana and 18th June 2021 at Kapurthala with a row to row spacing of 20 cm. The plot size at both locations was 6.5 m^2. The standard package of practices were followed to raise a healthy crop under direct seeded conditions

Observations were recorded on the following traits:

1. Days to 50% flowering

The number of days were recorded on the whole plot basis after the sowing of plant directly in field to the date when 50% tillers for each entry per plot flowered.

2. Plant height (cm)

Five plants were randomly chosen from the center of plots. The plant height data were recorded at maturity time. The measurement was done by taking the height of individual plant from the ground level to the tip of the tallest panicle excluding awns.

3. Ear bearing tillers (EBT/m^2)

Ear bearing tillers were calculated by counting the number of productive tillers in 1m row length per plot.

4. SPAD Value

The chlorophyll content per plant was measured and recorded at maximum tillering stage with the help of chlorophyll SPAD Value meter. Soil-Plant Analyses Development (SPAD) chlorophyll meter (SPAD-502, Minolta Co., Japan) measures chlorophyll content as ratio of transmittance radiation through a leaf at two wavelengths centered near 650 nm and 940 nm converts them into digital signals and then into SPAD value (Zhang *et al* 2019). The data were recorded from the terminal leaf of three random plants in each plot.

5. Thousand grain weight of paddy

Random grain sample was taken from each entry and 100 well developed whole

grains dried to 14% moisture were counted. They were weighed and used to calculate the thousand grain weight in grams.

6. Grain yield per plot

The harvest from each plot was cleaned, threshed, dried (14% moisture) and yield was weighed. The data was recorded in kg and converted to kg/ha.

7. Spikelet fertility

At physiological maturity five primary panicles were selected randomly avoiding the border plants. The total numbers of filled and sterile spikelets were counted separately and added which gives the total number of spikelets per panicle. Spikelet fertility percentage was calculated using the formula given below and expressed in percentage.

Spikelet Fertility (SF) (%) = Total number filled spikelets per panicle/Total number of spikelets per panicle x 100

8. Total Rice Recovery (TRR) and Milled Rice Recovery (MRR)%

The weighed samples (125g) of paddy were collected and were dehusked by using Satake Rubber Roll Laboratory Sheller (Satake Engineering Co., Japan) for direct seeded rice. The moisture content was between 13 – 14%. The brown rice was obtained after shelling and brown rice samples were milled in McGill Miller No.2, USA. The time of the milling was adjusted to obtain a 6% degree of polish in brown rice samples. The remaining rice sample after milling was total rice including broken rice grains and recovery percentage was calculated by using formula.

Total Rice Recovery % = (Weight of brown rice/ Weight of paddy) ×100%

Milled Rice Recovery % = (Weight of milled rice/ Weight of paddy) ×100%

9. Head Rice Recovery (HRR)%

The head rice is the milled rice which includes broken kernels that are 75% or more of the whole kernel. The total rice obtained after polishing was graded for 2 minutes by using test rice grader machine to separate the head rice from broken in direct seeded rice and transplanted rice (Plate 3.2). It was calculated by using formula.

Head Rice Recovery % = (Weight of head rice/ Weight of paddy) ×100%

3.3.1 Statistical Analysis

Shoot length and germination data recorded on seedlings grown at different depths in cups was subjected to analysis of variance with genotypes and depth of sowing as two factors of variation. Critical difference was worked out to test difference between different treatments The field trial was conducted using the Alpha Lattice field Design with two replications at both locations. Data recorded for all characters were subjected to analysis of variance (ANOVA) (Table 3.3).

The statistical software SAS 9.2 was used to test the performance of genotypes.

Analysis of variance

$Y_{ij} = m + g_i + b_j + e_{ij}$

Where,

Y_{ij}= phenotypic value of the i^{th}genotype of a generation grown in the j^{th}replication

m = general population mean

g_i = effect of the i^{th} genotype, where i=1g

b_j= effect of the j^{th} replication, where j=1r

e_{ij} = environmental effect

Analysis of variance based on the above model led to the following components of variance.

Table 3.3. Analysis of variance

Source of variation	DF	SS	MSS		F value
			Observed	Expected	
Replication	r - 1	$Sr=\Sigma r^2/g-(\Sigma x)^2/N$	$M_r= S_r/r-1$	$\sigma_e+ g\sigma_r$	M_r/ M_e
Genotypes	g - 1	$Sg=\Sigma g^2/r- (\Sigma x)^2/N$	$M_g= S_g/g-1$	$\sigma_e+ r\sigma_g$	M_g/ M_e
Error	(r – 1)(g – 1)	$S_e= S_t-S_r-S_g$	$M_e=S_e/(r-1)(g-1)$	σ_e	
Total	gr - 1	S_t			

Where,

r = number of replications

g = number of genotypes

N = total number of observations

S_r= replication sum of squares

S_g= genotype sum of squares

S_e= error sum of squares

S_t= total sum of squares

σ_r= replication variance

σ_g= genotypic variance

σ_e= error variance

The genotypic variance was tested against error variance by F test for (g-1) and (r-1)(g-1) degrees of freedom. Similarly, the replication variance was tested against error variance for (r-1) and (r-1)(g-1) degrees of freedom.

The standard error of difference between the genotypic means was estimated as follows:

$$SD\ (d) = \pm \sqrt{\frac{2Me}{r}}$$

Critical Difference (CD) = SE (d) × $t_{(r-1)(g-1)}$ at 5% level of significance.

3.4. Methodology for 'Identification of genes with differential expression from candidates associated with mesocotyl elongation under deep sowing'

The experiment was conducted in the School of Agricultural Biotechnology, Punjab

Agricultural University, Ludhiana

The plant material was selected from the previous results obtained from the genome wide association studies. The accession R 146 (IRGC 38606-1) with longer mesocotyl length and better germination ability from depth under direct seeded cultivation conditions was used as test entry, while PR126 served as control. The following steps were followed for this experiment:

a) The appropriate stages/time points for the sampling of mesocotyl tissue was standardized. RNA was isolated from the test and control genotypes at specific stages/time points
b) Mesocotyl samples from PR126 and IRGC 38606-1 sown at 4 cm and 10 cm sowing depths were used. Samples were collected every alternate day starting from 3 days after sowing (DAS) to 15 DAS. Mesocotyl lengths was measured using a vernier calliper. The significant variations in mesocotyl elongation were observed at 5 days, 10 days and 15 days.
c) Independent RNA samples were isolated for the test and control genotypes across all sampling points at the two sowing depths. Each RNA sample was used to construct the RNA-Seq libraries at 150-bp paired end reads using the Illumina TruSeq RNA library prep kit according to standard protocols (Illumina inc., USA).Sequencing was carried out on HiSEQ 4000 and data was generated.
d) In addition, *in-silico* candidate gene analysis was done and functional annotation of SNPs by extracting information from genome browsers and annotation databases like Gramene/RiceQTLpro/Q-TARO/QTL Network/etc. Functionally characterized potential candidate genes were identified from the already identified marker trait associations.

The workflow of processing of RNA seq data was as follows:

1. RNA isolation and sequencing
2. Raw data quality control and filtering
3. Raw data statistics, filtered data statistics
4. Mapping of the QC filtered reads to the reference genome using suitable software.
5. Differential gene expression profiling results between the samples with fold change, -value and FDR correction was generated.
6. Transcripts having a false discovery rate (FDR) > 0.05 and log2 (fold change) < 2 was filtered and not considered for the further analysis
7. Gene ontology and pathway analysis

Observations and analysis: Gene expression profile of candidate genes at relevant stages was subjected to comparative transcriptomic profiling for the identification of differentially expressed genes using bioinformatic tools and following analysis were

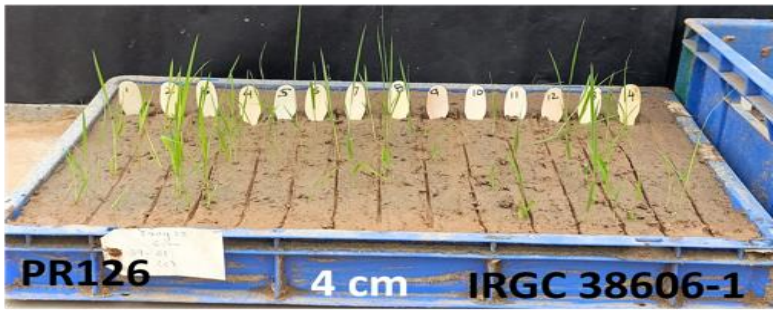


Plate 3: Seedling emergence in PR 126 and IRGC 38606-1 at 4 cm seeding depth

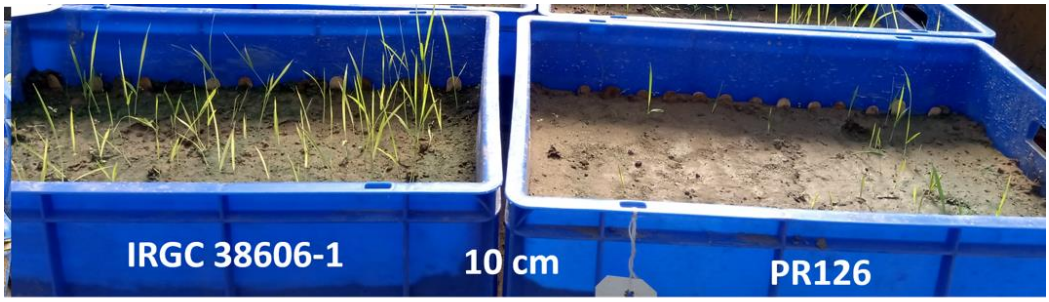


Plate 4: Seedling emergence in IRGC 38606-1 and PR 126 at 10 cm seeding depth

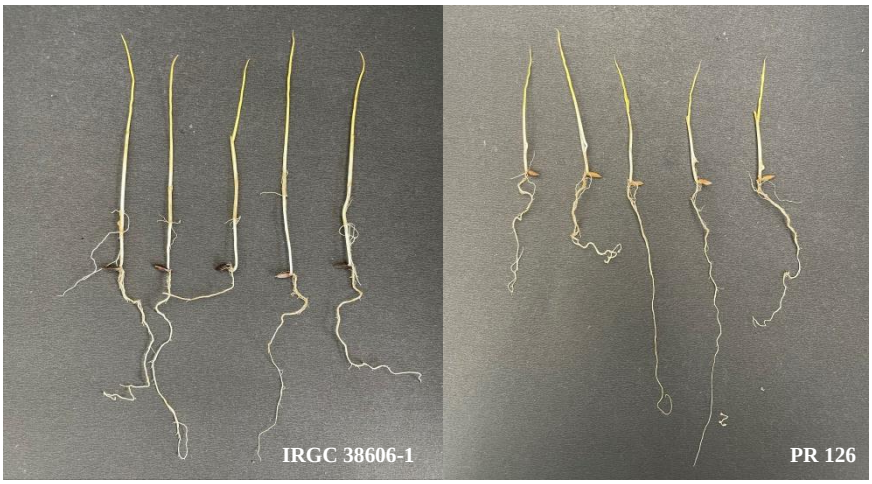


Plate 5: Differences of shoot length recorded at day 5 of sowing at 10 cm sowing depth

Plate 6: Sampling of shoot tissues for differential gene expression analysis

performed.

a) Raw data quality control and filtering
b) Raw data statistics, filtered data statistics
c) Mapping of the QC filtered reads to the reference genome using suitable software.
d) Differential gene expression profiling results between the samples with fold change, p-value and FDR correction were generated.
e) Gene ontology and pathway analysis was carried out.

CHAPTER IV

RESULTS AND DISCUSSION

The rice breeding programme at Punjab Agricultural University, Ludhiana has taken up identification and characterization of potential donors of DSR adaptive traits, followed by crossing and material generation. The breeding strategy employs screening of plant material in appropriate conditions as well as use of molecular markers linked to relevant traits. The present study contributes to this initiative by evaluating a set of advanced breeding lines including a set generated from a complex cross involving parents possessing specific DSR traits/QTLs. Attempts were also made to compare the transcriptome profile of a genotype with exceptional ability for seedling emergence from depth with that of a common cultivar in order to infer the underlying molecular genetic basis of this trait. The overall objectives of the study included, (i) morpho-physiological evaluation of advanced derivatives with DSR related traits in well adapted background, (ii) evaluation of advanced derivatives for yield performance and milling quality traits under direct seeded conditions, and (iii) identification of genes with differential expression from candidates associated with mesocotyl elongation under deep sowing

Short duration non-Basmati rice varieties such as PR 126, PR 128, PR 129 and *Basmati* varieties such as Pusa Basmati 1509, Pusa Basmati 1121 and Punjab Basmati 7 have shown encouraging results under DSR conditions and have been recommended to the farmers for cultivating under these conditions. These varieties, however, have been developed for puddled transplanted rice (PTR) system and lack several of the attributes required for better adaptation to direct seeding. Under Punjab conditions, the problems of anaerobic germination and emergence from depth are encountered if there is incidence of rain within 4-5 days of sowing, causing anaerobic environment for germinating seeds/seedlings besides adding to the soil layer atop the seed as soil is likely to flow into the seeded furrow with rain water. An ideal plant type for DSR should have the ability to germinate under anaerobic condition coupled with tolerance of early submergence and good seedling vigor. The set of 48 lines used in the study included dual purpose (DSR and PTR) varieties as checks. The advanced breeding lines representing the test entries, had been enriched not only for DSR related traits but also for disease and insect pest resistance. Some of these traits were contributed by tagged QTLs, which have been previously catalogued by using SNP markers and the allelic constitution of these lines are given in Table 3.2 of Materials and Methods.

The results obtained from different experiments are presented and discussed below.

4.1Morpho-physiological evaluation of advanced derivatives with DSR related traits in well adapted background

In order to address the problem related to uniform emergence and germination from

depth varieties with optimum seedling/shoot length are preferred. The coleoptile (the protective sheath that covers the emerging shoot) and mesocotyl (the structure between the scutellar and coleoptiler nodes) of rice are primarily responsible for the emergence of seedlings from deeper soil layers (Turner *et al* 1982, Dilday *et al* 1990). Most of the studies measure these structures on seedlings grown on germination paper (e.g., Huang *et al* 2010), but pot based protocols have been preferred in some studies (e.g., Kashiwagi *et al* 2018) as it is closer to field conditions. Wu *et al* (2015) used 5 cm sand culture for screening of large number of rice genotypes for mesocotyl length. In the present study the test entries and checks were sown in cups at 3 depths (3 cm, 6 cm & 9 cm). Observations on seedling emergence were recorded on day 7 after sowing. The length of the seedling was measured using vernier caliper. For each entry, three replicates of six seeds each were taken. In the sowing done at 3 cm depth, majority of the seedlings emerged above the soil surface when observed at day 5. This depth was thus not suitable for revealing the genotypic differences for emergence potential. Seedlings remained beneath the soil surface in pots with sowing at 6 cm and 9 cm depths and both provided similar results with respect to seedling shoot length. Data from 6 cm sowing depth was used for shoot length analysis. Among the three sowing depths, sowing at 9 cm is likely to generate partial anaerobic conditions. The proportion of successfully germinated seeds as compared to those with only rudimentary or arrested growth was taken to indicate potential for anaerobic germination.

4.1.1 Morphological evaluation for seedling length

The seedling lengths (in mm) observed at day 7, in the set sown in pots at 6 cm depth are given in table 4.1. The coleoptiler node was not prominent in most of the genotypes, and would have potentially added to errors in separate measurement of mesocotyl and coleoptile. Taken together these constitute the seedling shoot length which is directly responsible for emergence ability. The observed shoot length for different genotypes given in Table 4.1 ranged from 22.4 mm in Genotype 19 to 40.9 mm in Genotype 17. Most of the checks, including PR 126 were, however, statistically at par with the entry with longest shoot length.

For Genotypes 1 to 38, information on early uniform emergence QTLs, *EMM1.1 and EMM11.1* was known and is given along with shoot length in Table 4.1. A highly significant Pearson Correlation Coefficient of 0.618 was calculated for this set of lines by assigning score 1 and 0 for QTL presence and absence respectively. It was also evident that the check varieties possessed shoot length comparable to best lines with known QTLs. Transferring the QTLs *EMM 1.1*and *EMM 11.1* to these backgrounds may enhance the shoot length further. Small pot or cup based screening can be an effective screening tool for pre-selecting segregating material to be planted in the field while QTL information available from various studies (Sandhu *et al* 2015, Quilloy, 2021) can greatly facilitate choice of parents and donors, besides being used for marker assisted consolidation of QTLs and traits. The present study

also corroborates one of the important conclusions arrived at in the review paper by Zhan *et al* (2020) wherein humus soil culture with a burial depth of 6 cm was considered the optimal method for mesocotyl phenotyping.

