agronomy

MDPI

Article

Genetic Evaluation of a Diverse Rice Panel for Direct Seeded Adapted Traits Using Kompetitive Allele Specific Primer Assay

Harpreet Singh [1,†], Jasneet Singh [2,†], Pooja Ankush Ade [2], Om Prakash Raigar [2], Rupinder Kaur [1], Renu Khanna [1], Gurjit Singh Mangat [1] and Nitika Sandhu [2,*]

[1] Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, Punjab, India
[2] School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
* Correspondence: nitikasandhu@pau.edu
† These authors contributed equally to this work.

Abstract: Direct seeded rice (DSR) cultivation is an attractive non-conventional technology for growing rice. It saves labor, water, energy, and takes 5 to 7 days for early crop maturity. The yield advantage in DSR can be obtained by implementing various cultural practices including proper sowing time and seed rate, selection of suitable cultivars with appropriate management of weeds and water. The present study involves the agronomic and molecular screening of advanced breeding lines under direct seeded as well as transplanted conditions, so as to identify DSR adapted genotypes. Significant variations among genotypes have been observed for most of the traits measured in the present study. The yield under DSR was comparable to TPR but the grain quality was not comparable, and poor milling and head rice recovery were observed. Molecular characterization using 106 Kompetitive Allele-Specific PCR assays (KASP) was performed. The best performing genotypes with different allele combinations under DSR were PAU 6456-8-2-1-1-1, PAU 5187-RIL1649-F8, PAU 6456-8-1-1-1-3, PAU 6456-8-2-1-1-2, NVSR 2107, and PAU 6778-12-1-4-1-1. The selected genotypes performed better in terms of traits associated with seedling establishment, root architecture, yield, and yield-related traits. The identified promising breeding lines may serve as novel donors to be further used in a marker-assisted selection program which target improving the grain yield and adaptability under DSR.

Keywords: advanced breeding lines; direct seeded rice; grain yield; grain quality; KASP; root architecture

Citation: Singh, H.; Singh, J.; Ade, P.A.; Raigar, O.P.; Kaur, R.; Khanna, R.; Mangat, G.S.; Sandhu, N. Genetic Evaluation of a Diverse Rice Panel for Direct Seeded Adapted Traits Using Kompetitive Allele Specific Primer Assay. *Agronomy* **2022**, *12*, 2083. https://doi.org/10.3390/agronomy12092083

Academic Editors: Bhagirath S. Chauhan and Gulshan Mahajan

Received: 9 June 2022
Accepted: 5 July 2022
Published: 1 September 2022

1. Introduction

Rice is a major staple cereal crop for more than half of the global population, and is produced in more than 95 countries [1]. India is the second largest producer of rice, which contributes more than 24% of total global rice production [2]. In India, most of the paddy is planted following the traditional transplanted puddled rice system (TPR) of rice cultivation. An enhancement in the productivity of rice under direct seeded rice (DSR) cultivation conditions can be achieved through introgression of multiple attributes for abiotic and biotic stresses along with increasing adaptability to DSR conditions. A shortage of water, labor input, reduction in cultivation area, and fluctuating weather conditions lead to an increase in cost of paddy production and are unsustainable through TPR towards the near future [3]. An ongoing large-scale shift towards DSR necessitates great efforts to improve the efficiencies of a DSR breeding program. The molecular breeding approaches such as quantitative trait loci (QTL) or gene pyramiding and application of multiparents have been identified to be feasible in developing resistant or tolerant breeding lines against various biotic and abiotic stresses [4–6]. Direct seeded rice (DSR) is an alternative to traditional transplanted puddled rice (TPR) that has the potentiality to meet future rice demand through lower water requirement, reduced labor costs, adaptation to climatic

risks, mitigation of greenhouse gas emissions, and the yield in comparison to TPR [7]. The cultivation of direct seeded rice (DSR) gives advantage over the TPR by avoiding the very basic operations such as puddling, transplanting, and maintenance of standing water.

DSR is being practised in Punjab since the last 15 years. As per the Department of Agriculture, Chandigarh, DSR covered 5 lakh hectares in Punjab during 2021 with an estimated increase of 10 lakh acres from the previous year. The area under DSR has observed an increase of 34% from 2010–2019 [8]. However, there is still a tremendous scope to increase the area under DSR in Punjab. The unavailability of rice varieties suitable for direct seeded cultivation conditions demands the development of new DSR varieties with improved grain yield, early uniform germination and vigor for weed competitiveness, and better root architectural attributes that enhance uptake of nutrients [7]. In non-flooded conditions, uptake of water and nutrients were inefficient which resulted in poor root structure development [9–11]. The root architectural traits depend on water and nutrient availability, nutrient uptake, and signaling [9,11,12]. A clear picture about an ideal root architecture required for efficient water-nutrient uptake and utilization under DSR may offer a real possibility of higher grain yield under DSR. Non-uniform germination and poor emergence, seedling death, and very less weed competitiveness are the factors causing yield reduction under DSR. Therefore, it is very important for agricultural scientists to focus on breeding new varieties of direct seeded rice in order to ensure sustainable increase in yield. The cultivation of DSR has not gained more popularity due to the poor crop stand, lower yield, weed problem, less adaptability, reduction in nutrient uptake and utilization (especially of N, P, and Fe), and lodging. The cultivation of direct seeded rice system is generally very sensitive for weed growth that competes for moisture, nutrients, and sunlight, and ultimately causes great yield losses as compression to TPR [13].

In order to improve the rice crop establishment, especially during the early stages, and to minimize the risks associated with direct seeding, there is a need for direct seeded adapted rice varieties with better germination percent and faster and vigorous seedling growth. These traits could help to conserve soil moisture and accelerate uptake of soil water and nutrients through roots. Varietal development for DSR adaptable conditions requires the selection of desired traits, identification, and genomic region introgression linked with particular traits of interest in various genetic backgrounds. For yield stability and adaptability of DSR cultivation provided by different traits, viz., anaerobic germination (the ability of rice seeds to germinate under water), the early and uniform seedling emergence and seedling vigor, root plasticity for efficient nutrient uptake, and the lodging resistance are required.

To date, utilization of these QTL/genes through the marker-assisted breeding programs universally relied on and earlier identified SSR (simple-sequence repeats) marker systems. In a marker-assisted introgression breeding program involving multiple donors, SSRs are not so useful because there is a chance to obtain the same allelic pattern in case of multiple parents. Advances in genome sequencing technology leads to a development of low-cost genome resequencing approaches; now, these provide a great opportunity for the highly accurate single nucleotide polymorphism (SNP) marker systems' development. For rapid genotyping to be used in the targeted MAS (marker-assisted selection) through high-throughput SNP genotyping platforms, Kompetitive Allele-Specific PCR (KASP) assay is suggested. Genotyping through KASP allows a high precision biallelic characterization of SNPs as well as InDels in specific loci, and it is a simple, fast, and economical method.

Considering the importance of a direct seeded rice cultivation system, the present study involves (i) the screening of advanced breeding lines under direct seeded as well as transplanted conditions, so as to identify breeding lines/genotypes suitable for cultivation under DSR and (ii) molecular characterization of breeding lines to identify QTL/genes linked with the improved grain yield and adaptability of rice under DSR.