Table 4.1. Shoot length (mm) under deep sowing (6 cm) at 7 days after sowing in cups

Genotype	Shoot length (mm)	QTLs for early uniform emergence	Entry	Shoot length (mm)	QTLs for early uniform emergence
1	35.6	*EMM11.1*	**27**	35.3	Absent
2	34.9	*EMM11.1*	**28**	26.6	Absent
3	39.1	Absent	**29**	32.5	*EMM11.1*
4	31.2	Absent	**30**	35.9	*EMM1.1*
5	28.4	Absent	**31**	36.4	*EMM11.1*
6	33.9	*EMM1.1*	**32**	35.8	Absent
7	34.7	*EMM1.1*	**33**	31.5	Absent
8	32.5	Absent	**34**	34.4	Absent
9	33.1	Absent	**35**	32.9	*EMM11.1*
10	40.7	*EMM11.1*	**36**	34.9	*EMM11.1*
11	26.2	Absent	**37**	35.8	Absent
12	27.5	Absent	**38**	27.1	Absent
13	26.3	Absent	**39**	31.6	-
14	28.7	Absent	**40**	27.1	-
15	30.4	Absent	**41**	32.1	-
16	36.8	Absent	**42**	33.3	-
17	40.9	*EMM1.1*	**43**	36.1	-
18	32.7	*EMM1.1*	**44**	39.0	*EMM1.1*
19	22.4	Absent	PR126	36.9	-
20	38.9	*EMM11.1*	PR128	32.5	-
21	31.6	Absent	PR 130	32.1	-
22	32.9	Absent	PR 131	37.9	-
23	33.1	Absent	**Mean**	33.2	
24	32.9	Absent	CD	7.3	
26	32.6	Absent	CV	13.5	

+QTL present; - no information

4.1.2 Germination under partially anaerobic conditions

As proposed in the previous section, the seeds that failed to undergo shoot elongation and showed only rudimentary or arrested germination are likely to have encountered partially

anaerobic conditions, particularly in case of seeds sown in cups at depth of 9 cm. The successfully germinating seeds (percent) under these conditions for the entire set of genotypes is given in Table 4.2.

Table: 4.2. Germination (%) observed in seeds sown in cups at 9 cm depth

Genotype	Germination (%)	QTL for anaerobic germination (*AG9*)	Genotype	Germination (%)	QTL for anaerobic germination (*AG9*)
1	52.78	+	27	63.89	+
2	72.22	+	28	77.78	+
3	66.67	+	29	83.33	+
4	69.44	+	30	83.33	+
5	77.78	+	31	69.44	+
6	75.00	+	32	61.11	+
7	63.89	+	33	72.22	+
8	66.67	+	34	66.67	+
9	69.44	+	35	63.89	+
10	69.44	+	36	77.78	+
11	41.67	Absent	37	88.89	+
12	56.11	+	38	91.67	+
13	42.78	Absent	39	69.44	-
14	58.33	+	40	77.78	-
15	86.00	+	41	75.00	-
16	64.44	+	42	83.33	-
17	86.11	+	43	80.56	-
18	61.11	+	44	52.78	-
19	58.33	+	PR126	77.78	-
20	75.00	+	PR128	75.00	-
21	88.89	+	PR 130	66.67	-
22	72.22	+	PR 131	72.22	-
23	75.00	+	Mean	70.20	
24	83.33	+	CD	9.672	
25	75.00	+	CV	6.846	
26	86.11	+			

+QTL present; - no information

The germination percentage across genotypes showed a wide range from 42.78% (Genotype11) to 91.67% (Genotype 38) while the mean of entire set was 70.20%. Genotypic differences and presence of stress was indicated by these observations. PR 126 with 77.78% gemination was best among the check varieties. With respect to available information on anaerobic germination QTL *AG 9* in this set, it was known to be present in 36 of the 38 lines. Presence of other unknown genetic factors was evident as the *AG 9* positive lines showed significant variation in their germination. Lines lacking *AG 9*, namely Genotypes 11 and 13 however were at the bottom of the set with germination values of 41.67% and 42.78%. The Pearson Correlation Coefficient of *AG 9* presence with germination percentage in the set of 38 lines with known QTL information turned out to be highly positive (r= 0.906) and significant. The observed data pattern also confirmed the presence of anaerobic stress conditions as anticipated.

The stress caused by anaerobic conditions is mainly due to hypoxia and its impact on seed germination and growth of rice has been investigated by a large number of studies (Li *et al* 2018, Su *et al* 2022). Under field conditions, the problem of poor emergence is generally encountered if there is incidence of rain within 4-5 days of sowing, causing anaerobic environment for germinating seeds/seedlings and increase in depth of seeds as the soil flows into the seeded furrow with rain water. As pre monsoon showers are expected in Punjab during month of June, particularly the second half of the month, farmers tend to go for direct seeding early. Farmer field sowings as early as second half of May in order to avoid anaerobic conditions and failure of crop establishment due to pre-monsoon showers seriously undermines the water saving potential of DSR. Early DSR is also vulnerable to heat stress at flowering and the resultant floret sterility. Anaerobic germination ability is thus of great significance in this region for productivity as well as water saving under direct seeding of rice.

4.2 Evaluation of advanced derivatives for yield component traits, yield and milling quality traits under direct seeded conditions

A major focus of the present study is the field performance of advanced lines specifically developed and selected for DSR conditions. A replicated, multilocation trial of 48 entries (including checks) was conducted and observations recorded on days to 50% flowering, plant height, ear bearing tillers, fertility percentage, 1000 grain weight and grain yield. The experiment was carried out at two locations, Ludhiana and Kapurthala, representing rice growing areas of the state. This was followed by an evaluation of the milling attributes in order to have a perspective of the commercial potential of these genotypes.

4.2.1. Analysis of variance of field trials conducted at Ludhiana and Kapurthala

The analysis of variance for the trials conducted at Ludhiana and Kapurthala along with pooled analysis was carried out for all the characters. Analysis of observations recorded

at Ludhiana revealed significant genotypic variation for all traits except for ear bearing tillers (Table 4.3)

Table 4.3. Analysis of variance for observations recorded in the field trial at Ludhiana during 2021 crop season

Source of Variation	DF	DTF	PH	EBT	TGW	SF %	YD
Replication	1	4.17	0.26	2,062.76	0.382	3.250	339238.13
Genotypes	47	37.35**	133.69**	1856.33	8.692*	102.044*	1168011.86**
Error	47	3.50	7.47	1,154.78	1.221	9.013	96413.08

** Significant at 5% and ** Significant 1%; DF: degrees of freedom;*
DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2;
TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

Similar trend with respect to analysis of variance was seen for the trial conducted at Kapurthala with the exception that significant genotypic variation was also not evident for days to flowering, besides ear bearing tillers (Table 4.4)

Table 4.4. Analysis of variance for observations recorded in the field trial at Kapurthala during 2021 crop season

Source of Variation	DF	DTF	PH	EBT	TGW	SF%	YD
Replication	1	70.042*	135.469**	31.51	0.988	9.052*	170138.35
Genotypes	47	30.765	200.645**	1195.34	6.520*	104.424**	1438656.52**
Error	47	14.914	13.041	643.21	1.571	2.074	123493.10

** Significant at 5% and ** Significant 1%; DF: degrees of freedom;*
DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2;
TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

The pooled analysis of variance for both the locations (Table 4.5) showed that location effects were highly significant for all traits. Genotypic variation was also highly significant for all traits except ear bearing tillers (in which case the genotypic variation was non-significant at individual locations itself). The location x genotypic variation was also highly significant for all traits except ear bearing tillers.

Table 4.5. Pooled analysis of variance for observations recorded in the field trials at Ludhiana and Kapurthala during 2021 crop season

Source of Variation	DF	DTF	PH	EBT	TGW	SF%	YD
Location	1	1734.01**	3913.25**	9,562.63**	16.68**	123.44**	10858344.02**
Genotypes	47	116.19**	280.21**	1,704.39	12.75**	84.13**	1809818.68**
Interaction Loc x Geno	47	49.03**	54.12**	1,578.32	2.46**	122.34**	796865.88**
Error	95	9.23	10.92	947.56	1.57	5.61	114008.54

** Significant at 5% and ** Significant 1%; DF: degrees of freedom;*
DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2;
TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

The pooled analysis clearly brings out the importance of location differences. As location x genotype differences for all the traits showing genetic variation was significant, it becomes imperative for varietal development for direct seeded conditions to adopt wider multilocation evaluation and aim at combining high productivity with stability of performance.

4.2.2. Mean values of observations recorded in the field trials conducted at Ludhiana and Kapurthala

The mean values of observations recorded in the field trial conducted at Ludhiana during 2021 crop season are given in Table 4.6. The corresponding data recorded in the field trial conducted at Kapurthala are given in Table 4.7. The pooled mean values of the trait means recorded at the two locations with respect to the set of traits and genotypes are given in Table 4.7.

Table 4.6. Mean values of observations recorded in the field trial conducted at Ludhiana during 2021 crop season

Genotype	Designation	DTF	PH	EBT	TGW	SF	YD
1	IR 129477-4139-439-1-1-2	79.5	115.0	212.5	23.1	76.3	2408.0
2	IR 129477-1813-87-5-2-2	81.5	100.5	222.5	26.7	73.8	3700.0
3	IR 129477-1232-81-2-1-3	77.0	110.5	247.5	20.3	76.7	2885.0
4	IR 129477-3343-109-13-1-1	82.5	105.0	237.5	23.0	84.4	4292.0
5	IR 129477-1629-210-2-2-2	77.5	107.5	240.0	19.8	67.1	2962.0
6	IR 129477-3873-201-5-1-5	76.0	105.0	255.0	21.7	72.2	4515.0
7	IR 129477-1629-210-4-4-4	79.0	105.5	230.0	22.1	62.0	3508.0
8	IR 129477-3873-297-2-6-4	85.0	97.0	262.5	22.1	69.8	3246.0
9	IR 129477-991-430-1-9-4	84.0	105.0	217.5	24.5	55.7	3346.0
10	IR 129477-1813-191-5-7-6	82.5	109.5	260.0	23.1	73.8	4846.0
11	IR 129477-3425-97-4-3-3	81.0	117.5	247.5	20.1	78.4	4200.0
12	IR 129477-4197-209-2-2-2	79.5	98.0	255.0	23.0	66.0	3854.0
13	IR 129477-4139-439-2-4-2	80.0	107.0	215.0	21.9	78.4	3231.0
14	IR 129477-3873-297-3-3-2	78.5	108.0	222.5	24.3	81.8	4431.0
15	IR 129477-3873-297-3-3-4	88.5	98.0	290.0	20.1	70.9	5231.0
16	IR 129477-1629-14-1-4-1	85.5	105.5	212.5	21.3	78.8	3169.0
17	IR 129477-1629-14-1-4-2	85.0	112.5	217.5	24.5	77.9	3292.0
18	IR 129477-2815-185-1-7-3	77.5	89.5	227.5	22.2	81.3	3569.0
19	IR 129477-2815-185-2-1-1	74.0	107.0	227.5	20.6	67.4	3585.0
20	IR 129477-4026-249-15-1-5	82.0	116.0	285.0	19.9	65.3	3592.0
21	IR 129477-709-375-3-5-7	82.0	112.5	275.0	21.1	72.5	4854.0

22	IR 129477-2815-179-3-1-1	79.0	106.0	192.5	24.9	80.2	3877.0
23	IR 129477-991-456-6-4-5	75.5	99.5	240.0	19.7	76.8	4508.0
24	IR 129477-902-121-10-1-3	77.0	107.0	232.5	25.1	64.6	3462.0
25	IR 129477-3343-500-36-5-1	79.0	113.5	250.0	19.0	69.5	3938.0
26	IR 129477-1510-100-7-5-2	81.5	100.0	180.0	23.1	85.8	4508.0
27	IR 129477-4026-249-15-1-2	85.0	106.0	182.5	19.0	65.1	3346.0
28	IR 129477-2815-179-3-1-5	78.0	101.0	245.0	23.4	72.4	3685.0
29	IR 129477-902-121-10-1-4	75.5	105.0	195.0	26.0	77.7	3785.0
30	IR 129477-4026-249-15-1-7	82.0	111.0	252.5	19.9	61.2	3969.0
31	IR 129477-1629-14-1-1-5	82.0	114.0	240.0	23.6	67.0	3046.0
32	IR 129477-1510-100-7-5-6	80.5	97.0	190.0	20.4	72.0	2331.0
33	IR 129477-991-456-6-4-4	73.5	104.5	267.5	18.7	76.5	3931.0
34	IR 129477-1510-100-7-5-4	84.5	97.0	272.5	25.4	73.4	3246.0
35	IR 129477-709-375-3-5-6	79.5	113.0	272.5	21.1	83.9	3546.0
36	IR 129477-4026-249-15-1-4	76.5	110.0	205.0	20.2	61.2	3285.0
37	IR 129477-3343-500-36-5-4	91.0	86.0	225.0	19.6	64.5	2377.0
38	IR 129477-902-121-10-1-1	79.5	102.0	207.5	21.0	67.2	3477.0
39	RYT-3872	74.5	91.5	300.0	18.4	83.3	4708.0
40	RYT-4008	76.5	106.0	200.0	20.1	71.8	2115.0
41	RYT-4009	80.5	96.5	222.5	23.4	65.7	2908.0
42	DRR Dhan 44	84.0	110.0	210.0	19.9	83.7	4823.0
43	Sahbhagi Dhan	89.5	119.5	175.0	19.8	62.9	3838.0
44	MTU 1010	82.0	86.5	257.5	21.1	75.4	3423.0
PR126	PR 126	76.5	90.0	220.0	22.0	70.9	5100.0
PR128	PR 128	101.1	96.5	237.5	24.3	79.1	4800.0
PR130	PAU 6260-3-1-6 (PR 130)	104.5	93.5	242.5	21.3	72.8	2708.0
PR131	PAU 6707-10-2-2-1 (PR 131)	103.5	94.0	305.0	21.0	72.4	2785.0
Mean		81.9	103.9	235.0	21.8	72.6	3671.7
CD		3.802	5.517	70.8	2.23	6.059	626.6
CV		2.351	2.629	14.548	5.046	4.133	8.46

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

Table 4.7. Mean values of observations recorded in the field trial conducted at Kapurthala during 2021 crop season

Genotype	Designation	**DTF**	**PH**	**EBT**	**TGW**	**SF**	**YD**
1	IR 129477-4139-439-1-1-2	90	110	245	25.3	63	2431
2	IR 129477-1813-87-5-2-2	85	101	228	26.1	64	4785
3	IR 129477-1232-81-2-1-3	91	95	228	20.9	70	2708
4	IR 129477-3343-109-13-1-1	90	92	243	23.7	83	2577
5	IR 129477-1629-210-2-2-2	86	112	215	21.3	84	4046
6	IR 129477-3873-201-5-1-5	90	99	218	23.6	77	3892
7	IR 129477-1629-210-4-4-4	88	101	210	22.9	79	5154
8	IR 129477-3873-297-2-6-4	89	82	235	23.2	62	2415
9	IR 129477-991-430-1-9-4	91	94	265	25.5	81	3762
10	IR 129477-1813-191-5-7-6	81	104	243	22.8	67	4138
11	IR 129477-3425-97-4-3-3	95	112	278	23.8	70	4615
12	IR 129477-4197-209-2-2-2	94	78	268	22.1	64	2131
13	IR 129477-4139-439-2-4-2	95	96	183	22.2	80	2346
14	IR 129477-3873-297-3-3-2	93	95	255	25.0	67	2569
15	IR 129477-3873-297-3-3-4	100	100	241	21.4	66	4385
16	IR 129477-1629-14-1-4-1	91	92	213	22.3	74	2754
17	IR 129477-1629-14-1-4-2	94	103	178	24.9	79	3738
18	IR 129477-2815-185-1-7-3	84	84	258	23.1	59	2831
19	IR 129477-2815-185-2-1-1	92	81	233	21.2	71	2308
20	IR 129477-4026-249-15-1-5	92	90	228	20.5	86	2715
21	IR 129477-709-375-3-5-7	88	108	225	21.9	76	4669
22	IR 129477-2815-179-3-1-1	94	97	235	25.5	71	3215
23	IR 129477-991-456-6-4-5	94	92	215	20.5	70	2408
24	IR 129477-902-121-10-1-3	90	102	220	25.0	84	2638
25	IR 129477-3343-500-36-5-1	91	111	215	20.3	82	3208
26	IR 129477-1510-100-7-5-2	95	89	205	23.8	81	2631
27	IR 129477-4026-249-15-1-2	95	99	195	20.3	83	3708
28	IR 129477-2815-179-3-1-5	95	93	170	24.4	74	2400
29	IR 129477-902-121-10-1-4	90	99	238	25.2	73	3569
30	IR 129477-4026-249-15-1-7	94	91	208	20.7	81	3046
31	IR 129477-1629-14-1-1-5	91	115	223	24.5	72	4638
32	IR 129477-1510-100-7-5-6	88	75	198	22.0	77	1369

33	IR 129477-991-456-6-4-4	91	94	235	19.8	88	3015
34	IR 129477-1510-100-7-5-4	91	86	228	23.9	69	2485
35	IR 129477-709-375-3-5-6	90	102	225	22.2	82	3662
36	IR 129477-4026-249-15-1-4	85	99	218	21.1	86	3077
37	IR 129477-3343-500-36-5-4	92	84	205	20.4	77	3031
38	IR 129477-902-121-10-1-1	91	86	238	22.3	71	2331
39	RYT-3872	93	80	245	18.7	72	3308
40	RYT-4008	83	108	203	20.0	65	1831
41	RYT-4009	87	87	183	23.9	73	2969
42	DRR Dhan 44	88	111	185	21.3	75	3662
43	Sahbhagi Dhan	92	96	238	21.3	66	2369
44	MTU 1010	89	91	200	21.8	64	3569
PR126	PR 126	84	84	205	21.2	76	4723
PR128	PR 128	96	92	225	23.3	75	4392
PR130	PAU 6260-3-1-6 (PR 130)	96	84	210	22.1	79	3115
PR131	PAU 6707-10-2-2-1 (PR 131)	98	84	286	21.3	75	3169
Mean		91	95	224	22	74	3219
CD		NS	7.288	51.2	2.53	2.906	709.2
CV		4.3	3.803	15.22	5.575	1.94	10.99

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

Table 4.8. Pooled mean values of observations recorded in the field trials conducted at Ludhiana and Kapurthala during 2021 crop season

Genotype	DTF	PH	EBT	TGW	SF	YD
1	86	113	229	23.63	70	2419
2	88	101	225	23.33	69	4242
3	87	103	238	20.90	73	2796
4	87	99	240	23.78	84	3435
5	84	110	228	20.78	76	3504
6	85	102	236	23.13	75	4204
7	89	103	220	22.50	71	4331
8	84	90	249	24.30	66	2831
9	91	100	241	25.23	68	3554
10	81	107	251	22.98	70	4492

11	90	115	263	22.63	74	4408
12	92	88	261	22.00	65	2992
13	88	101	199	22.15	79	2788
14	87	101	239	24.43	74	3500
15	99	99	266	21.08	68	4758
16	88	99	213	22.30	76	2962
17	89	108	198	24.78	78	3515
18	81	87	243	24.45	70	3200
19	84	94	230	22.40	69	2946
20	93	103	256	20.45	76	3154
21	88	110	250	20.90	74	4762
22	89	102	214	25.55	75	3546
23	85	96	228	19.45	74	3458
24	83	105	226	24.73	74	3050
25	85	112	233	19.98	76	3573
26	92	95	193	23.18	83	3569
27	92	103	189	19.98	74	3527
28	89	97	208	23.80	73	3042
29	83	102	216	25.53	75	3677
30	93	101	230	20.40	71	3508
31	87	115	231	22.50	70	3842
32	84	86	194	21.90	75	1850
33	84	99	251	20.03	82	3473
34	86	91	250	23.95	71	2865
35	88	107	249	22.43	83	3604
36	82	105	211	20.13	73	3181
37	95	85	215	19.38	71	2704
38	84	94	223	22.68	69	2904
39	86	86	273	18.55	78	4008
40	72	107	201	19.23	69	1973
41	84	92	203	21.78	69	2938
42	89	111	198	21.05	79	4242
43	94	108	206	22.18	64	3104
44	89	89	229	21.93	70	3496
PR126	82	87	213	20.75	74	4412

PR128	99	94	231	23.43	77	4596
PR130	100	89	226	21.43	76	2912
PR131	101	89	296	21.25	74	2977
CD Locations (A)	1	1	9	0.36	1	97
CD Varieties (B)	4	5	43	1.762	3	475
CD Interaction(AXB)	6	7	61	2.492	5	671

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

4.2.3. Days to 50 percent flowering

As mentioned in the previous section, the days to 50% flowering showed significant genotypic variation at Ludhiana as well as in pooled analysis of variance over Ludhiana and Kapurthala. The means for days to 50% flowering are given in Tables 4.6 to 4.8. The box plot of means recorded for days to 50 percent flowering is given in Figure 4.1.

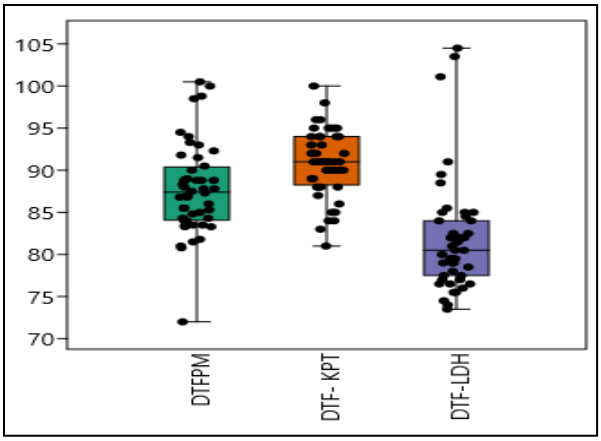


Figure 4.1. Box plot representation of observations recorded for days to 50 percent flowering at Ludhiana and Kapurthala

The days to 50 % flowering ranged from 73.5 to 91 days at Ludhiana and from 81 to 100 at Kapurthala. The days to flowering had higher mean at Kapurthala than Ludhiana. The trend however remained same with respect to checks and some other cultivars. PR130, PR131 and PR128 were found to have taken longest to flower at both the locations and Sahbhagi Dhan was the earliest at both the locations. Amongst the checks, PR 126 was among early flowering genotypes at both the locations with an average of 82 days. The lines with minimum and maximum duration however differed at the two locations. Genotype 33 was earliest to flower at Ludhiana while Genotype 37 took the most days to flower. At Kapurthala Genotype 10 was earliest and Genotype 15 took longest to flower.

Counting the days from seed to seed, the flowering is earlier under the DSR conditions by 7-10 days as compared to PTR conditions (Ginigaddara and Ranamukhaarachchi 2011, Singh 2022). Further, early to medium maturing genotypes are

preferred for DSR conditions as these are resource use efficient and also vacate the field timely for the timely sowing of wheat crop. The entire set of lines in the trial conformed to this requirement.

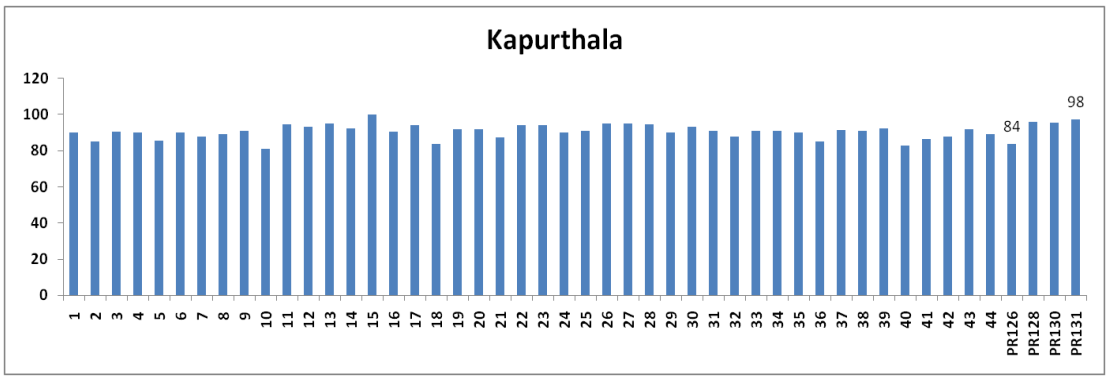


Figure 4.2. Days to 50 percent flowering at Kapurthala

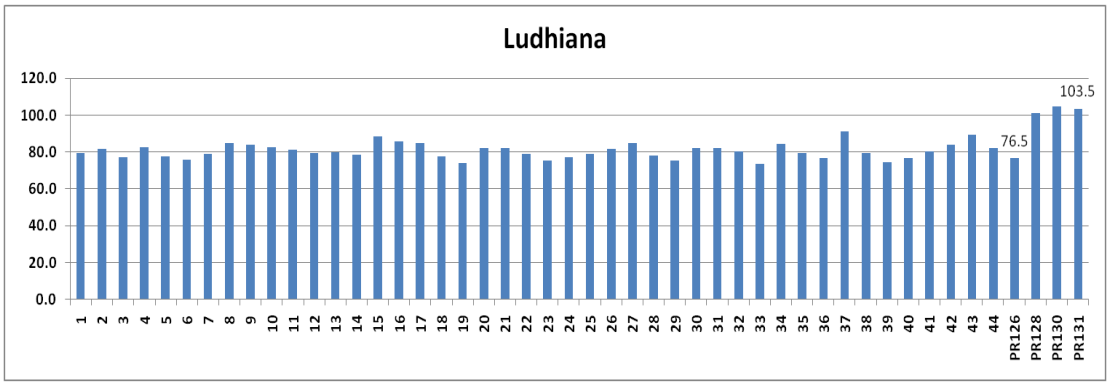


Figure 4.3. Days to 50 percent flowering at Ludhiana

It is evident from the graphical representation (Figures 4.2 and 4.3)that a few genotypes as early as PR 126 which is considered a desirable duration for DSR are available in this set and further interventions would be needed in this regard. However, most of the genotypes belonged to mid early to medium maturity group.

4.2.4. Plant height

Plant height is a pivotal feature of varietal adaptation and acceptance (Bhadru *et al* 2011). Though plant height under DSR is less than PTR (Rampson and Rehman 2006, Soriana *et al* 2018, Singh 2022), the tendency to lodge is greater under DSR. Further several donors of DSR traits are derived from landraces of the pre-dwarfing era. It is also expected that longer coleoptile or seedling shoot length will be correlated with greater plant height. All these considerations make it very important to select stringently for appropriate plant height in the materials developed for DSR adaptive traits. Significant genotype variation was observed in the field trials at both Ludhiana and Kapurthala and the means are given in Table 4.6 to 4.8. The distribution of means is represented as a box plot in Figure 4.4.

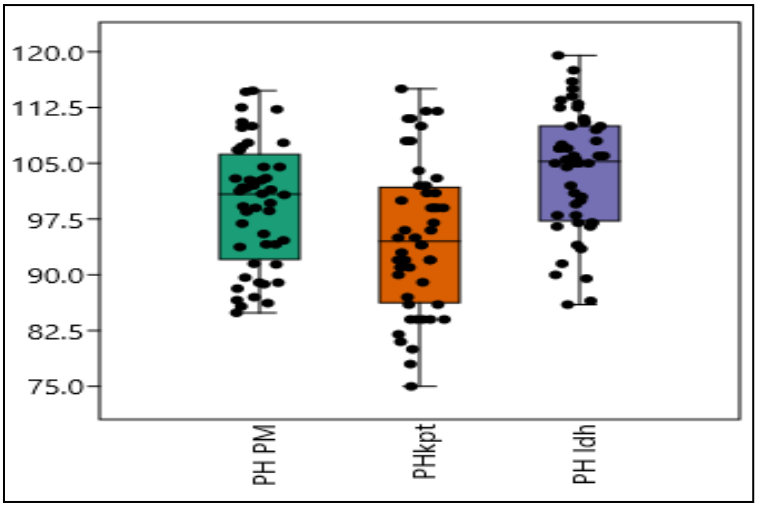


Figure 4.4. Box plot representation of observations recorded for plant height at Ludhiana and Kapurthala

The average plant height Ludhiana (103.99 cm) was more than the average plant height at Kapurthala (94.96 cm). The plant height varied from 86.5 to 119.5 cm at Ludhiana while at Kapurthala it ranged from 78.3 cm to 112 cm. Most of the genotype did fall under the semi dwarf category but some such as Genotypes 5, 11, 25, 31, RYT 3872 and RYT 4008 were taller than 110 cm.

The graphical representation given in Figure 4.5 also indicates the distribution of height is inclined towards tallness though a full range of categories are seen. The shortest lines at both location were Genotype 18 and 37 which were similar in height to PR126. Also both of these have similar height at both the locations. The tallest entry is RYT4008 at Ludhiana and entry 31 at Kapurthala. Further, Genotype 31, Genotype 5, Sahbhagi Dhan and RYT 3872 are taller at Kapurthala than at Ludhiana and show a greater location influence.

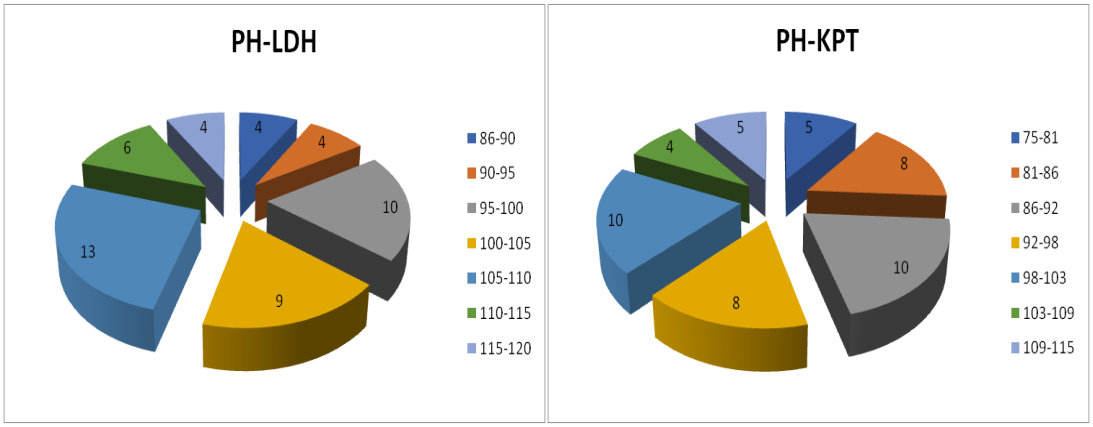


Figure 4.5. Pie chart representation of distribution of genotypes for plant height at Ludhiana and Kapurthala

4.2.5. Ear bearing tillers

Ear bearing tillers are an important yield component but the genotypic variation in the present study was non-significant. The mean ear bearing tillers at Ludhiana and Kapurthala

are given in Tables 4.6 to 4.8. In the present experiment greater proneness of this trait to environmental influence probably overwhelmed the relatively smaller genotypic differences that may have existed in this set. The box plot representation of mean observations is given in Figure 4.6

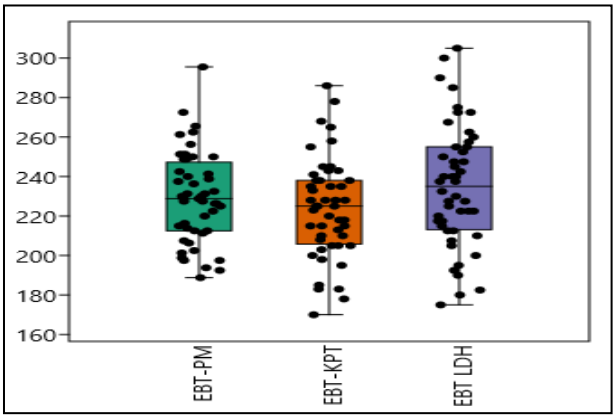


Figure 4.6. Box plot representation of observations recorded for plant height at Ludhiana and Kapurthala

In a recent study at our centre (Singh 2022) the number of ear bearing tillers was similar under DSR and PTR conditions. With a greater flexibility to fine tune plant stand through adjustment of seed rate, obtaining an optimal number of ear bearing tillers may not pose a serious challenge under DSR conditions. Better field management for ensuring optimal germination provides another avenue for maintaining tiller number per meter square. The graphical representation (Figure 4.6) shows consistently higher ear bearing tillers at Ludhiana as compared to Kapurthala with 233 and 188 being the respective location means. PR130 showed maximum number of tillers at Ludhiana followed by Genotypes 20, 21 and 15. At Kapurthala Genotypes 10, 7 and 37 showed higher number of ear bearing tillers/m^2. The numerical differences observed were however statistically non-significant.