2. Materials and Methods

This field experiment was conducted at Rice Experimental Area, B Block, Department of Plant Breeding and Genetics, PAU, Ludhiana (30_54′ N, 75_48′ E) for two consecutive years *viz. kharif* 2020 and 2021. The experimental material panel comprised of 27 advanced breeding lines, which were bulked in F_6/F_7 generation from diverse crosses and 13 donors possessing traits providing adaptability under DSR and two control checks (PR126 and PR121), and were evaluated in the randomized complete block design (RCBD) with three replications (Figure 1A).

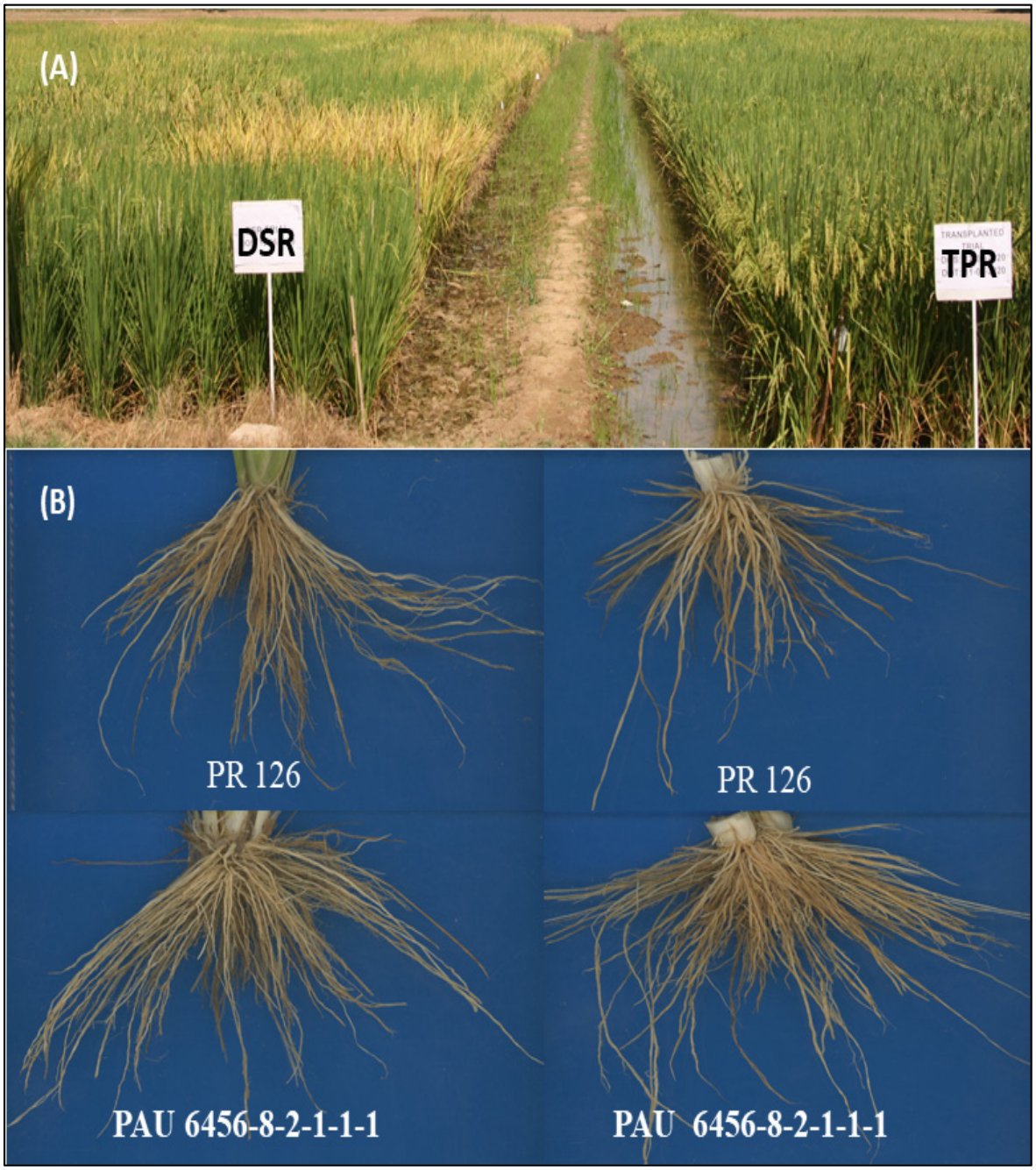

Figure 1. (**A**) The field view of the phenotypic evaluation of the advanced breeding lines under direct seeded cultivation conditions (DSR) and transplanted puddled system of rice cultivation (TPR). (**B**) Root architecture of control check variety (PR126) and one of the selected breeding lines (PAU 6456-8-2-1-1-1) under direct seeded cultivation conditions (DSR, left side) and transplanted Puddled system of rice cultivation (TPR, right side) using WinRhizo PRO root scanner.

The nursery was sown for cultivation under puddled transplanted conditions in the month of May. The thirty-day-old seedlings were transplanted in the field in an RBD design with three replications having plant to plant and row to row spacing of 15 and 20 cm, respectively, in plot size 7.02 m^2 (2020) and 5.4 m^2 (2021). On the same day of sowing of the nursery, seeding was done in the field under DSR condition. The plot size was 5.44 m^2 and 4.64 m^2 under DSR during *kharif* 2020 and 2021. The standard package of practices was followed to raise a healthy crop. The details of the tested genotypes along with their parentage are given in Table 1. The identification of desirable traits and genomic regions linked with the traits that improve grain yield and adaptability of rice under direct-seeded cultivation conditions have been studied in this experiment.

2.1. Irrigation

Irrigation was given prior to sowing under DSR followed by 21 days of sowing. Further irrigation was based on need so that cracks did not appear in the field. Water was continuously standing for two weeks under TPR after transplanting to enable the proper establishment of crop. Subsequently, irrigation was given after two weeks therefore, ponded conditions should be present [14].

2.2. Fertilizer

Fertilizer under DSR was mainly urea (130 $kgacre^{-1}$) in three equal split doses at 4, 6, and 9 weeks of sowing. Phosphorous and potash can be applied on the basis of soil test. Neem coated urea, DAP (diammonium phosphate) and muriate of potash was applied in doses of 90, 27, and 20 $kgacre^{-1}$ under TPR. Nitrogen was applied under 3 equal split doses at 7, 21, and 35 days after transplanting and it should not be applied in standing water [14].

2.3. Weeding

Pre and post herbicides in recommended doses were used for control of pre and post-emergence weeds as per recommended by Punjab Agricultural University, Ludhiana [14].

2.4. Phenotypic Characterization of the Breeding Panel

The genotypes used in the present study were evaluated for morpho-physiological traits such as seedling vigor (according to SES, IRRI Philippines), root traits (root shoot ratio (length and biomass), root length (in cm), average root diameter (mm), root volume (in cm^3), forks, tips, crossing, and root surface area), agronomical traits (days to 50% flowering (in days), plant height (in cm)), grain yield (kg ha^{-1}), yield contributing traits (tiller number ($/m^2$), SPAD value (Soil Plant Analysis Development, nmol cm^{-1}), thousand grain weight (g), spikelet fertility (%), quality parameters such as total rice recovery (%), milled rice recovery (%), and head rice recovery (%)).

A total of 17 parameters were observed under field conditions in all experiments across both seasons. The seedling vigor was scored on a visual basis during seedling stage of the plant on a plot basis (Supplementary Materials Table S1). Destructive sampling was done at the stage of 60 days after sowing (DAS) using six plants per plot to evaluate early root architectural and shoot traits. The shoot and root fresh weight were measured separately. Roots were thoroughly cleaning and stored in 70% alcohol at 4 °C for root trait evaluation. The shoot samples were dried at 70 °C in oven until constant dry shoot weight (DSW) was observed. Measurement of total root length (RL), total root diameter (RD), total root surface area (SA), total root volume (RV), number of forks (NF), and number of tips (Ntips) were recorded using the WinRhizo PRO [Figure 1B]. After scanning, the roots were dried at 70 °C in the oven until constant dry root weight (DRW) was observed. Root shoot ratio in terms of biomass was calculated by dry root weight divided by dry shoot weight, while in terms of length, it was calculated as root length divided by shoot length [15].

Table 1. The detailed information on the parentage and the QTL/genes identified in the breeding panel.

Genotypes	Number	PARENTAGE	Combination of Gene/QTL
PAU 7180-36-5-0-0-0	1	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*xa13* + *Gm4* + $qGY_{10.1}$ + $qDTY_{3.1}$ + $qDTY_{2.1}$ + *BPH3*
PAU 7180-8-13-0-0-0	2	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*xa13* + *Gm4* + $qGY_{10.1}$ + *BPH3*
PAU 7180-9-17-0-0-0	3	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*Xa21* + *xa13* + *Gm4* + $qGY_{10.1}$ + $qGY_{1.1}$ + $qDTY_{3.1}$ + $qDTY_{2.1}$ + $qAG_{9.1}$ + *BPH3*
PAU 7180-3-9-0-0-0	4	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*Xa21* + *Gm4* + $qGY_{10.1}$ + $qDTY_{3.1}$ + *BPH3*
PAU 7180-3-15-0-0-0	5	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*xa13* + *Gm4* + $qGY_{10.1}$ + $qDTY_{12.1}$ + *BPH3* + $qLDG_{3.1}$ + $qEUE_{11.1}$
PAU 7180-4-2-0-0-0	6	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*Xa21* + *Gm4* + $qGY_{10.1}$ + *BPH3*
PAU 7180-113-14-0-0-0	7	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*Xa21* + *xa13* + *Gm4* + $qGY_{10.1}$ + $qGY_{1.1}$ + $qDTY_{2.1}$ + $qNR_{5.1}$ + *BPH3* + $qEUE_{11.1}$
PAU 7180-5-14-0-0-0	8	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*xa13* + *Gm4* + $qGY_{10.1}$ + $qGY_{1.1}$ + $qDTY_{3.1}$ + *BPH3* + $qEUE_{11.1}$
PAU 7180-9-15-0-0-0	9	PR 121/IR 96321//PR 121///PR 121/IR 71033-121-15-B//PR 121	*Xa21* + *xa13* + *Gm4* + $qGY_{10.1}$ + $qDTY_{3.1}$ + *BPH3*
PAU 5187-RIL1649-F8	10	PR115/CRR 615-PR 27699-D-808-4-4	*Xa4* + $qGY_{10.1}$ + $qRHD_{5.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$
PAU 5567-32-3-1-5	11	PR 120//PAU 201/UPR 1561-6-3	*Xa4* + *Gm4* + $qGY_{10.1}$
PAU 5729-60-5-4-1	12	IRBB 60/PAU 3699-13-2-2-4	*Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + *BPH3* + $qEUE_{11.1}$
RP 6273-HHZ4-DT3-LI1-LI1	13	Huang-Hua-Zhan*2/IR 64	*Xa4* + $qGY_{10.1}$ + $qNR_{5.1}$ + $qAG_{9.1}$ + $qRHD_{1.1}$ + $qEUE_{11.1}$
RP 6314-GSR IR 1-DQ 150-R5-Y1	14	IRRI 209/IRRI 192	*Xa4* + $qNR_{5.1}$ + $qAG_{9.1}$
NVSR 2107	15	Gurjari/PAU 201	*Xa4* + *Gm4* + $qGY_{10.1}$ + $qNR_{5.1}$ + $qAG_{9.1}$ + $qRHD_{8.1}$ + $qRHD_{1.1}$ + $qRHD_{5.1}$ + *BPH3* + $qLDG_{3.1}$ + $qEUE_{11.1}$
PAU 6778-12-1-4-1-1	16	CSR2720-2-IR82590-B-B-32-2-150/CR2702-185-16-1-1-1//IR71033-121-15-B	*Xa4* + $qGY_{10.1}$ + $qDTY_{2.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$
PAU 6456-8-1-1-1-3	17	PAU3699-13-2-2-4/IR78908-81-B-4-8//HKR07-95	*xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$
PAU 6456-8-2-1-1-1	18	PAU3699-13-2-2-4/IR78908-81-B-4-8//HKR07-95	*Gm4* + $qGY_{10.1}$ + $qEUE_{11.1}$ + $qRHD_{5.1}$ + $qDTY_{1.1}$
PAU 6456-8-2-1-1-2	19	PAU3699-13-2-2-4/IR78908-81-B-4-8//HKR07-95	*xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$
PAU 5533-56-3-1-2-3-1-2	20	PR120/MASARB 868	*Xa4* + *Gm4* + $qGY_{10.1}$
PAU 5533-56-3-1-3-1-1-1	21	PR120/MASARB 868	*Xa4* + $qGY_{10.1}$
CR 4116-3-2-1-1-1	22	CR 4043-3-1-1-1/CR Dhan 204	*xa5* + *Xa4* + *Gm4* + $qGY_{10.1}$ + $qDTY_{12.1}$ + $qRHD_{8.1}$ + $qRHD_{1.1}$ + *BPH3* + $qEUE_{11.1}$
PR 121	23		-
PR 126	24		-
PAU 9562-1-1	25	BC_3F_2 [PR 122/*O. punctata* IRGC105137(amphi)]//3*PR 122	*xa13* + *Xa4* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + *BPH3* + $qLDG_{4.1}$ + $qEUE_{11.1}$ + $qEUE_{1.1}$
PAU 9562-2-1	26	BC_3F_2 PR 122/*O. punctata* IRGC105137(amphi)]//3*PR 122	*Xa21* + *Xa4* + *Gm4* + $qGY_{10.1}$ + *BPH3* + $qLDG_{4.1}$ + $qEUE_{11.1}$ + $qEUE_{1.1}$
PAU 9562-3-1	27	BC_3F_2 [PR 122/*O. punctata* IRGC105137(amphi)]//3*PR 122	*xa13* + *Xa4* + *Gm4* + $qGY_{10.1}$ + $qRHD_{8.1}$ + $qEUE_{11.1}$
PAU 9563-1-1	28	BC_3F_2 (PR 121/*O. longistaminata* IR104151)//2*PR 121	*Xa21* + *xa13* + *Gm4* + $qGY_{10.1}$ + *BPH3* + $qLDG_{4.1}$ + $qEUE_{11.1}$
IR 11L101	29		$qGY_{10.1}$ + $qDTY_{3.1}$ + $qRHD_{8.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{4.1}$ + $qEUE_{11.1}$