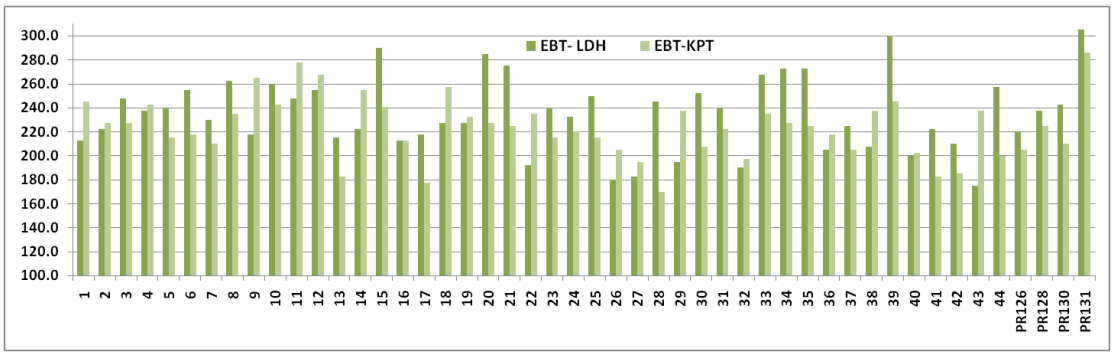


Figure 4.7. Ear bearing tillers recorded in the field trials conducted at Ludhiana and Kapurthala

4.2.6. Thousand grain weight

Within the optimal range of the length and breadth ratio for a particular category of

rice, thousand grain weight is an important yield component (Xu *et al* 2015). More dense grains like in case of PR 121 add to yield as well as quality by lowering broken percentage. The mean thousand grain weight recorded in the field trials at two locations is given in Tables 4.6 to 4.8. The box plot representation of the means is given in Figure 4.8.

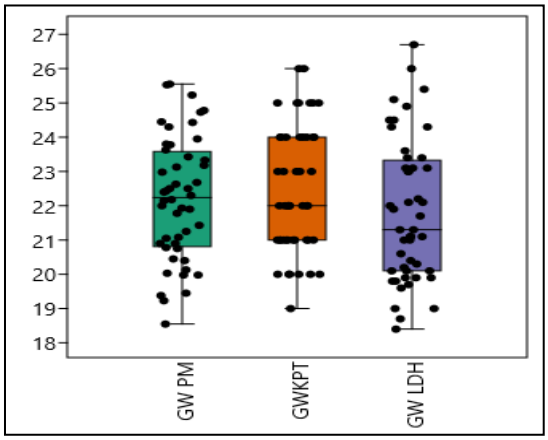


Figure 4.8. Box plot representation of observations recorded for plant height at Ludhiana and Kapurthala

The graphical representation of this data (Figure 4.9) shows slight edge of Kapurthala location for majority of the genotypes. The means grain weight was found to be 21.9gms at Ludhiana and 22.5gms at Kaputhala. The check PR126 shows 22 and 21.2 grams grain weight for 1000 seeds at Ludhiana and Kapurthala respectively. PR130 and PR131 are similar to PR126 for grain weight while PR128 has higher grain weight than PR126. Genotype 2, 9,17, 22, 29 and 34 had sigificantly superior thousand grain weight than PR126 at both the locations with Genotype 2 having the highest grain weight (26.7 g). DRR Dhan 44 had the lowest grain weight at both the locations.

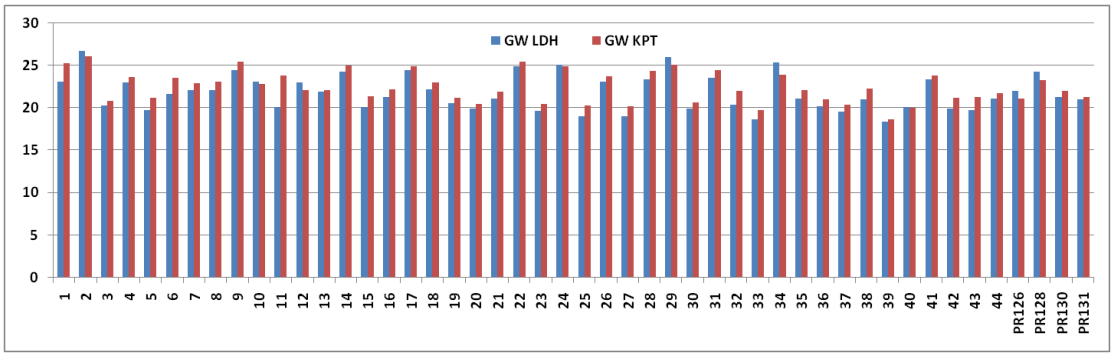


Figure 4.9. Thousand grain weight recorded in the field trials conducted at Ludhiana and Kapurthala

Aslam *et al (*2008) and Ehsanullah *et al* (2000) reported significantly higher thousand grain weight in conventional planting than in direct seeding of rice. Similar results were obtained by Singh (2022). However, Sharma *et al* 2005 had reported that different establishment methods had no significant effect on thousand grain weight.

4.2.7. Spikelet fertility

Related cereal wheat is able to exercise plasticity in grain formation through modulating the formation of central florets. In case of rice where the panicle has a preset number of florets the plasticity is expressed through fertility or filling of the florets. Higher sterlity is also indicative of yield constraints that became severe during course of growth and developemnt of a crop. Spikelet fertility thus is one of the yield components along with number of productive tillers, number of potential grains per panicle and grain weight (Sheehy *et al* 2001). The trait is highly variable and is most affected trait by environmental conditions. Location and genotypes both showed significant difference and interaction was also higly significant. The spikelet fertility percentage recorded in the field trials is given in Table 4.6 to 4.8. The box plot of means is given in Figure 4.10.

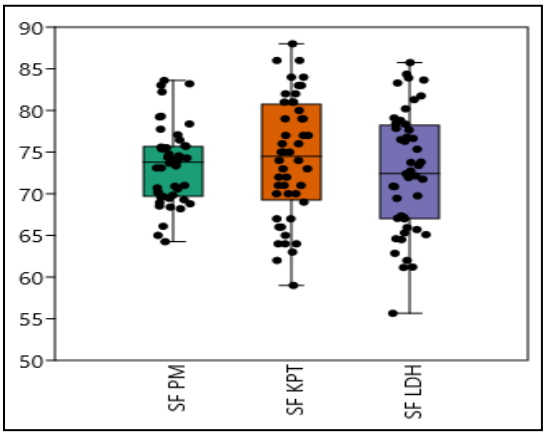


Figure 4.10. Box plot representation of observations recorded for spikelet fertility at Ludhiana and Kapurthala

The mean fertility was 72.6 % for Ludhiana and 74.2 % at Kapurthala. Genotype 4, 13,17 and 35 were similar at both the locations while Genotypes 5, 7, 9,20, 24, 27, 30, 33, 36 and 37 were significantly higher at Kaputhala than Ludhiana (Figure 4.11). Likewise 1,2,10,14,18 and 22 were significantly higher at Ludhiana. PR126 had 71% fertile spikelets at Ludhiana and 76.5 % at Kapurthala. PR130 and PR131 had lower fertility at both the locations.

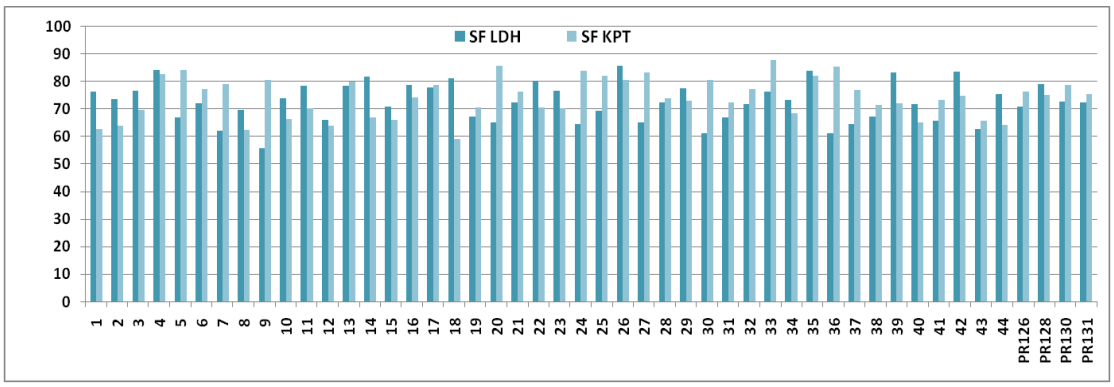


Figure 4.11. Spikelet fertility (%) recorded in the field trials conducted at Ludhiana and Kapurthala

Amegan *et al* (2020) reported high phenotypic variation in a set of 130 rice accessions from South Korea and Africa for spikelet number and fertility. Gathala *et al* (2011) found a higher count of sterile spikelet in direct seeded rice than transplanted rice. Singh (2022), reported similar spikelet fertility under DSR and PTR conditions but a small number of genotypes performed poorly under DSR conditions as compared to transplanted crop.

4.2.8. SPAD values

The mean SPAD values for the various entries are given in Table 4.9 and represented in Figure 4.12. It is a measure of leaf chlorophyll content which supports photosynthetic rate (Miah *et al* 1997) and is in turn responsible for higher biomass production. DSR shows lower SPAD values than PTR (Singh 2022)

Table 4.9. SPAD value for field trial conducted at Ludhiana during 2021 crop season

Genotype	Mean	Genotype	Mean
1	29.8	27	28.6
2	33.5	28	30
3	33.0	29	30.2
4	32.0	30	33.4
5	34.4	31	34.3
6	31.5	32	37.1
7	32.2	33	29.6
8	33.3	34	33.9
9	35.4	35	32.5
10	35.0	36	28.7
11	36.6	37	35.7
12	36.5	38	33.0
13	36.2	39	27.2
14	26.1	40	32.8
15	35.2	41	30.1
16	33.8	42	34.9
17	34.0	43	35.5
18	32.4	44	37.1
19	31.6	PR126	32.3
20	33.8	PR128	39.9
21	34.8	PR 130	34.5
22	33.7	PR 131	35.2
23	34.0	Mean	33.25
24	35.1	C.D.	4.645
25	30.2	C.V.	6.922
26	34.4		

The SPAD score values for PR126 was 32.3 while the mean was 33.25. Genotypes 9, 10,11, 12,13, and 15 were found to have high SPAD score viz, 35.45, 35.1, 36.65, 36.5, 36.25 and 35.2 respectively. Genotype 11, 12 and 13 were significantly spoerior over PR126. The highest SPAD value (39.9) was observed for PR 128. As iron deficiency commonly occurs in direct seeded rice, the genotypes with higher SPAD value are preferred.

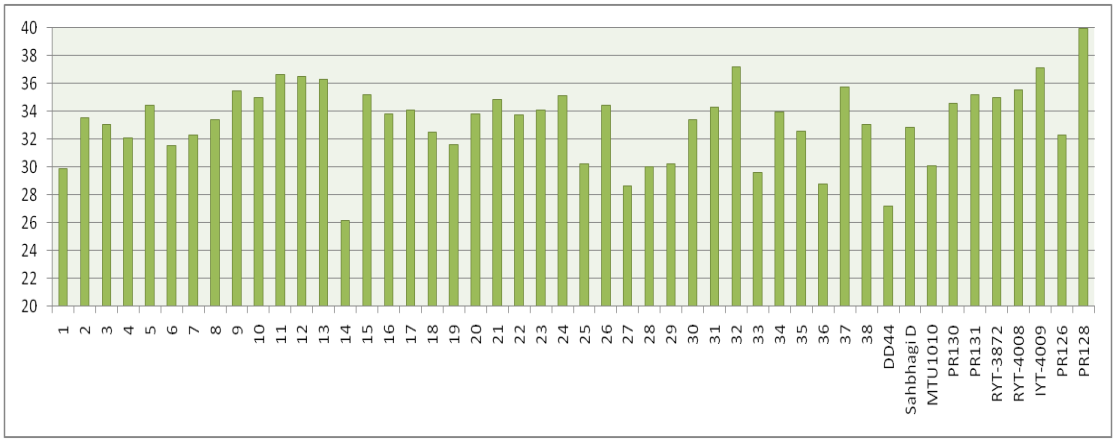


Figure 4.12. SPAD values recorded in the field trial at Ludhiana

4.2.9. Yield

The yield is the composite and often the ultimate trait for identifying the actual worth of a test entry. The yield observations for the field trial at both locations are given in Tables 4.6 to 4.8. The graphical representation of distribution of data and genotype means at Ludhiana and Kapurthala are given in Figures 4.13 and 4.14.

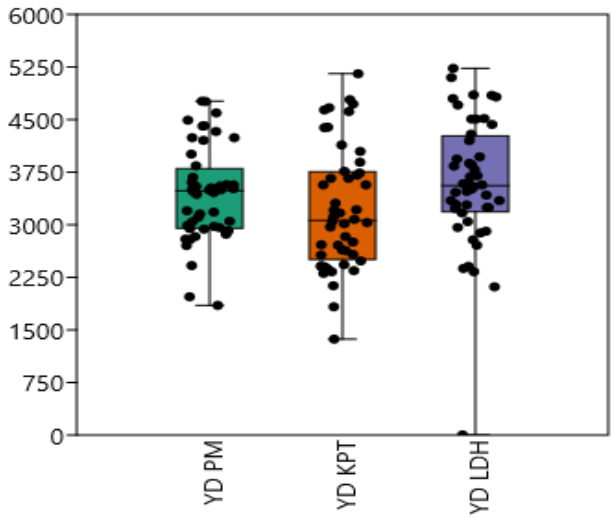


Figure 4.13. Box plot representation of yield recorded in the field trial conducted at Ludhiana and Kapurthala

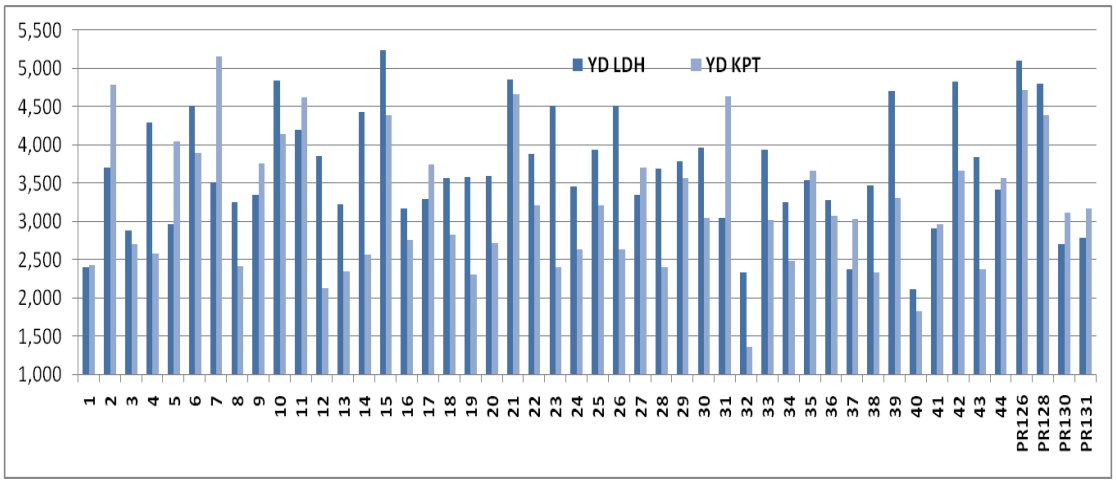


Figure 4.14. Yield (kg/ha) recorded in the field trials at Ludhiana and Kapurthala

The paddy grain yield data observed shows that PR126 as check had higher yield at both Ludhiana and Kapurthala. It yielded 5100 kg/ha at Ludhiana and 4723 kh/ha at Kapurthala while both PR 130 and PR131 were having low yield as compared to PR126. Among the test entries, Genotype 15 has higher yield (5230kg/ha) than PR126 at Ludhiana though yield at Kapurthala was lower as compared with Ludhiana. Genotype 21 has yieled 4853 kg/ha at Ludhiana and 4669 kg/ha at Kapurthala. The highest yielding entry at Kapurthala was Genotype 7 with 5153.85 kg/ha yield. Other high yielding entries were Genotype 10,11, 14, 23, 26 and DRR Dhan 44 at Ludhiana while Genotype 2, 7, 11, 31 at Kapurthala. Lowest yielding entry was Sahbhagi Dhan at Ludhiana and Genotype 32 at Kapurthala. Average yield was found to be higher for Genotype10, 15 and 21 as compared to best check PR126. Xu (2019) had shown that DSR conferred a yield penalty of about 12% and similar results were obtained by Singh (2022). Kaur and Singh (2017) had however shown higher yields for DSR.