Table 1. *Cont.*

Genotypes	Number	PARENTAGE	Combination of Gene/QTL
IR 91648-B-32-B	30		$qGY_{10.1} + qRHD_{8.1} + qRHD_{1.1} + qLDG_{3.1} + qLDG_{4.1} + qEUE_{11.1} + qEUE_{1.1}$
IR 13L500	31		$qGY_{10.1} + qGY_{1.1} + qDTY_{12.1} + qDTY_{3.1} + qDTY_{2.1} + qNR_{5.1} + qRHD_{8.1} + qRHD_{1.1} + BPH3 + qLDG_{4.1} + qEUE_{11.1}$
IR 87707-446-B-B-B	32		$Xa4 + qGY_{10.1} + qDTY_{2.1} + qRHD_{1.1} + BPH3 + qLDG_{3.1} + qLDG_{4.1} + qEUE_{11.1}$
Vandana	33		$qGY_{10.1} + qAG_{9.1} + qRHD_{8.1} + qRHD_{1.1} + BPH3 + BPH17 + qLDG_{4.1} + qEUE_{11.1}$
Kali aus	34		$Xa4 + qGY_{10.1} + qDTY_{12.1} + qDTY_{2.1} + qNR_{5.1} + qRHD_{8.1} + qRHD_{1.1} + BPH3 + qLDG_{3.1} + qLDG_{4.1} + qEUE_{11.1}$
MTU 1010	35		$xa13 + xa5 + Xa4 + qGY_{10.1} + qRHD_{1.1} + BPH3 + qLDG_{4.1}$
Abhaya	36		$Gm4 + Xa4 + qGY_{10.1} + qNR_{5.1} + qRHD_{5.1} + BPH3 + qLDG_{3.1} + qLDG_{4.1}$
Tadukan	37		$Gm4 + qLDG_{4.1} + qEUE_{11.1}$
IRBB60	38		$Xa21 + xa13 + xa5 + Xa4 + Gm4 + qGY_{10.1} + qLDG_{4.1} + qEUE_{11.1}$
IR 93312-30-101-2013-30-66-6	39		$Xa4 + qGY_{10.1} + qAG_{9.1} + qNR_{5.1} + qRHD_{5.1} + qRHD_{1.1} + BPH3 + qLDG_{3.1} + qLDG_{4.1} + qEUE_{11.1}$
IR 94226-B-177-B	40		$Xa4 + qGY_{10.1} + qGY_{1.1} + qNR_{5.1} + qRHD_{1.1} + qLDG_{4.1} + qNR_{4.1}$
IR 96322-34-223	41		$xa5 + Xa4 + qGY_{10.1} + qDTY_{2.1} + qDTY_{3.1} + qLDG_{4.1}$
IR 94225-D-82-B	42		$qGY_{10.1} + qGY_{1.1} + qRHD_{5.1} + qRHD_{8.1} + qRHD_{1.1} + qLDG_{4.1} + qEUE_{11.1} + qNR_{4.1}$

Days to 50% flowering (DTF) was recorded when around 50% of the plants exerted their panicles in a plot. The number of productive tillers (NPT) were counted manually in 0.5 m row length under DSR whereas the data from 5 random plants were collected under TPR conditions. The plant height (PH) in cm was measured from the randomly selected five plants for each entry as the mean height, measured from the base of the plant to the top panicle at the maturity stage. The chlorophyll content per plant was measured and recorded at maximum tillering stage with the help of chlorophyll SPAD value meter from the terminal leaf of plant [16]. The genotypes were harvested when the panicles turned to golden yellow, harvested grains were threshed, then dried, and weighed to determine the grain yield (GY) [17]. For thousand grain weight, 100 well-developed and whole grains dried to 13% moisture were counted. They were weighed and used to calculate the thousand grain weight in grams (g). Spikelet fertility was obtained from panicle which was taken after maturity. The total numbers of filled and sterile spikelets were counted separately and added, which gives the total number of spikelets/panicles [18]. It can be calculated by using the following formula:

$$\text{Spikelet Fertility (SF) (\%)} = \text{Total number filled spikelets per panicle/Total number of spikelets per panicle} \times 100 \quad (1)$$

Quality parameters were calculated by taking 125 g of paddy as a sample. The weighed samples (125 g) of paddy were collected and were dehusked by using Satake Rubber Roll Laboratory Sheller (Satake Engineering Co., Japan). The moisture content was between 13 to 14%. The brown rice was obtained after shelling and brown rice samples were milled in McGill Miller No. 2, USA. The adjustment of milling time obtains a 6% degree of polish in brown rice samples. The remaining rice sample after milling was total rice including broken rice grains. 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 min by using a test rice grader machine to separate the head rice from the broken in direct seeded rice and transplanted rice [19]. It was calculated by using the following formulas:

$$\text{Total Rice Recovery \%} = (\text{Weight of brown rice/Weight of paddy}) \times 100\% \quad (2)$$

$$\text{Milled Rice Recovery \%} = (\text{Weight of milled rice/Weight of paddy}) \times 100\% \quad (3)$$

$$\text{Head Rice Recovery \%} = (\text{Weight of head rice/Weight of paddy}) \times 100\% \quad (4)$$

2.5. Statistical Analysis

Analysis of variance, and experiment-wise and season-wise mean for each season were analyzed using a mixed model analysis in SAS 9.2 [20]. Fisher's t test was performed to estimate the significant difference among the genotypes constituting the breeding panel, treatments, seasons, and to estimate the interactions.

2.6. Genotyping

The already reported 106 KASP markers [21], Supplementary Materials Table S2, associated traits such as bacterial blight resistance, brown plant hopper resistance, gall midge resistance, seedling vigor, early and uniform emergence, anaerobic germination, lodging resistance, root traits improving nutrient uptake such as number of nodal roots, root hair density, and grain yield under DSR and drought condition were used in the present study to identify the QTL/genes in the breeding panel constituting 27 advanced breeding lines, 13 DSR adapted checks, and two control checks.

2.7. DNA Extraction and Quantification

The genomic DNA of the selected genotypes was isolated from fresh and young leaves from the fifteen-day-old seedlings using the CTAB method described by Murray

and Thompson [22]. Extracted DNA was treated with RNAase A enzyme, and quantified using 0.8% agarose gel and nanodrop spectrometer.

2.8. KASP Assay

KASP markers associated with various important DSR traits [21] were used for the molecular profiling of the selected rice panel. The genomic DNA of the advanced breeding lines was normalized for 25 nano-grams per micro-liter. A total of 106 KASP markers were used for the molecular screening.

2.9. KASP Assay

The KASP genotyping assays were performed as mentioned by Sandhu et al. [21]. KASP genotyping assays constitute 2 µL of template DNA (25 ng), 0.056 µL of the primer mix and 1.944 µL of the Kasp mix. The touchdown PCR was performed involving the following steps: the initial denaturation at 95 °C for 15 min, 10 touchdown cycles (95 °C for 20 s, touchdown at 65 °C, −1 °C per cycle, 25 s) followed by 20 cycles of DNA amplification (95 °C for 10 s, 57 °C for 60 s).