A summary of performance of entries as per yield rank in the field trials along with other agronomic traits is given in table 4.10 to obtain an overview of the results of this experiment. Genotype 15 and 21 have yield levels above PR 128 and these two entries along with Genotype 10 rank above PR 126 for yield. It is important to note that Genotype 10 has a short duration as indicated by average of 81 days taken to 50% flowering compared to 82 days taken by the early check PR 126, considered to be optimal for DSR conditions. Entry 21 is tallest (110 cm) among these top yielding entries though lodging was not observed in the present trial. Interestingly Entry 15 is able to yield high even at a relatively low spikelet fertility. The ear bearing tillers and grain weight parameters for the top three entries are similar and in an acceptable range. A column in Table 4.10 lists the grain yield QTLs known in the entries short listed here. The contribution of the known yield QTLs, however does not seem to be critical as the yield based ranking is apparently independent of their presence.

Table 4.10. High yielding genotypes on basis of pooled mean and their trait profile

Rank	Genotype	YD	Grain yield QTLs	DTF	PH	EBT	GW	SF	HRR
1	15	4808	-	98	99	265	21	68.40	50.17
2	21	4762	*GY6.1*	87	110	250	20	74.38	36.72
3	PR128	4596	-	99	94	231	23	77.08	59.01
4	10	4492	-	81	107	251	22	70.15	41.67
5	PR126	4412	-	82	87	212	21	73.68	52.5
6	11	4408	*GY10.1*	90	114	262	23	74.40	45.41
7	7	4331	-	89	103	220	22	70.60	36.21
8	2	4242	*GY6.1*	88	101	225	23	68.80	43.51
9	RYT 3872	4242	-	89	110	197	21	79.30	53.56
10	6	4204	-	85	102	236	23	74.75	46.87
11	DRR Dhan 44	4008	-	85	86	272	19	77.75	41.4
12	31	3842	-	87	115	231	22	69.65	42.29
13	29	3677	*GY6.1+GY10.2*	83	101	216	26	75.40	23.39
14	35	3604	*GY6.1*	88	107	249	22	83.03	34.71
15	25	3573	-	85	112.	232	20	75.78	49.8

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; TGW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

4.2.10. Inter trait correlations

The correlations between various trait observations recorded at Ludhiana and Kapurthala as well as on pooled data basis were worked out and are given in Tables 4.11, 4.12 and 4.13 respectively. For Ludhiana shoot length and anaerobic germination were also included in the correlation matrix (4.11). Shoot length was seen to be uncorrelated with plant height. Thus benefit of longer coleoptile and mesocotyl may not carry a negative cost in form of taller plants.

Table 4.11. Inter trait correlations for observations recorded at Ludhiana

	AG	DTF	PH	EBT	GW	SF	YD
SL	0.147	0.260	0.000	0.055	0.057	-0.067	-0.086
AG		0.072	0.006	-0.068	-0.022	-0.169	0.197
DTF			-0.234	0.141	0.040	-0.049	-0.120
PH				-0.155	-0.058	-0.091	0.036
EBT					-0.204	0.051	0.235
GW						0.119	0.010
SF							0.314*

SL: shoot length; AG: anaerobic germination; DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; GW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

At Ludhiana the only significant correlation was a positive correlation between spikelet fertility and yield (Table 4.11, Figure 4.15). Ear bearing tillers per square metre showed a high though non-significant correlation with yield but a negative correlation with grain weight.

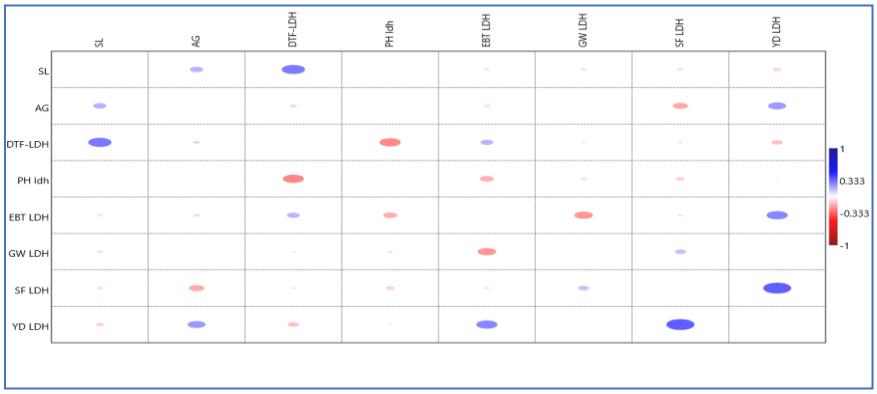


Figure 4.15. Inter trait correlation dot plot for pooled observations recorded at Ludhiana

At Kapurthala location plant height was correlated with yield (Table 4.12, Figure 4.16). The growth conditions at Kapurthala led to lower yield than Ludhiana for the entire trial and the taller genotypes seem to be less affected by the yield suppression. Further along expected lines at lower soil fertility, spikelet fertility was negatively correlated with yield.

Table 4.12. Inter trait correlations for observations recorded at Kapurthala

	PH	EBT	GW	SF	YD
DTF	-0.185	0.114	-0.048	0.131	-0.100
PH		-0.092	0.180	0.117	0.482**
EBT			0.130	-0.318*	0.055
GW				-0.201	0.153
SF					0.107

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; GW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

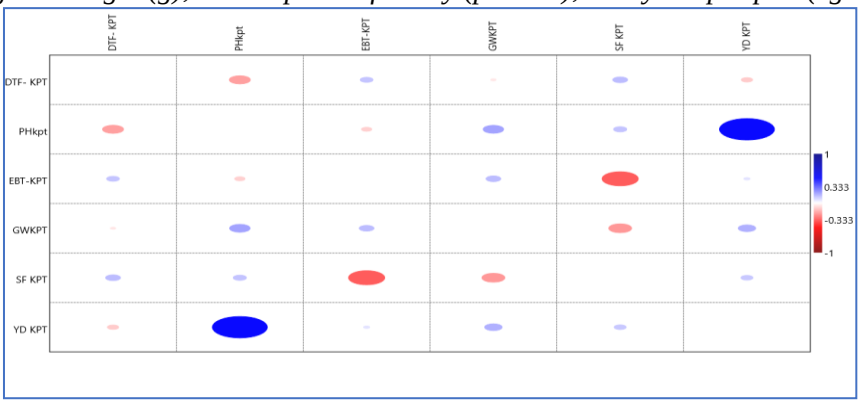


Figure 4.16. Inter trait correlation dot plot for pooled observations recorded at Kapurthala

As the correlation trends at the two locations were different, the pooled data tended to cancel out these trends and no significant correlation was observed (Table 4.13).

Table 4.13. Inter trait correlations for pooled observations recorded at Ludhiana and Kapurthala

	PH	EBT	TGW	SF	YD
DTF	-0.153	0.230	-0.013	0.066	0.213
PH		-0.107	0.059	0.129	0.276
EBT			-0.054	-0.097	0.264
TGW				-0.050	0.033
SF					0.171

DTF: days to 50% flowering (days); PH: plant height (cm); EBT: ear bearing tillers/m^2; GW: thousand grain weight (g); SF%: spikelet fertility (percent); YD: yield per plot (kg/ha)

4.2.11. Quality traits

The harvest of field trial at Ludhiana was analyzed for basic grain quality attributes. Rice quality characteristic are major determinant of market prices and consumer acceptability. Milling traits are a major concern for viability of the processing industry and norms tend to be stringent. These traits include brown rice yield, milled rice yield and head rice yield. While brown rice results from primary cleaning and shelling operation of removing grain from the husk, milling yield is the estimate of the quantity of total milled rice obtained from a unit of rough rice (paddy) and produced by removing the hulls, germ, and most of the bran. It includes intact and broken kernels and generally expressed as percentage. Head rice is the intact or "whole" kernels and includes milled kernels having equal to or more than three-fourth length. The market value of broken kernels is only 50-60% that of head rice, and thus has major economic implications for the processing industry.

In the present study all three milling related quality traits were evaluated. It was observed that the check rice varieties have similar brown rice percentage (about 78-80 percent) while test entries vary in their response ranging from 75 to 81 percent (Figure 4.17 and 4.18)

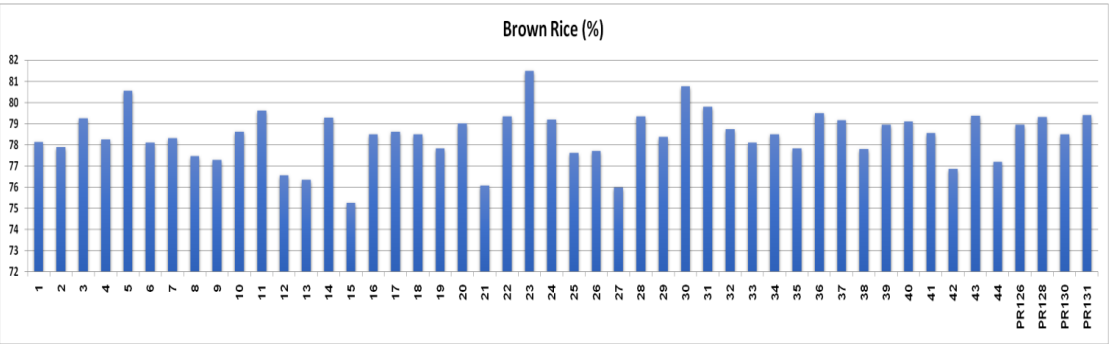


Figure 4.17. Brown rice (%) recovery recorded in the trial conducted at Ludhiana

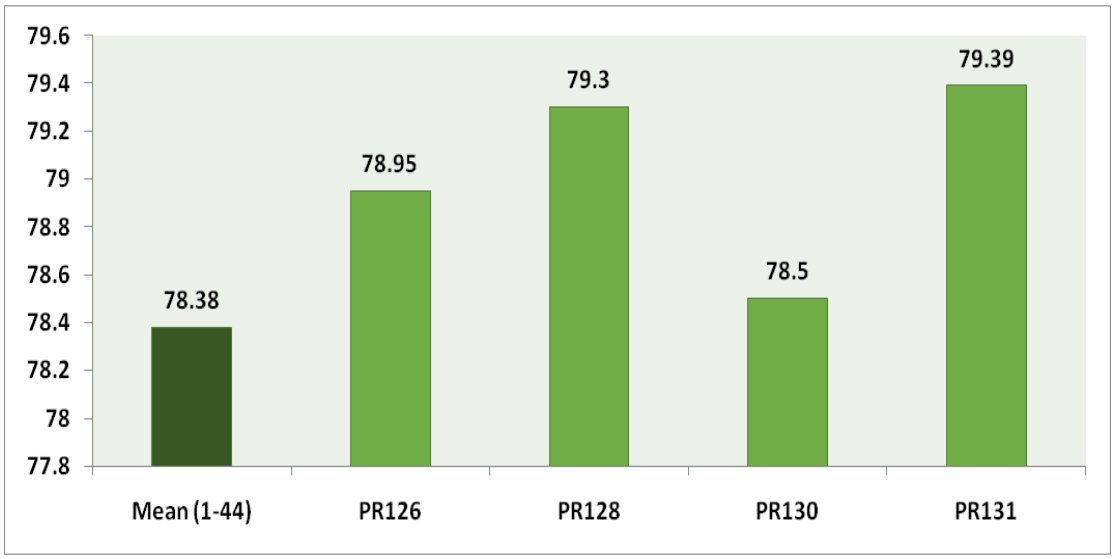


Figure 4.18. Mean of Genotypes 1 to 44 and checks for brown rice percentage

The milling percentage recorded for the entries is depicted in Figure 4.19 and 4.20. The milling percentage was similar across checks and other entries and ranged from 66 to 69 percent.

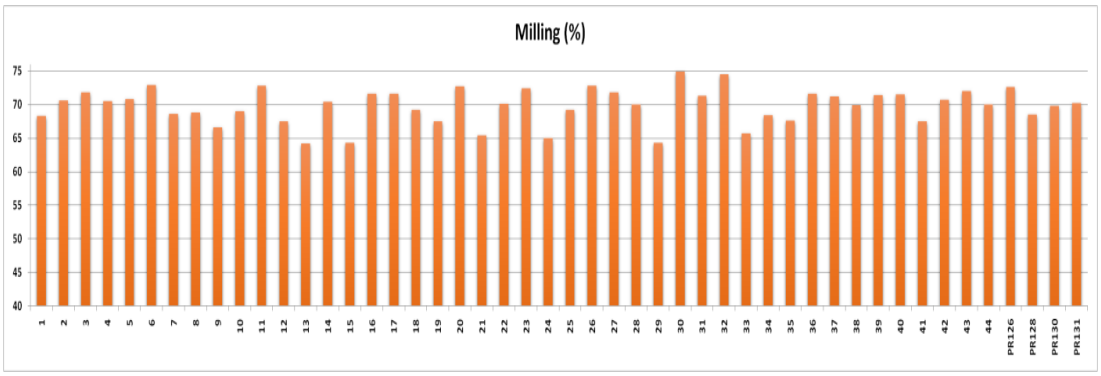


Figure 4.19. Milling recovery (%) recorded in the trial conducted at Ludhiana

Figure 4.20. Mean of Genotypes 1 to 44 and checks for milling percentage

The head rice recovery showed greater variation. High head rice recovery was observed for PR128, PR30, PR131 and RYT 3872 while PR126 showed medium head rice recovery. Among the test entries, Entry 15, 20, 26 and 30 had high head rice recovery. Head rice recovery percentage information is depicted in Figures 4.21 and 4.22

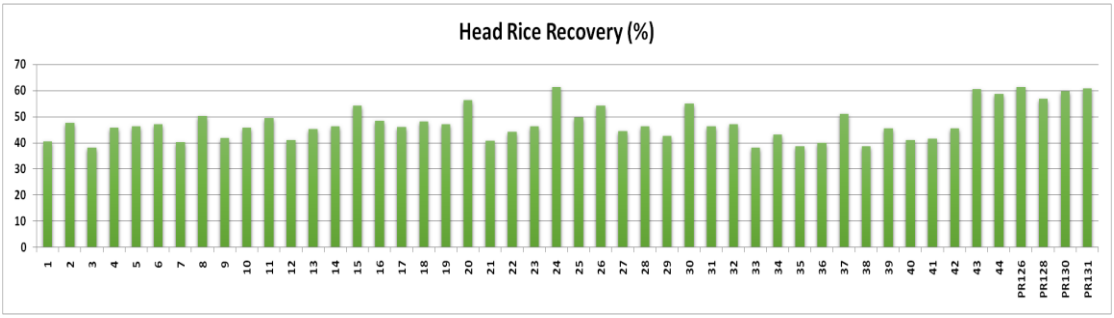


Figure 4.21: Head rice recovery (%) recorded in the trial conducted at Ludhiana

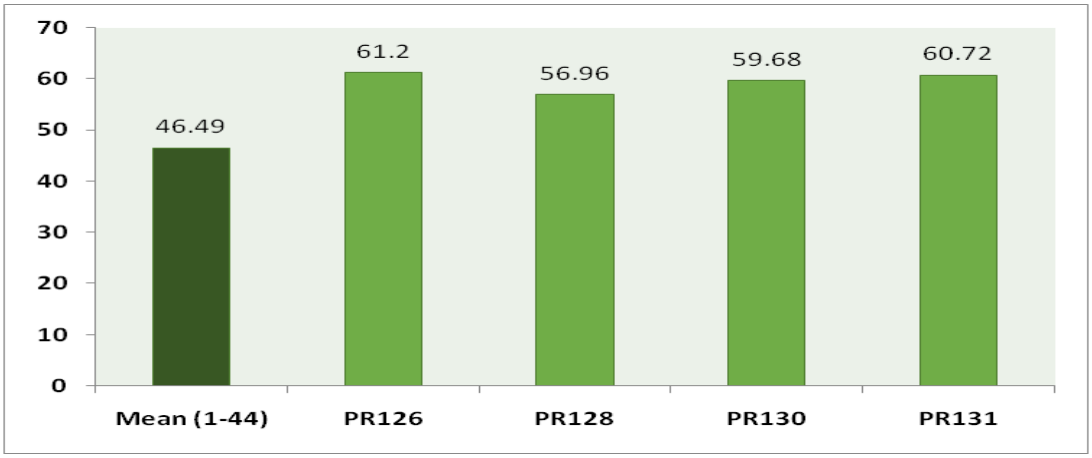


Figure 4.22. Mean of Genotypes 1 to 44 and checks for Head Rice Recovery

Among grain quality traits, chalkiness and consequently head rice yield have been considered the two that are most significantly affected by environment (Zhao and Fitzgerald 2013). DSR represents a change in the conventional rice field environment affecting physical as well as physiological parameters. Chen *et al* (2020) compared the physical quality of direct seeded rice and hand transplanted rice and reported lower milled rice and head rice recovery in direct seeded as compared to transplanted rice. Several studies corroborate this trend. The quality related issues for DSR might also get accentuated by the nature of donors used in the breeding of DSR varieties. Thus it becomes very important to complement field trial of the test entries with evaluation of quality parameters. The milling response of entries shortlisted on basis of yield is given in Table 4.14.