Data was collected using an infinite F200 pro micro-plate reader on the basis of fluorescence and the data was analyzed using the Tecan i-control 1.11 software. The clusters were marked as XX, XY, YY based on their graphical location using the KlusterCaller.

2.10. Diversity Studies of Breeding Lines

DARwin 6.0.013 software was used to estimate pair-wise distance matrix through calculating the dissimilarity matrix [23]. For the construction of a neighbor joining tree, we used an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and it was followed by the bootstrap analysis with 1000 permutations.

3. Results

3.1. Phenotypic Characterization of Breeding Panel

The present study was conducted to evaluate a set of advanced breeding lines of rice for yield and quality traits under direct seeded and transplanted conditions. Based on the comprehensive information on the DSR-related traits, associated QTL were also investigated in a set of selected genotypes using molecular markers. The analysis of variance revealed significant variations for genotypes, treatments, and seasons for the traits associated with root architecture, grain yield, and yield-related traits. Significant interactions of genotypes with treatment and seasons were observed for the traits measured in the present study (Table 2). Most of the advanced breeding lines showed improved seedling vigor under DSR than TPR conditions (Supplementary Materials Tables S3 and S4). PR126 showed improved seedling vigor under DSR.

Table 2. Analysis of variance (ANOVA) for plant morphological, grain quality and root architecture traits under direct seeded and transplanted puddled system of rice cultivation.

Plant morphological and quality traits										
Source of Variation	**DTF**	**TN**	**PH**	**YLD**	**SPAD**	**TGW**	**FER %**	**TRR**	**MRR**	**HRR**
Genotype	40.256 ***	13.984 ***	101.731 ***	233.795 ***	4.519 ***	54.578 ***	35.208 ***	22.684 ***	6.355 ***	624.661 ***
Replication	2.376	4.815 **	1.645	2.423	0.132	0.79	0.653	1.784	1.502	0.646
Treatment	45.134 ***	9.009 **	317.063 ***	5072.625 ***	42.008 ***	334.761 ***	124.275 ***	76.696 ***	11.534 ***	3673.681 ***
Season	228.49 ***	0.399	39.835 ***	284.334 ***	0.65	177.04 ***	6.892 ***	2.459	0.009	2362.003 ***
Genotype × Replication	1.049	0.756	1.173	1.112	1	1.146	0.766	2.274 ***	1.21	0.745
Genotype × Treatment	6.365 ***	4.475 ***	12.889 ***	37.763 ***	1.234	12.637 ***	14.427 ***	1.123	4.378 ***	143.564 ***
Genotype × Season	7.727 ***	4.961 ***	11.523 ***	64.499 ***	3.693 ***	6.601 ***	1.877 **	18.804 ***	2.732 ***	47.87 ***
Treatment × Season	96.572 ***	6.136 *	23.503 ***	301.025 ***	33.363 ***	13.259 ***	6.116 *	66.403 ***	5.851 *	1437.944 ***
Replication × Treatment	0.192	0.319	0.428	1.178	1.015	0.751	0.925	0.366	1.116	2.157
Replication × Season	0.65	1.421	1.691	1.123	0.297	0.687	0.692	0.039	0.016	1.487
Genotype × Treatment × Season	5.637 ***	4.121 ***	4.577 ***	33.465 ***	1.708 *	5.628 ***	1.911 **	1.477	2.34 ***	49.228 ***
Genotype × Replication × Treatment	1.076	0.992	1.186	0.947	1.057	1.094	0.819	0.363	0.947	1.284
Replication × Treatment × Season	0.077	0.231	2.607	1.093	1.178	0.102	0.417	0.185	0.411	1.406
Root architecture traits										
Source of Variation	**RL**	**AD**	**RV**	**SA**	**Tips**	**Forks**	**Crossing**	**RSRL**	**RSRB**	
Genotype	19.35 ***	3.202 ***	10.462 ***	14.083 ***	33.941 ***	20.311 ***	22.389 ***	1.002	3.036 ***	
Replication	0.365	0.959	0.925	0.883	0.227	0.2	33.478 ***	1.006	1.016	
Treatment	18.279 ***	188.855 ***	788.187 ***	206.714 ***	1.983	4.105 *	673.809 ***	1.704	4.568 *	
Season	3137.355 ***	11,092.418 ***	1009.157 ***	88.271 ***	4633.28 ***	4319.686 ***	4542.341 ***	1.689	4.022 *	
Genotype × Replication	1.085	1.05	1.082	1.069	1.01	1.055	1.006	0.998	0.995	
Genotype × Treatment	2.222 ***	3.343 ***	3.726 ***	2.201 ***	0.056	0.081	1.464 *	0.99	3.049 ****	
Genotype × Season	16.189 ***	5.778 ***	5.259 ***	10.239 ***	33.984 ***	19.761 ***	22.37 ***	1.018	2.979 ***	
Treatment × Season	2.961	8.16 **	13.522 ***	44.525 ***	1.886	0.576	679.464 ***	0.323	2.963	
Replication × Treatment	0.336	0.67	0.527	0.308	0.27	0.242	38.689 ***	1.092	1.065	
Replication × Season	0.448	0.947	1.183	1.352	0.244	0.253	35.021 ***	1.086	1.088	
Genotype × Treatment × Season	2.038 ***	4.817 ***	4.701 ***	1.535 *	0.032	0.089	1.754 **	0.985	3.005 ***	
Genotype × Replication × Treatment	1.012	0.994	1.033	1.12	0.99	1.007	0.999	1.004	1.002	
Replication × Treatment × Season	0.364	0.471	0.534	0.679	0.27	0.183	39.105 ***	0.916	0.991	

DTF: days to 50% flowering (days), TN: tiller number (m^{-2}), PH: plant height (cm), YLD: yield (kg ha^{-1}), SPAD: Soil Plant Analysis Development Meter Value (nmol cm^{-1}), TGW: thousand grain weight (g), TRR: total rice recovery (%), MRR: milled rice recovery (%), HRR: head rice recovery (%), FER %: Spikelet fertility (%), RL: root length (cm), AD: average root diameter (mm), RV: root volume (cm^3), SA: surface area (cm^2), RSR L: Root shoot ratio (length), RSR B: Root shoot ratio (biomass). * Significant at <0.05 level, ** significant at <0.01 level, *** significant at <0.001 level.

The root shoot ratio (biomass) for the genotypes varied from 0.27 to 0.55 with an average value of 0.39 under DSR, and from 0.18 to 0.42 with an average value of 0.30 under transplanted conditions (Tables 3 and 4). The root shoot ratio of the genotypes (length) ranged from 0.31 to 0.59 with an average value of 0.38 under DSR, and 0.22 to 0.43 with an average value of 0.33 under TPR. Most of the genotypes showed better root architecture in terms of root length and root shoot ratio under DSR compared to the TPR conditions (Tables 3 and 4). The maximum root length under DSR was from 665 to 2744 cm with an average of 1542 cm, whereas under TPR, it varied from 578 to 2346 cm with an average of 1387 cm (Supplementary Materials Tables S3 and S4). The average root volume was 0.82 cm^3 under DSR and it ranged from 0.50 to 1.21 cm^3, whereas under TPR, the average root volume was 1.37 cm^3 and volume ranged from 0.85 to 1.94 cm^3. The average diameter ranged from 0.306 to 0.389 mm with the average of 0.34 mm under direct seeded conditions whereas under TPR, it varied from 0.335 to 0.437 mm with an average of 0.382 mm. On average, surface area was less under DSR. It ranged from 50.53 to 144.47 cm^2 with an average of 102.63 cm^2 but it ranged from 88.42 to 161.01 cm^2 having a mean of 128.31 cm^2. The average number of tips was 14,566 in DSR and it ranged from 5388 to 27,465. Similarly, under TPR, the average number of tips was 15,113 with the range of 5451 to 27,829. The forks for the panel varied from 9674 to 50,088 with an average value of 30,777 under DSR, and it varied under TPR from 11,734 to 51,423 with an average value of 32,343. The average number of root crossing was 11,100 under DSR. The root volume ranged from 2973 to 24,496 under DSR, whereas in TPR, the average root volume ranged from 7314 to 40,321 cm^3 with an average of 23,694 cm^3.

Days to 50% flowering for the breeding panel varied from 88 to 110 with an average value of 100 days, and it varied under transplanted conditions from 92 to 110 with an average value of 102 days. The breeding panel showed early flowering under DSR compared to TPR conditions. The plant height was less under DSR compared to the TPR conditions. The plant height of the breeding panel ranged from 84 to 115 cm with a mean plant height of 99 cm under DSR, whereas the plant height ranged from 93 cm to 124 cm with an average plant height of 105 cm under TPR. In DSR, 7 breeding lines belonged to the dwarf category, 28 breeding lines to the intermediate, and 1 to the tall plant category.

The average grain yield of the breeding panel was lower under DSR compared to the TPR conditions. The grain yield of the breeding panel ranged from 1721 to 6504 $kgha^{-1}$ with an average of 4616 kg ha^{-1} under DSR, and from 3686–7334 kg ha^{-1} with an average 6095 kg ha^{-1} under TPR. On average, a 24% decrease in grain yield was observed under DSR compared to TPR. The average spikelet fertility was 86% and 89% under DSR and TPR, respectively. The average thousand grain weight of the breeding panel was higher under TPR. As compared to the TPR, 8.66% decrease in the thousand grain weight was observed under DSR.

The grain quality of the breeding panel was comparable under DSR and TPR in terms of total rice recovery and milled rice recovery in contrast to the head rice recovery which was better under TPR. The average total rice recovery was 80.07% under DSR, and 79.43% under TPR. The milled rice recovery of the breeding panel varied from 51.34 to 72.81% with an average of 68.32% under DSR, and from 46.73 to 56.63% with an average of 52.34% under TPR. A total of a 30% improvement in milled rice recovery was observed under DSR compared to the TPR conditions. The 14% decrease in head rice recovery rate was observed under DSR compared to the TPR conditions.

Table 3. Mean value of plant morpho-physiological and grain quality traits across different seasons under direct seeded and transplanted puddled system of rice cultivation.

Traits	Season	Mean	Max	Min	Std. Dev.	S.E.	F Value
DTF	DSR 2020	97	109	82	7.289	1.220	70.728 ***
	TPR 2020	102	109	93	4.393	0.964	40.164 ***
	DSR 2021	104	113	91	7.092	3.143	8.321 ***
	TPR 2021	103	112	89	6.290	2.739	8.694 ***
TN	DSR 2020	285	357	204	40.410	14.997	12.741 ***
	TPR 2020	287	353	209	43.407	11.636	26.243 ***
	DSR 2021	281	343	216	41.760	21.677	5.429 ***
	TPR 2021	293	332	242	32.144	22.936	1.90 *
PH	DSR 2020	101	128	84	10.432	2.365	37.534 ***
	TPR 2020	105	124	93	9.094	2.224	31.939 ***
	DSR 2021	98	136	81	12.162	3.686	20.131 ***
	TPR 2021	104	128	93	10.771	2.189	47.305 ***
YLD	DSR 2020	4971	7310.05	1365.71	1824.31	217.51	141.316 ***
	TPR 2020	6090	7521.00	3236.00	969.05	122.73	124.880 ***
	DSR 2021	4260	6214.00	2075.43	846.86	141.72	70.653 ***
	TPR 2021	6100	7469.00	4136.00	882.19	208.89	34.294 ***
SPAD	DSR 2020	37	44	33	3.589	2.086	3.975 ***
	TPR 2020	37	41	34	2.526	1.716	2.358 ***
	DSR 2021	36	41	23	4.722	3.222	2.320 ***
	TPR 2021	39	44	34	3.254	2.076	2.951 ***
TGW	DSR 2020	24.08	32.07	19.03	2.93	1.32	7.997 ***
	TPR 2020	25.84	34.77	18.10	3.60	1.38	11.729 ***
	DSR 2021	22.06	30.23	13.20	3.34	0.55	72.020 ***
	TPR 2021	24.68	34.63	18.69	3.44	0.57	73.212 ***
FER %	DSR 2020	86	94	70	6.716	2.308	23.980 ***
	TPR 2020	89	95	80	4.053	2.308	4.228 ***
	DSR 2021	86	95	70	6.791	1.230	60.088 ***
	TPR 2021	88	95	78	4.410	2.172	6.347 ***
TRR	DSR 2020	80.42	82.36	78.50	1.07	0.42	10.767 ***
	TPR 2020	79.20	81.01	75.88	1.22	0.40	16.417 ***
	DSR 2021	79.72	82.97	76.03	1.89	0.77	10.217 ***
	TPR 2021	79.68	82.97	76.03	1.89	0.87	7.555 ***
MRR	DSR 2020	67.94	73.34	35.42	7.97	4.88	3.389 ***
	TPR 2020	69.83	72.50	64.89	2.14	1.14	5.078 ***
	DSR 2021	68.69	72.57	51.98	4.29	2.32	4.903 ***
	TPR 2021	69.01	73.74	65.25	2.01	0.92	7.622 ***
HRR	DSR 2020	51.62	67.71	26.26	12.21	1.11	245.971 ***
	TPR 2020	62.12	68.47	43.16	6.34	1.17	57.322 ***
	DSR 2021	50.49	65.31	27.73	10.68	0.59	674.510 ***
	TPR 2021	52.90	64.25	33.28	6.84	0.55	317.877 ***

DTF: days to 50% flowering (days), TN: tiller number (m^{-2}), PH: plant height (cm), YLD: yield (kg ha^{-1}), SPAD: Soil Plant Analysis Development Meter Value (nmol cm^{-1}), TGW: thousand grain weight (g), FER %: Spikelet fertility (%), TRR: total rice recovery (%), MRR: milled rice recovery (%), HRR: head rice recovery (%), DSR 2020: *Kharif* 2020 under direct seeded condition, DSR 2021: *Kharif* 2021 under direct seeded condition, TPR 2020: *Kharif* 2020 under transplanted condition, TPR 2021: *Kharif* 2020 under transplanted condition. F value: the F distribution value determining whether the test is statistically significant or not, * Significant at <0.05 level, *** significant at <0.001 level.

Table 4. Mean value of root architectural traits across different seasons under direct seeded and transplanted puddled system of rice cultivation.