Milling and head rice recovery constraints are evident from Table 4.14. The need for greater focus on grain quality attributes in ongoing and future DSR breeding work is indicated. The modified management practices can also prove useful in this regard.

Table 4. 14. The milling response of the high yielding test genotypes

Yield rank	Genotype	Yield (kg/ha)	1000-Grain weight (gms)	Brown rice %	Milling %	Head Rice Recovery %
1	15	4808	21.08	75.24	60.3	50.17
2	21	4762	20.90	76.06	61.44	36.72
3	PR 128	4596	23.43	79.34	68.65	56.96
4	10	4492	22.98	78.62	65.05	41.67
5	PR 126	4412	20.75	78.95	72.56	61.2
6	11	4408	22.63	79.62	68.77	45.41
7	7	4331	22.50	78.3	64.58	36.21
8	2	4242	23.33	77.87	66.65	43.51
9	RYT 3872	4242	21.05	78.95	68.56	53.56
10	6	4204	23.13	78.1	72.88	46.87
11	DRR Dhan 44	4008	18.55	78.95	67.37	41.4
12	31	3842	22.50	79.78	67.28	42.29
13	29	3677	25.53	78.38	60.26	23.39
14	35	3604	22.43	77.82	63.59	34.71
15	25	3573	19.98	77.60	69.20	49.8

4.3. Identification of genes with differential expression from candidates associated with mesocotyl elongation under deep sowing

In our previous genome wide association mapping (Menard *et al* 2021), we found that the main QTL for mesocotyl and coleoptile length, percentage seedling emergence and shoot biomass in the 684 accessions selected from 3K-RGP (rice genome project) co-located on 4.84 Mb region on the short arm of chromosome 7. The candidate genes associated with seedling vigour in the earlier identified 4.84 Mb genomic regions in the 3K-RG subset were identified.

The sequencing data obtained was filtered and low-quality bases were trimmed and removed. Data statistics obtained after filtering is in terms of number of reads, total bases and size of reads in GB as shown in table 4.15.

Table 4.15: Filtered and trimmed reads of all the samples after sequencing

Sample name	No. of Reads	Total Bases	Data in GB	Sample name	No. of Reads	Total Bases	Data in GB
Rice-10.R1	14558965	4396807430	4.40	Rice-15.R2	15906621	4803799542	4.80
Rice-10.R2	14511865	4382583230	4.38	Rice-15.R3	15828646	4780251092	4.78
Rice-10.R3	14606067	4411032234	4.41	Rice-16.R1	16051083	4847427066	4.85
Rice-11.R1	16352468	4938445336	4.94	Rice-16.R2	15938101	4813306502	4.81
Rice-11.R2	16212400	4896144800	4.90	Rice-16.R3	16164067	4881548234	4.88
Rice-11.R3	16492537	4980746174	4.98	Rice-17.R1	15681771	4735894842	4.74
Rice-12.R1	14549218	4393863836	4.39	Rice-17.R2	15501848	4681558096	4.68
Rice-12.R2	14480018	4372965436	4.37	Rice-17.R3	15861695	4790231890	4.79
Rice-12.R3	14618418	4414762236	4.41	Rice-18.R1	15896748	4800817896	4.80
Rice-13.R1	14191032	4285691664	4.29	Rice-18.R2	15916741	4806855782	4.81
Rice-13.R2	14852853	4485561606	4.49	Rice-18.R3	15936736	4812894272	4.81
Rice-13.R3	15629211	4720021722	4.72	Rice-1.R1	15295126	4619128052	4.62
Rice-14.R1	14264276	4307811352	4.31	Rice-1.R2	15305011	4622113322	4.62
Rice-14.R2	14264276	4307811352	4.31	Rice-1.R3	15285243	4616143386	4.62
Rice-14.R3	14264276	4307811352	4.31	Rice-30.R1	16888342	5100279284	5.10
Rice-15.R1	16017633	4837325166	4.84	Rice-30.R2	16912311	5107517922	5.11
Rice-30.R3	16864374	5093040948	5.09	Rice-31.R1	17680898	5339631196	5.34
Rice-20.R1	13916990	4202930980	4.20	Rice-31.R3	17475884	5.28E+09	5.28
Rice-20.R2	14016905	4233105310	4.23	Rice-32.R1	16262510	4.91E+09	4.91
Rice-20.R3	14116822	4263280244	4.26	Rice-32.R2	16162113	4.88E+09	4.88
Rice-21.R1	16885083	5099295066	5.10	Rice-32.R3	16362909	4.94E+09	4.94
Rice-21.R2	16800000	5073600000	5.07	Rice-33.R1	15030330	4.54E+09	4.54
Rice-21.R3	16760168	5061570736	5.06	Rice-33.R2	14911129	4.5E+09	4.50
Rice-22.R1	16198004	4891797208	4.89	Rice-33.R3	15149533	4.58E+09	4.58
Rice-22.R2	16210110	4895453220	4.90	Rice-35.R1	16151496	4.88E+09	4.88
Rice-22.R3	16221898	4899013196	4.90	Rice-35.R2	16046172	4.85E+09	4.85
Rice-23.R1	15665515	4730985530	4.73	Rice-35.R3	16256821	4.91E+09	4.91
Rice-23.R2	15677411	4734578122	4.73	Rice-37.R1	13292016	4.01E+09	4.01
Rice-23.R3	15689309	4738171318	4.74	Rice-37.R2	13310016	4.02E+09	4.02
Rice-24.R1	16523120	4989982240	4.99	Rice-37.R3	13380000	4.04E+09	4.04
Rice-24.R2	16720000	5049440000	5.05	Rice-3.R1	17573295	5.31E+09	5.31
Rice-24.R3	16926242	5111725084	5.11	Rice-3.R2	17473261	5.28E+09	5.28

Rice-25.R1	17103770	5165338540	5.17	Rice-3.R3	17673331	5.34E+09	5.34
Rice-25.R2	17002130	5134643260	5.13	Rice-4.R1	13315606	4.02E+09	4.02
Rice-25.R3	17205410	5196033820	5.20	Rice-4.R2	13325271	4.02E+09	4.02
Rice-26.R1	16389454	4949615108	4.95	Rice-4.R3	13261234	4E+09	4.00
Rice-26.R2	16099494	4862047188	4.86	Rice-5.R1	13321528	4.02E+09	4.02
Rice-26.R3	16679415	5037183330	5.04	Rice-5.R2	13251528	4E+09	4.00
Rice-27.R1	15647070	4725415140	4.73	Rice-5.R3	13380018	4.04E+09	4.04
Rice-27.R2	15541870	4693644740	4.69	Rice-6.R1	13376900	4.04E+09	4.04
Rice-27.R3	15752270	4757185540	4.76	Rice-6.R2	13256271	4E+09	4.00
Rice-28.R1	15500772	4681233144	4.68	Rice-6.R3	13497529	4.08E+09	4.08
Rice-28.R2	15631876	4720826552	4.72	Rice-7_R1	14710404	4.44E+09	4.44
Rice-28.R3	15369669	4641640038	4.64	Rice-7_R2	14601400	4.41E+09	4.41
Rice-29.R1	17566794	5305171788	5.31	Rice-7_R3	14710405	4.44E+09	4.44
Rice-29.R2	17186714	5190387628	5.19	Rice-8.R1	15220252	4.6E+09	4.60
Rice-29.R3	17946874	5419955948	5.42	Rice-8.R2	15020221	4.54E+09	4.54
Rice-2.R1	13427930	4055234860	4.06	Rice-8.R3	15420284	4.66E+09	4.66
Rice-2.R2	13327833	4025005566	4.03	Rice-9.R1	15208548	4.59E+09	4.59
Rice-2.R3	13227737	3994776574	3.99	Rice-9.R2	15251241	4.61E+09	4.61
Rice-41.R1	13252924	4002383048	4.00	Rice-9.R3	15165856	4.58E+09	4.58
Rice-41.R2	13311100	4019952200	4.02	Rice-41.R3	13396120	4.05E+09	4.05

Rice-1, Rice-3, Rice-5 (PR126, Stage1, 4cm); Rice-7, Rice-9, Rice-10 (IR5797, Stage1, 4cm); Rice-2, Rice-6, Rice-41 (PR126, Stage1, 10cm); Rice-4, Rice-8, Rice-37 (IR5796, Stage1, 10cm); Rice-15, Rice-18, Rice-21, Rice-22 (PR126, Stage2, 4cm); Rice-12, Rice-14, Rice-20 (IR5797, Stage2, 4cm); Rice-16, Rice-23 (PR126, Stage2, 10cm); Rice-11, Rice-13, Rice-17 (IR5797, Stage2, 10cm); Rice-26, Rice-27, Rice-30 (PR126, Stage3, 4cm); Rice-29, Rice-31, Rice-35 (IR5797, Stage3, 4cm); Rice-32, Rice-33 (PR126, Stage3, 10cm); Rice-24, Rice-25, Rice-28 (IR5797, Stage3, 10cm); R1, R2, R3 represents technical replicates

Filtered reads were then aligned on to the reference genome and samples having alignment rate more than 80% were used for further analysis. Data for alignment of reads were obtained in form of number and percentage of uniquely aligned reads, reads aligned multiple times and reads that were not aligned onto the reference genome as shown in table 4.16. Considering all the reads, 7133 features (12.74% of reads) were not aligned in any of the sample. Features with low counts provides very little evidence for differential expression so they were filtered out to improve further analysis.

Table 4.16. Data of mapping of reads on reference genome after filtering

Input Reads		Aligned Reads					
Sample	Total Records	Feature	No Feature	Ambiguous	Alignment not Unique	Low Alignment Quality	Not aligned
Sort_15.3	17,934,809	4,070,458 / 22.70%	4,938,747 / 27.54%	22,489 / 0.13%	0	7,509,440 / 41.87%	1,393,937 / 7.77%
Sort_5.3	13,580,656	384,059 / 2.83%	1,087,536 / 8.01%	2,057 / 0.02%	0	11,216,366 / 82.59%	890,668 / 6.56%
Sort_11.1	17,137,195	1,180,852 / 6.89%	1,767,683 / 10.31%	5,421 / 0.03%	0	13,269,522 / 77.43%	913,755 / 5.33%
Sort_9.1	15,993,684	1,568,265 / 9.81%	2,450,666 / 15.32%	7,274 / 0.05%	0	10,659,959 / 66.65%	1,307,592 / 8.18%
Sort_11.2	16,989,377	1,178,355 / 6.94%	1,767,361 / 10.40%	5,266 / 0.03%	0	13,144,404 / 77.37%	894,035 / 5.26%
Sort_1.2	15,764,921	815,125 / 5.17%	1,479,526 / 9.38%	4,655 / 0.03%	0	11,379,538 / 72.18%	2,086,109 / 13.23%
Sort_11.3	17,276,105	1,203,498 / 6.97%	1,808,889 / 10.47%	5,458 / 0.03%	0	13,359,550 / 77.33%	898,742 / 5.20%
Sort_1.3	15,741,340	825,348 / 5.24%	1,494,580 / 9.49%	4,475 / 0.03%	0	11,332,336 / 71.99%	2,084,625 / 13.24%
Sort_30.1	19,149,492	3,543,758 / 18.51%	4,360,833 / 22.77%	16,156 / 0.08%	0	9,368,687 / 48.92%	1,860,186 / 9.71%
Sort_5.1	13,592,994	387,302 / 2.85%	1,090,022 / 8.02%	2,054 / 0.02%	0	11,219,620 / 82.54%	894,017 / 6.58%
Sort_8.3	17,686,986	3,176,241 / 17.96%	3,743,603 / 21.17%	14,475 / 0.08%	0	10,003,694 / 56.56%	749,092 / 4.24%
Sort_4.3	0	0	0	0	0	0	0
Sort_35.3	20,015,620	5,529,811 / 27.63%	6,220,370 / 31.08%	26,618 / 0.13%	0	6,951,058 / 34.73%	1,287,920 / 6.43%
Sort_16.1	20,302,051	6,499,949 / 32.02%	7,054,162 / 34.75%	35,515 / 0.17%	0	5,961,446 / 29.36%	751,201 / 3.70%
Sort_16.2	20,125,781	6,442,333 / 32.01%	6,990,183 / 34.73%	34,261 / 0.17%	0	5,913,472 / 29.38%	745,733 / 3.71%
Sort_8.1	17,503,416	3,167,354 / 18.10%	3,700,753 / 21.14%	14,576 / 0.08%	0	9,871,968 / 56.40%	748,912 / 4.28%
Sort_16.3	20,364,477	6,525,027 / 32.04%	7,091,791 / 34.82%	35,106 / 0.17%	0	5,952,966 / 29.23%	759,845 / 3.73%
Sort_8.2	17,250,081	3,103,553 / 17.99%	3,653,583 / 21.18%	14,128 / 0.08%	0	9,745,741 / 56.50%	733,218 / 4.25%
Sort_31.3	18,303,213	1,289,162 / 7.04%	2,309,081 / 12.62%	5,423 / 0.03%	0	12,286,185 / 67.13%	2,413,416 / 13.19%
Sort_30.3	19,087,819	3,555,414 / 18.63%	4,378,334 / 22.94%	16,006 / 0.08%	0	9,265,746 / 48.54%	1,872,429 / 9.81%
Sort_1.1	15,755,390	804,966 / 5.11%	1,460,380 / 9.27%	4,531 / 0.03%	0	11,399,929 / 72.36%	2,085,613 / 13.24%
Sort_30.2	19,147,208	3,558,176 / 18.58%	4,387,774 / 22.92%	16,284 / 0.09%	0	9,305,089 / 48.60%	1,879,975 / 9.82%
Sort_26.1	19,787,502	5,909,768 / 29.87%	6,673,049 / 33.72%	27,413 / 0.14%	0	6,153,524 / 31.10%	1,023,927 / 5.17%
Sort_26.2	19,395,089	5,801,597 / 29.91%	6,567,164 / 33.86%	26,769 / 0.14%	0	5,994,473 / 30.91%	1,005,253 / 5.18%
Sort_22.2	17,954,896	2,551,611 / 14.21%	3,382,175 / 18.84%	10,997 / 0.06%	0	11,417,611 / 63.59%	592,575 / 3.30%
Sort_26.3	20,051,636	6,009,204 / 29.97%	6,800,873 / 33.92%	28,156 / 0.14%	0	6,174,078 / 30.79%	1,039,490 / 5.18%