Traits	Season	Mean	Max	Min	Std. Dev.	S.E	F Value
RL	DSR 2020	2322.77	4755.69	862.33	954.53	408.84	6.018 ***
	TPR 2020	2107.15	4019.78	677.43	647.03	26.13	1250.837 ***
	DSR 2021	763.03	1172.16	467.99	230.25	115.48	2.846 ***
	TPR 2021	666.79	866.82	478.45	202.13	111.36	1.603 *
RV	DSR 2020	0.54	0.85	0.18	0.23	0.10	6.210 ***
	TPR 2020	1.02	1.62	0.46	0.32	0.01	1331.454 ***
	DSR 2021	1.10	1.62	0.78	0.41	0.21	2.482 ***
	TPR 2021	1.73	2.66	1.03	0.56	0.25	4.886 ***
AD	DSR 2020	0.18	0.22	0.14	0.03	0.01	7.268 ***
	TPR 2020	0.22	0.34	0.15	0.04	0.00	419.597 ***
	DSR 2021	0.50	0.59	0.43	0.07	0.04	1.637 *
	TPR 2021	0.55	0.62	0.47	0.07	0.03	4.599 ***
SA	DSR 2020	116.98	193.31	33.84	48.32	20.47	6.268 ***
	TPR 2020	130.74	200.47	54.72	34.66	1.42	1214.425 ***
	DSR 2021	88.28	131.27	56.95	30.36	15.16	2.954 ***
	TPR 2021	125.88	176.12	83.39	33.04	17.33	2.301 ***
Tips	DSR 2020	27,519.87	53,375.83	9634.50	16,897.24	6494.08	8.719 ***
	TPR 2020	28,600.38	54,046.56	9760.27	13,461.89	325.14	3505.043 ***
	DSR 2021	1612.40	2544.17	949.33	740.36	385.00	2.429 ***
	TPR 2021	1626.04	2350.00	974.00	656.83	344.06	2.263 ***
Forks	DSR 2020	55,875.60	94,177.50	15,593.50	29,069.93	12,849.73	5.336 ***
	TPR 2020	58,027.58	95,830.71	18,184.42	20,273.16	638.83	2057.025 ***
	DSR 2021	5679.29	11,114.33	3028.67	3276.95	1722.86	2.193 ***
	TPR 2021	6658.14	9295.83	4293.00	2644.53	1506.90	1.163
Crossing	DSR 2020	21,126.42	47,983.83	5360.50	16,071.81	7560.85	4.109 ***
	TPR 2020	46,367.65	79,948.34	13,979.57	21,785.48	2907.52	68.252 ***
	DSR 2021	1073.71	3434.17	365.33	1423.28	788.29	1.532 *
	TPR 2021	1020.98	2264.00	648.00	955.85	553.53	0.963
RSR L	DSR 2020	0.68	0.57	0.27	3.24	2.65	0.995
	TPR 2020	0.39	0.55	0.22	0.10	0.05	6.375 ***
	DSR 2021	0.39	0.48	0.29	0.08	0.06	1.575
	TPR 2021	0.27	0.35	0.20	0.05	0.04	1.756 *
RSR B	DSR 2020	0.36	0.79	0.19	0.10	0.03	27.746 ***
	TPR 2020	0.27	0.39	0.15	0.08	0.04	5.978 ***
	DSR 2021	1.18	0.61	0.30	0.85	3.71	3.014 ***
	TPR 2021	0.33	0.56	0.18	0.10	0.06	3.264 ***

RL: Root length (cm), AD: average root diameter (mm), RV: root volume (cm^3), SA: Surface area (cm^2), RSR L: Root shoot ratio (length), RSR B: Root shoot ratio (biomass), DSR 2020: *Kharif* 2020 under direct seeded condition, DSR 2021: *Kharif* 2021 under direct seeded condition, TPR 2020: *Kharif* 2020 under transplanted condition, TPR 2021: *Kharif* 2020 under transplanted condition. F value: the F distribution value determining whether the test is statistically significant or not, * Significant at <0.05 level, *** significant at <0.001 level.

3.2. DNA Fingerprinting of the Breeding Panel

The KASP assay data was generated using a total of 106 KASP markers associated with traits such as biotic stress tolerance/resistance, early and uniform germination, root traits, and yield and yield-related traits. The 106 KASP assays include 32 KASP assays for biotic stress tolerance/resistance, 10 KASP assays for early and uniform germination, 19 KASP assays for root traits, and 45 KASP assays for yield and yield-related traits (Figure 2). The genetic relationship among the breeding panel as determined by the UPGMA cluster analysis and two-dimensional PCA scaling showed that the 42 advanced breeding lines constituting the breeding panel were divided into two distinct groups (Figure 3A,B). The group I had three advanced breeding lines and 6 donors. The group II was further divided into subgroups having remaining donors and the advanced breeding lines. The advanced breeding lines in PR121 background and the check variety PR121 constitute one subgroup in the major group II. The advanced breeding lines possessing same pedigree represented the same subgroup in the cluster analysis.