Sort_22.3	17,960,892	2,553,786 / 14.22%	3,375,863 / 18.80%	10,854 / 0.06%	0	11,428,638 / 63.63%	591,840 / 3.30%
Sort_22.1	17,979,455	2,565,052 / 14.27%	3,379,258 / 18.80%	10,839 / 0.06%	0	11,437,619 / 63.61%	586,764 / 3.26%
Sort_41.2	14,085,027	1,245,377 / 8.84%	2,212,473 / 15.71%	5,664 / 0.04%	0	8,195,876 / 58.19%	2,425,658 / 17.22%
Sort_41.1	14,026,479	1,237,728 / 8.82%	2,200,567 / 15.69%	5,666 / 0.04%	0	8,160,111 / 58.18%	2,422,430 / 17.27%
Sort_41.3	0	0	0	0	0	0	0
Sort_18.3	18,340,838	3,799,784 / 20.72%	4,777,190 / 26.05%	20,683 / 0.11%	0	9,534,959 / 51.99%	208,346 / 1.14%
Sort_18.2	18,326,515	3,789,630 / 20.68%	4,770,431 / 26.03%	20,932 / 0.11%	0	9,539,640 / 52.05%	206,021 / 1.12%
Sort_14.2	19,557,991	7,516,625 / 38.43%	8,191,106 / 41.88%	37,884 / 0.19%	0	3,673,886 / 18.78%	138,770 / 0.71%
Sort_37.2	14,367,056	1,751,352 / 12.19%	2,361,363 / 16.44%	9,098 / 0.06%	0	9,507,166 / 66.17%	738,155 / 5.14%
Sort_37.1	14,345,978	1,744,700 / 12.16%	2,338,101 / 16.30%	9,184 / 0.06%	0	9,520,172 / 66.36%	733,896 / 5.12%
Sort_6.1	15,209,644	2,659,954 / 17.49%	3,380,203 / 22.22%	13,873 / 0.09%	0	8,168,130 / 53.70%	987,586 / 6.49%
Sort_6.2	15,061,362	2,630,710 / 17.47%	3,342,337 / 22.19%	14,151 / 0.09%	0	8,096,543 / 53.76%	977,702 / 6.49%
Sort_14.3	19,546,725	7,516,018 / 38.45%	8,193,249 / 41.92%	36,994 / 0.19%	0	3,662,584 / 18.74%	138,180 / 0.71%
Sort_6.3	15,284,257	2,684,368 / 17.56%	3,418,588 / 22.37%	14,010 / 0.09%	0	8,167,472 / 53.44%	999,898 / 6.54%
Sort_18.1	18,318,820	3,791,485 / 20.70%	4,759,903 / 25.98%	20,965 / 0.11%	0	9,541,327 / 52.08%	205,274 / 1.12%
Sort_37.3	14,916,942	2,478,223 / 16.61%	3,194,357 / 21.41%	13,315 / 0.09%	0	8,772,713 / 58.81%	458,406 / 3.07%
Sort_10.2	16,383,201	3,616,928 / 22.08%	4,335,378 / 26.46%	18,012 / 0.11%	0	7,787,029 / 47.53%	626,009 / 3.82%
Sort_33.2	17,495,427	3,985,288 / 22.78%	4,581,156 / 26.18%	17,870 / 0.10%	0	8,349,454 / 47.72%	561,794 / 3.21%
Sort_33.1	17,685,850	4,034,123 / 22.81%	4,628,142 / 26.17%	18,454 / 0.10%	0	8,443,305 / 47.74%	561,969 / 3.18%
Sort_2.1	15,994,439	4,285,088 / 26.79%	4,963,559 / 31.03%	22,260 / 0.14%	0	6,455,432 / 40.36%	268,267 / 1.68%
Sort_2.2	15,848,333	4,262,031 / 26.89%	4,942,070 / 31.18%	22,023 / 0.14%	0	6,355,247 / 40.10%	267,123 / 1.69%
Sort_10.3	16,476,788	3,636,016 / 22.07%	4,350,518 / 26.40%	17,993 / 0.11%	0	7,845,462 / 47.62%	626,905 / 3.80%
Sort_2.3	15,714,713	4,234,399 / 26.95%	4,912,615 / 31.26%	21,633 / 0.14%	0	6,282,511 / 39.98%	263,744 / 1.68%
Sort_14.1	19,591,253	7,515,822 / 38.36%	8,198,690 / 41.85%	38,362 / 0.20%	0	3,699,182 / 18.88%	139,505 / 0.71%
Sort_33.3	17,763,821	4,049,378 / 22.80%	4,642,334 / 26.13%	18,718 / 0.11%	0	8,482,826 / 47.75%	570,703 / 3.21%
Sort_29.3	20,388,994	3,438,240 / 16.86%	4,396,847 / 21.56%	14,432 / 0.07%	0	10,221,266 / 50.13%	2,318,288 / 11.37%
Sort_10.1	16,482,986	3,636,225 / 22.06%	4,348,052 / 26.38%	18,183 / 0.11%	0	7,847,380 / 47.61%	633,302 / 3.84%
Sort_25.3	18,404,946	1,665,409 / 9.05%	2,945,963 / 16.01%	7,578 / 0.04%	0	12,036,606 / 65.40%	1,749,447 / 9.51%
Sort_5.2	13,520,409	384,483 / 2.84%	1,088,557 / 8.05%	2,042 / 0.02%	0	11,156,246 / 82.51%	889,102 / 6.58%
Sort_15.1	18,193,697	4,090,419 / 22.48%	4,963,624 / 27.28%	23,247 / 0.13%	0	7,677,344 / 42.20%	1,439,375 / 7.91%
Sort_15.2	18,054,242	4,069,765 / 22.54%	4,947,562 / 27.40%	23,246 / 0.13%	0	7,593,680 / 42.06%	1,420,268 / 7.87%

Sort_29.2	19,539,844	3,289,468 / 16.83%	4,210,519 / 21.55%	13,608 / 0.07%	0	9,803,468 / 50.17%	2,222,866 / 11.38%
Sort_29.1	19,989,324	3,366,169 / 16.84%	4,293,683 / 21.48%	13,978 / 0.07%	0	10,051,453 / 50.28%	2,264,136 / 11.33%
Sort_25.2	18,137,111	1,629,006 / 8.98%	2,717,559 / 14.98%	7,374 / 0.04%	0	12,096,125 / 66.69%	1,687,094 / 9.30%
Sort_21.2	18,839,208	3,324,009 / 17.64%	4,373,686 / 23.22%	18,169 / 0.10%	0	8,737,059 / 46.38%	2,386,397 / 12.67%
Sort_24.1	20,495,117	6,162,832 / 30.07%	6,870,310 / 33.52%	29,004 / 0.14%	0	6,491,195 / 31.67%	941,942 / 4.60%
Sort_21.3	18,750,611	3,314,004 / 17.67%	4,361,161 / 23.26%	17,995 / 0.10%	0	8,698,133 / 46.39%	2,359,421 / 12.58%
Sort_24.2	20,686,590	6,205,627 / 30.00%	6,935,805 / 33.53%	29,090 / 0.14%	0	6,559,198 / 31.71%	957,041 / 4.63%
Sort_20.1	14,868,491	1,339,800 / 9.01%	2,045,976 / 13.76%	5,366 / 0.04%	0	10,648,695 / 71.62%	828,746 / 5.57%
Sort_20.2	14,974,888	1,349,866 / 9.01%	2,063,655 / 13.78%	5,692 / 0.04%	0	10,720,427 / 71.59%	835,325 / 5.58%
Sort_25.1	18,246,370	1,640,057 / 8.99%	2,728,408 / 14.95%	7,126 / 0.04%	0	12,169,319 / 66.69%	1,701,510 / 9.33%
Sort_20.3	15,064,797	1,354,422 / 8.99%	2,075,940 / 13.78%	5,556 / 0.04%	0	10,786,160 / 71.60%	842,817 / 5.59%
Sort_28.1	18,547,589	4,880,260 / 26.31%	5,572,023 / 30.04%	22,345 / 0.12%	0	5,865,971 / 31.63%	2,207,142 / 11.90%
Sort_17.3	21,386,107	8,488,196 / 39.69%	9,049,079 / 42.31%	39,581 / 0.19%	0	3,685,230 / 17.23%	124,260 / 0.58%
Sort_21.1	18,948,512	3,345,824 / 17.66%	4,396,271 / 23.20%	18,733 / 0.10%	0	8,784,175 / 46.36%	2,403,622 / 12.69%
Sort_7.1	16,618,173	3,052,503 / 18.37%	3,796,724 / 22.85%	14,904 / 0.09%	0	9,169,439 / 55.18%	584,754 / 3.52%
Sort_13.3	15,750,356	3,748,885 / 23.80%	4,368,356 / 27.73%	14,452 / 0.09%	0	7,219,979 / 45.84%	398,820 / 2.53%
Sort_7.2	16,475,351	3,037,826 / 18.44%	3,787,849 / 22.99%	15,174 / 0.09%	0	9,052,653 / 54.95%	581,977 / 3.53%
Sort_17.1	21,200,417	8,419,508 / 39.71%	8,982,220 / 42.37%	39,679 / 0.19%	0	3,640,090 / 17.17%	119,141 / 0.56%
Sort_7.3	16,581,305	3,049,146 / 18.39%	3,801,724 / 22.93%	14,904 / 0.09%	0	9,127,711 / 55.05%	587,967 / 3.55%
Sort_17.2	20,942,622	8,310,770 / 39.68%	8,873,276 / 42.37%	39,192 / 0.19%	0	3,600,736 / 17.19%	118,902 / 0.57%
Sort_32.3	16,805,208	750,520 / 4.47%	1,639,370 / 9.76%	3,325 / 0.02%	0	13,843,710 / 82.38%	568,336 / 3.38%
Sort_3.1	18,664,275	1,711,218 / 9.17%	2,624,427 / 14.06%	10,614 / 0.06%	0	12,382,977 / 66.35%	1,935,126 / 10.37%
Sort_32.2	16,618,310	740,018 / 4.45%	1,620,552 / 9.75%	3,255 / 0.02%	0	13,692,603 / 82.39%	561,931 / 3.38%
Sort_3.2	18,557,288	1,725,891 / 9.30%	2,639,312 / 14.22%	10,598 / 0.06%	0	12,247,224 / 66.00%	1,934,327 / 10.42%
Sort_13.1	15,633,750	3,723,720 / 23.82%	4,336,526 / 27.74%	14,327 / 0.09%	0	7,165,167 / 45.83%	394,114 / 2.52%
Sort_3.3	18,766,377	1,759,817 / 9.38%	2,691,669 / 14.34%	10,763 / 0.06%	0	12,343,310 / 65.77%	1,960,893 / 10.45%
Sort_13.2	17,434,892	4,153,148 / 23.82%	4,841,194 / 27.77%	16,370 / 0.09%	0	7,978,772 / 45.76%	445,561 / 2.56%
Sort_28.3	18,336,110	4,827,252 / 26.33%	5,519,419 / 30.10%	22,099 / 0.12%	0	5,776,024 / 31.50%	2,191,475 / 11.95%
Sort_32.1	16,726,805	735,891 / 4.40%	1,618,472 / 9.68%	3,214 / 0.02%	0	13,813,476 / 82.58%	555,793 / 3.32%
Sort_35.2	19,794,069	5,460,954 / 27.59%	6,149,396 / 31.07%	26,670 / 0.13%	0	6,889,706 / 34.81%	1,267,526 / 6.40%

Sort_28.2	18,688,014	4,919,934 / 26.33%	5,623,411 / 30.09%	22,155 / 0.12%	0	5,894,890 / 31.54%	2,227,794 / 11.92%
Sort_12.1	16,134,902	2,834,381 / 17.57%	3,527,027 / 21.86%	12,862 / 0.08%	0	8,011,734 / 49.65%	1,749,042 / 10.84%
Sort_24.3	20,887,619	6,257,494 / 29.96%	6,996,690 / 33.50%	29,329 / 0.14%	0	6,634,564 / 31.76%	969,731 / 4.64%
Sort_4.1	15,708,871	4,078,104 / 25.96%	4,603,169 / 29.30%	25,096 / 0.16%	0	6,400,042 / 40.74%	602,591 / 3.84%
Sort_12.2	16,046,850	2,826,430 / 17.61%	3,521,651 / 21.95%	12,890 / 0.08%	0	7,959,742 / 49.60%	1,726,267 / 10.76%
Sort_12.3	16,187,449	2,852,677 / 17.62%	3,557,214 / 21.98%	12,835 / 0.08%	0	8,040,676 / 49.67%	1,724,174 / 10.65%
Sort_35.1	19,947,202	5,500,643 / 27.58%	6,186,445 / 31.01%	27,392 / 0.14%	0	6,959,278 / 34.89%	1,273,639 / 6.39%
Sort_31.2	18,434,701	1,303,064 / 7.07%	2,327,696 / 12.63%	5,520 / 0.03%	0	12,372,898 / 67.12%	2,425,585 / 13.16%
Sort_4.2	15,664,971	4,083,091 / 26.07%	4,637,421 / 29.60%	25,213 / 0.16%	0	6,324,354 / 40.37%	595,020 / 3.80%
Sort_27.1	17,849,260	3,694,994 / 20.70%	4,245,364 / 23.78%	17,786 / 0.10%	0	8,200,013 / 45.94%	1,691,424 / 9.48%
Sort_31.1	18,553,232	1,310,226 / 7.06%	2,337,719 / 12.60%	5,561 / 0.03%	0	12,464,745 / 67.18%	2,435,024 / 13.12%
Sort_27.3	17,975,828	3,740,807 / 20.81%	4,291,053 / 23.87%	17,841 / 0.10%	0	8,240,269 / 45.84%	1,686,126 / 9.38%
Sort_23.1	15,739,469	3,242,977 / 20.60%	3,898,989 / 24.77%	16,224 / 0.10%	0	7,634,100 / 48.50%	947,302 / 6.02%
Sort_27.2	17,709,735	3,688,132 / 20.83%	4,233,274 / 23.90%	17,830 / 0.10%	0	8,097,464 / 45.72%	1,673,301 / 9.45%
Sort_23.2	17,931,850	3,724,402 / 20.77%	4,476,669 / 24.96%	18,905 / 0.11%	0	8,634,375 / 48.15%	1,077,659 / 6.01%
Sort_23.3	15,742,186	3,243,011 / 20.60%	3,893,356 / 24.73%	16,486 / 0.10%	0	7,642,639 / 48.55%	946,817 / 6.01%
Sort_9.3	15,915,800	1,578,324 / 9.92%	2,468,127 / 15.51%	7,194 / 0.05%	0	10,552,299 / 66.30%	1,309,919 / 8.23%
Sort_9.2	16,030,556	1,584,386 / 9.88%	2,471,772 / 15.42%	7,283 / 0.05%	0	10,649,098 / 66.43%	1,318,079 / 8.22%
Sort_5.2	13,520,409	384,483 / 2.84%	1,088,557 / 8.05%	2,042 / 0.02%	0	11,156,246 / 82.51%	889,102 / 6.58%
Sort_15.1	18,193,697	4,090,419 / 22.48%	4,963,624 / 27.28%	23,247 / 0.13%	0	7,677,344 / 42.20%	1,439,375 / 7.91%
Sort_15.2	18,054,242	4,069,765 / 22.54%	4,947,562 / 27.40%	23,246 / 0.13%	0	7,593,680 / 42.06%	1,420,268 / 7.87%

Mapped reads were further used to evaluate differential genes expression between the samples as shown in volcano plot in figure 4.23. Genes having fold change expression two folds higher or lower than the control and false discovery rate less than 0.5 were used and all other genes were discarded.

Genes having green colour are the optimum whereas genes having grey, black and red colour were all discarded on basis of fold change and false discovery rate.

Further functional annotation of differentially expressed genes was done and genes were found to involved in different biological processes, molecular function, important KEGG and reactome pathways, along with subcellular location at which these genes were found to be located. Graph summarizing the percentage of reads performing a specific function are shown in figure 4.24.

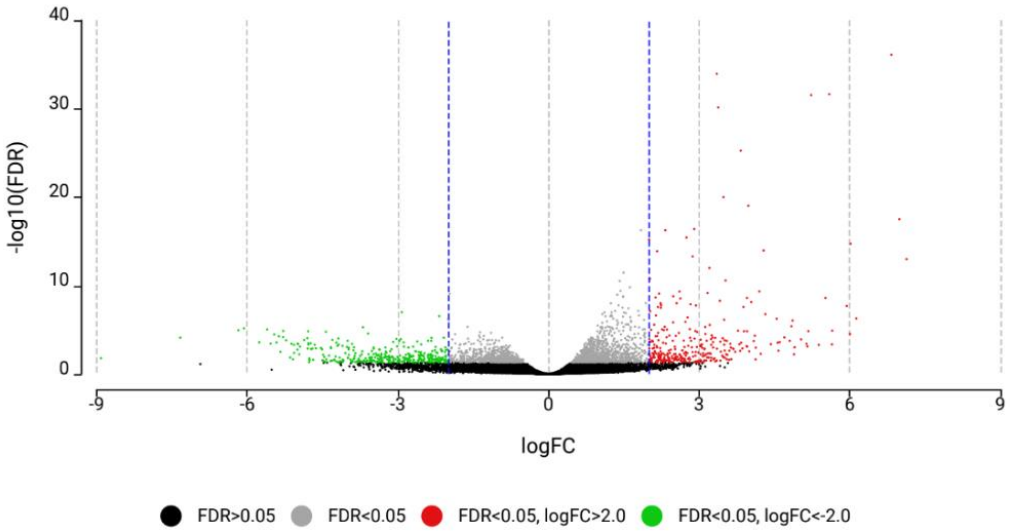


Figure 4.23. Volcano plot showing differentially expressed genes

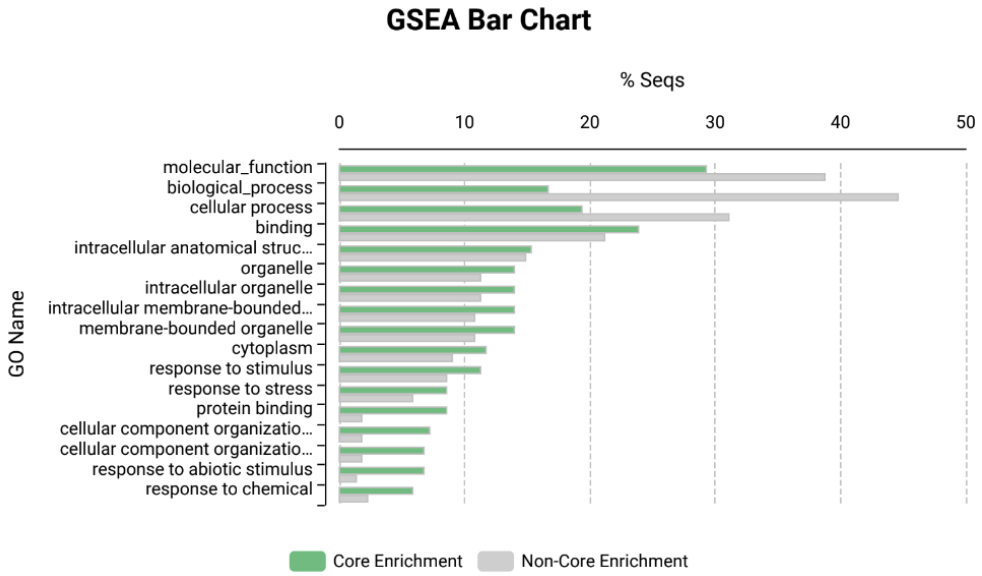


Figure 4.24. Functional annotation of differentially expressed genes

There was a total of 937 genes present in the region mapped on chromosome 7 in previous GWAS studies. After Differential expression analysis, we identified 4 genes that showed differential expression at stage of 5 days after sowing (DAS), 33 genes at stage of 10 days after sowing (DAS), 41 genes at stage of 15 days after sowing (DAS) being upregulated/downregulated in IRGC 128442. Further, we selected 1 differential genes having a log fold change value of 2 and FDR 0.05 (Table 4.17). The table represents gene name, along with the position of gene, their expression, fold change and function identified after annotation. Genes having positive fold change value are upregulated whereas genes with negative fold change value are downregulated.

Table 4.17: List of identified putative genes on chromosome 7

Gene ID	Position (bp)	Expression	Fold change	Annotation
LOC_Os07g15440	8930409	Up	2935.93462	alanyl-tRNA synthetase family protein, expressed
LOC_Os07g17184	10115273	Up	353.30917	expressed protein
LOC_Os07g17689	10440993	Up	339.46754	expressed protein
LOC_Os07g17770	10499765	Up	135.08079	tryptophanyl-tRNA synthetase, cytoplasmic, putative, expressed
LOC_Os07g18944	11222033	Up	815.79848	expressed protein
LOC_Os07g19310	11430564	Down	-359.44752	hypothetical protein
LOC_Os07g19320	11431985	Down	-90.07353	stripe rust resistance protein Yr10, putative, expressed
LOC_Os07g20310	11722281	Up	824.14272	expressed protein
LOC_Os07g24100	13659839	Down	-112.78247	retrotransposon protein, putative, unclassified, expressed
LOC_Os07g25150	14368174	Down	-124.63186	myb-related protein 306, putative, expressed

On the base of the current study, Genotype 15 (IR 129477-3873-297-3-3-2) and 21(IR 129477-709-375-3-5-7) were higher yielding than the check varieties. Genotype 21 (IR 129477-709-375-3-5-7) is early maturing. It possessed good germination under partially anaerobic conditions which is of great significance in Punjab region for good crop establishment and achieving higher productivity. However, it had very low Head Rice Recovery. Genotype 15 (IR 129477-3873-297-3-3-2) is a medium maturing semi dwarf genotype with good tillering ability. It also has good germination under partial anaerobic conditions and relatively better Head Rice Recovery. Milling quality is a major constraint under DSR conditions demanding a greater focus in the future DSR breeding program. However, these genotypes can be further evaluated in multi-location trial. Further functional annotation of differentially expressed genes for mesocotyl elongation under deep sowing conditions revealed different genes involved in various biological processes and molecular functions.

Confirmation and cloning of genes responsible for mesocotyl elongation from this list using RT PCR and gene knockout strategies would ease transfer and tracking of this important DSR adaptive trait.

CHAPTER V

SUMMARY

Direct seeded rice (DSR) is now widely recognized as a water conserving as well as less labour intensive option for rice cultivation. Improving rice varieties for better adaptation to non-puddled, direct-seeded conditions is critical for success of DSR on farmers' fields. The present study contributes to this initiative by evaluating a set of advanced breeding lines including a set generated from a complex cross involving parents possessing specific DSR traits/QTLs. Attempts were also made to compare the transcriptome profile of a genotype with exceptional ability for seedling emergence from depth with that of a common cultivar in order to infer the underlying molecular genetic basis of this trait.

Under Punjab conditions, the problems of anaerobic germination and emergence from depth are encountered if there is incidence of rain within 4-5 days of sowing, causing anaerobic environment for germinating seeds/seedlings besides adding to the soil layer atop the seed as soil is likely to flow into the seeded furrow with rain water. An ideal plant type for DSR should have the ability to germinate under anaerobic condition coupled with tolerance of early submergence and good seedling vigor. The set of 48 lines used in the study included dual purpose (DSR and PTR) varieties as checks. The test entries comprised advanced breeding lines representing the test entries that, had been genetically enhanced not only for DSR related traits but also for disease and insect pest resistance. Some of these traits were contributed by tagged QTLs, which has been previously catalogued by using SNP markers and the allelic constitution of these lines is given in the Materials and Methods.

In order to address the problem related to uniform emergence and germination from depth varieties with optimum seedling/shoot length are preferred. The coleoptile (the protective sheath that covers the emerging shoot) and mesocotyl (the structure between the scutellar and coleoptiler nodes) of rice are primarily responsible for the emergence of seedlings from deeper soil layers In the present study the test entries and checks were sown in cups at 3 depths (3 cm, 6 cm & 9 cm). In the sowing done at 3 cm depth, majority of the seedlings had emerged above the soil surface when observed at day 7 and thus pre and post emergence seedling growth got confounded. This depth was thus not suitable for revealing the genotypic differences for emergence potential. Data from 6 cm sowing depth was used for shoot length analysis. As frequent watering of cups was performed to keep soil wet, partial anaerobic conditions were expected, particularly for seed sown at 9 cm depth. In cups with seed sown at 9 cm depth, the proportion of successfully germinated seeds as compared to those with only rudimentary or arrested growth was taken to indicate potential for anaerobic germination.

The coleoptiler node was not prominent in most of the genotypes, and would have

potentially added to errors in separate measurement of mesocotyl and coleoptile. Taken together these constitute the seedling shoot length which is directly responsible for emergence ability. The observed shoot length for different entries ranged from 22.4 mm in Genotype 19 to 40.9 mm in Genotype 17. The checks PR 126 and PR 128 recorded a shoot length of 36.9 mm and 32.5 mm respectively. In case of 38 test entries, information on presence of early uniform emergence QTLs, EMM1.1 and EMM11.1 was known. Presence/absence of early uniform emergence QTLs showed a highly significant Pearson Correlation Coefficient of 0.618 with the observed shoot lengths. It was also evident that the check varieties possessed shoot length comparable to best lines with known QTLs. Transferring the QTLs *EMM 1.1*and *EMM 11.1* to these backgrounds may enhance the shoot length further. Small pot or cup based screening can be an effective screening tool for pre-selecting segregating material to be planted in the field while QTL information available from various studies can greatly facilitate choice of parents and donors, besides being used for marker assisted consolidation of QTLs and traits.

The germination percentage under partial anaerobic conditions showed a wide range from 42.78% (Genotype 11) to 91.67% (Genotype 38) while the mean of entire set was 70.20%. PR 126 with 77.78% gemination was best among the check varieties. With respect to available information on anaerobic germination QTL *AG 9* in this set, it was known to be present in 36 of the 38 lines. Presence of other unknown genetic factors was evident as the *AG 9* positive lines showed significant variation in their germination. Lines lacking *AG 9*, namely Genotypes 11 and 13 however had lowest germination values in the set at 41.67% and 42.78% respectively. The Pearson Correlation Coefficient of *AG 9* presence with germination percentage in the set of 38 lines with known QTL information turned out to be highly positive (r= 0.906) and significant. The observed data pattern also confirmed the presence of anaerobic stress conditions as anticipated.

A major focus of the present study is the field performance of advanced lines specifically developed and selected for DSR conditions. A replicated, multilocation trial of 48 entries (including checks) was conducted and observations recorded on days to 50% flowering, plant height, ear bearing tillers, fertility percentage, 1000 grain weight and grain yield. The experiment was carried out at two locations, Ludhiana and Kapurthala, representing rice growing areas of the state. This was followed by an evaluation of the milling attributes in order to have a perspective of the commercial potential of the material developed.

The analysis of variance for the trials conducted at Ludhiana and Kapurthala along with pooled analysis was carried out for all the characters. Analysis of observations recorded at Ludhiana revealed significant genotypic variation for all traits except for ear bearing tillers. Similar trend with respect to analysis of variance was seen for the trial conducted at Kapurthala with the exception that significant genotypic variation was also not evident for

days to flowering, besides ear bearing tillers. The pooled analysis of variance for both the locations showed highly significant location effects for all traits. Genotypic variation was also highly significant for all traits except ear bearing tillers. The location x genotypic variation was also highly significant for all traits except for ear bearing tillers. As location x genotype differences for all the traits showing genetic variation was significant, it becomes imperative for varietal development for direct seeded conditions to adopt wider multilocation evaluation and aim at combining high productivity with stability of performance.

The days to 50 % flowering ranged from 73.5 to 91 days at Ludhiana and from 81 to 100 at Kapurthala. The days to flowering had higher mean at Kapurthala than Ludhiana. Check variety PR 126 was among early flowering genotypes at both the locations with an average of 81.5 days. Early to medium maturing genotypes are preferred for DSR conditions as these are resource use efficient and also vacate the field timely for the timely sowing of wheat crop. The entire set of lines in the trial conformed to this requirement.

The average plant height Ludhiana (103.99 cm) was more than the average plant height at Kapurthala. The plant height varied from 86.5 to 119.5 cm at Ludhiana while at Kapurthala it ranged from 78.3 cm to 112 cm. The shortest lines at both location were Genotype 18 and Genotype 37 which are similar in height to PR126. Most of the genotypes were in the semi dwarf category.

Ear bearing tillers are an important yield component but the genotypic variation in the present study was non-significant. In the present experiment greater proneness of this trait to environmental influence probably overwhelmed the relatively smaller genotypic differences that may have existed in this set. Within the optimal range of the length and breadth ratio for a particular category of rice, thousand grain weight is an important yield component. Genotypes 2,9,17, and 22 in the present study had high grain weight at both the locations and thus showed promise for this trait under direct seeded conditions. Spikelet fertility is one of the yield components which is highly variable and is affected trait by environmental conditions. The mean fertility was 72.6 % for Ludhiana and 74.2 % at Kapurthala.

The paddy grain yield data observed shows that PR126 as check had higher yield at both Ludhiana and Kapurthala. Among the test entries, Genotype 15 has higher yield (5230kg/ha) than PR126 at Ludhiana. However it showed reduced yield at Kapurthala as compared with Ludhiana. The highest yielding entry at Kapurthala Genotype 7. Other high yielding entries included Genotypes 10,11, 14, 23, 26 and DRR Dhan 44 at Ludhiana and Genotypes 2, 7, 11, 31 at Kapurthala. Average yield was found to be higher for Entry10, 15 and 21 as compared to best check PR126. Entry 10 and Entry 15 also had higher SPAD value which is a measure of leaf chlorophyll content, a basic parameter for photosynthetic rate and biomass accumulation.

Pooled analysis over Ludhiana and Kapurthala shows Entry 15 and 21 to have yield

levels above PR 128 and these two entries along with Entry 10 rank above PR 126 for yield. It is important to note that Entry 10 has a short duration as indicated by average of 80.80 days taken to 50% flowering compared to 81.50 days taken by the early check PR 126, considered to be optimal for DSR conditions. Entry 21 is tallest (110 cm) among these top yielding entries though lodging was not observed in the present trial. Interestingly Entry 15 is able to yield high even at a relatively low spikelet fertility. The ear bearing tillers and grain weight parameters for the top three entries are similar and in an acceptable range. The yield QTL information available for several of the test lines did not show a strong correspondence with yield performance.

Milling traits including brown rice yield, milled rice recovery and head rice recovery were evaluated. It was observed that the check rice varieties have similar brown rice percentage (about 78-80 percent) while test entries vary in their response ranging from 75 to 81 percent. The milling percentage was similar across checks and other entries and ranged from 66 to 69 percent. The head rice recovery showed greater variation. Among the test entries, Entry 15, 20, 26 and 30 had high head rice recovery.

An important aspect of the study dealt with identification of genes with differential expression from candidates associated with mesocotyl elongation under deep sowing. Differential gene expression in a genetic stock, R 146 (IRGC 38606-1) with exceptional mesocotyl elongation vis-a vis check cultivar PR 126 was analysed. Mesocotyl samples from PR126 and IRGC 38606-1 sown at 4 cm and 10 cm sowing depths were used. RNA was isolated from the test and control genotypes at specific stages/time points. Each RNA sample was used to construct the RNA-Seq libraries at 150-bp paired end reads using the Illumina TruSeq RNA library prep kit according to standard protocols (Illumina inc., USA). Sequencing was carried out on HiSEQ 4000 and data was generated.

The sequencing data obtained was filtered and low-quality bases were trimmed and removed. Filtered reads were then aligned on to the reference genome and samples having alignment rate more than 80% were used for further analysis. Mapped reads were further used to evaluate differential genes expression between the samples Genes having two folds higher or lower expression than the control were used for functional annotation. There was a total of 937 genes present in the region mapped on chromosome 7 in previous GWAS studies. After Differential expression analysis, we identified 4 genes that showed differential expression at stage of 5 days after sowing (DAS), 33 genes at stage of 10 days after sowing (DAS), 41 genes at stage of 15 days after sowing (DAS) being upregulated/downregulated in IRGC 38606-1 Further, differential genes having a log fold change value of 2 and FDR 0.05 were identified. Confirmation and cloning of genes responsible for mesocotyl elongation from this list using RT PCR and gene knockout strategies would ease transfer and tracking of this important DSR adaptive trait.

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