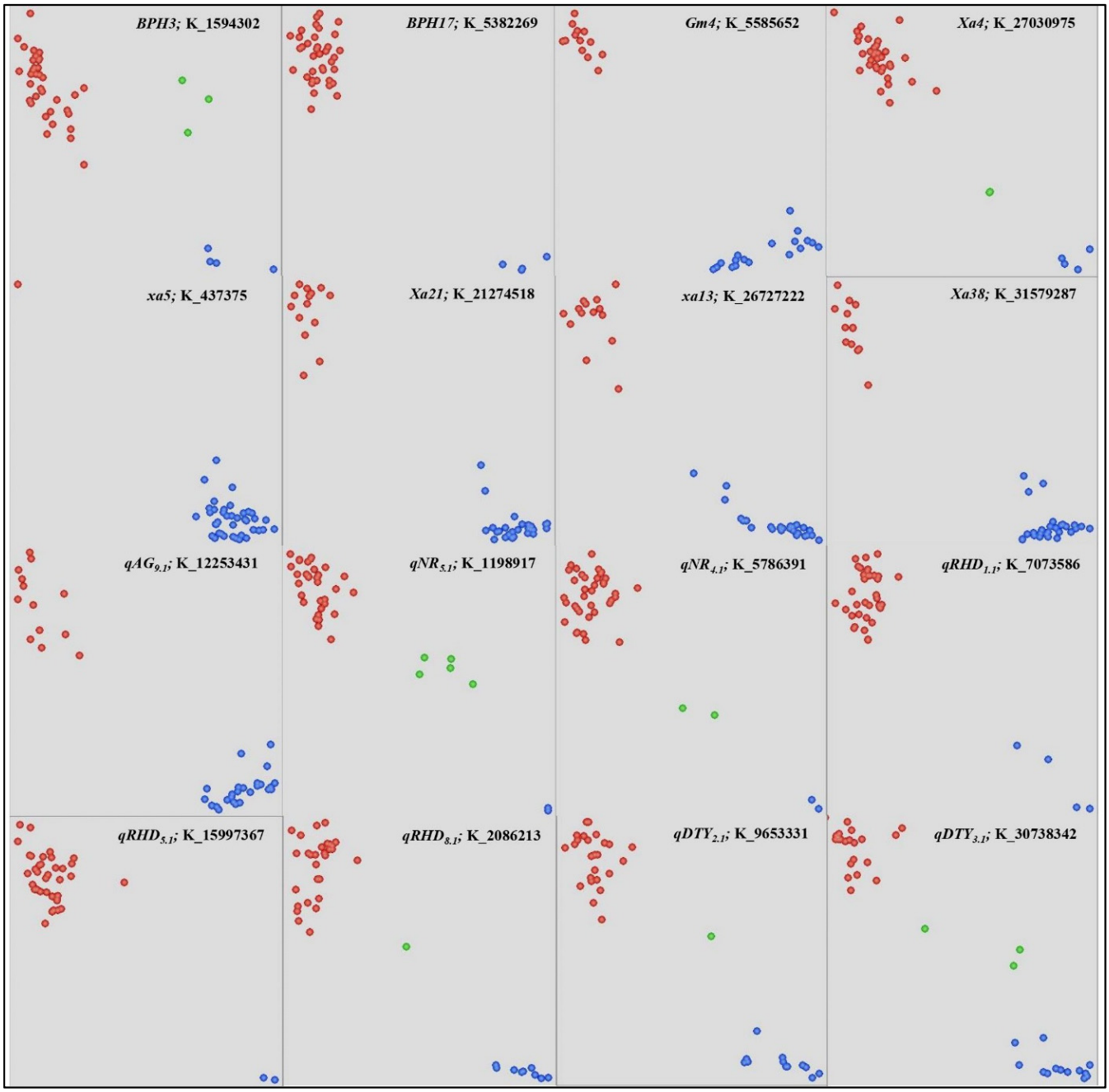

Figure 2. The pictorial representation of the KASP assay conducted on the advanced breeding lines panel including 13 DSR adapted donor checks, 2 control checks, and 27 advanced breeding lines. The blue color indicates the donor allele, the red color indicates the alternate allele, and green color indicates the heterozygotes.

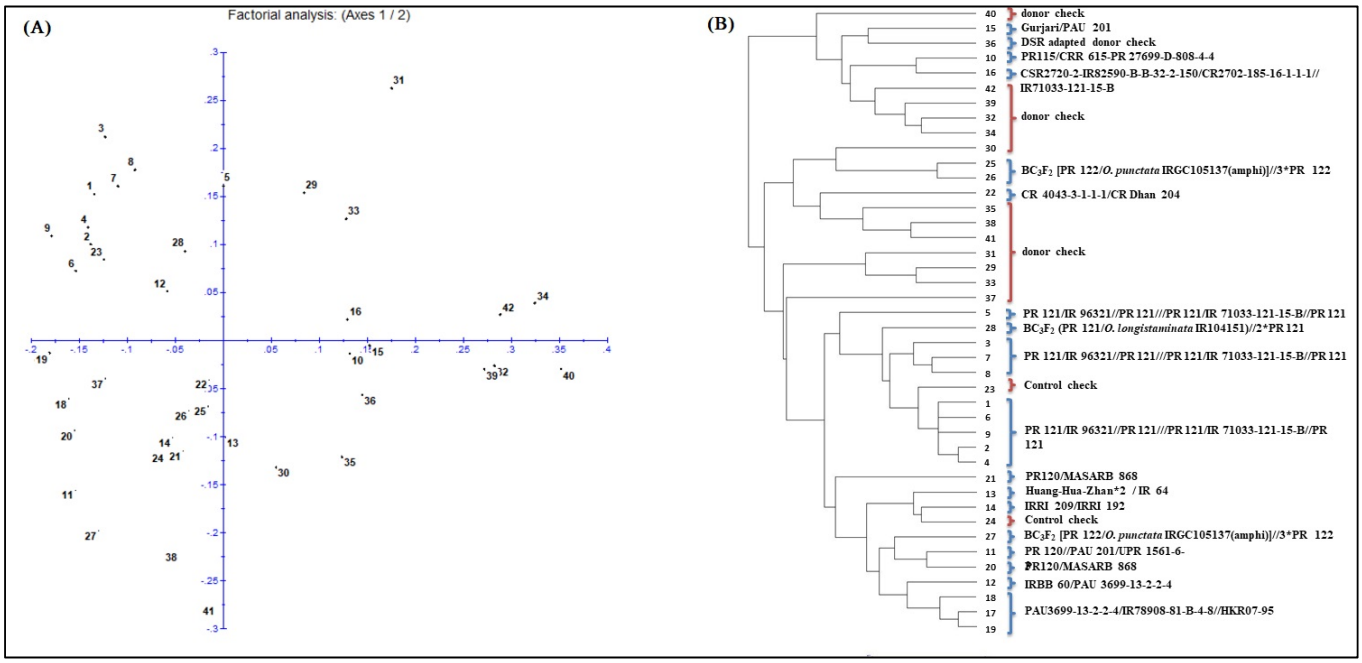

Figure 3. (**A**) Principal component analysis of the advanced breeding panel (13 DSR adapted donor checks, 2 control checks, and 27 advanced breeding lines). (**B**) Phylogenetic analysis of the 42 accessions (13 DSR adapted donor checks, 2 control checks, and 27 advanced breeding lines) using 116 KASP assay DNA fingerprinting database. The parentage of the advanced breeding lines is indicated in the phylogenetic tree (Figure 3B, right side). The numerical code represents the breeding panel constituting the advanced breeding lines/donor check/control checks in the breeding panel. The detailed information on the numerical codes has been provided in Table 1.

3.3. Molecular Profiling of the Breeding Panel

To make rice suitable for cultivation under direct seeded cultivation conditions, various traits such as early and uniform emergence, nodal roots, root hair density, resistance to brown planthopper, gall midge and bacterial blight, lodging resistance, anaerobic germination, and grain yield under direct seeded and drought conditions are required. The molecular profiling of the breeding panel for the above-mentioned traits was carried out using earlier identified KASP markers (Sandhu et al., 2022). The molecular profiling showed that the QTL associated with the traits improving rice grain yield and adaptability under DSR ranged from 2 to 11. Most of the breeding lines possess the favorable alleles associated with the *GM4*, *BPH3*, *Xa4*, $qGY_{10.1}$ (Figure 4). The breeding lines in the background of PR121 and PR126 had alleles associated with resistance to bacterial blight (*xa13* and *Xa21*). A total of 10 breeding lines possessed a combination of alleles specific for the traits associated with the root architecture, biotic stress resistance/tolerance, and grain yield under DSR. Eleven breeding lines possessing at least one QTL provided improved yield under DSR conditions and one QTL under reproductive stage drought stress conditions. Only three breeding lines (PAU 7180-9-17-0-0-0, PAU7180-113-14-0-0-0, and PAU 7180-5-14-0-0-0) having two QTL ($qGY_{1.1}$ + $qGY_{10.1}$) contributing to yield improvement under DSR and two breeding lines (PAU 7180-36-5-0-0-0 and PAU 7180-9-17-0-0-0) having QTL ($qDTY_{2.1}$ + $qDTY_{3.1}$) contributing to yield improvement under reproductive stage drought stress conditions. A total of 14 breeding lines possessed alleles associated with early and uniform emergence under DSR conditions. Eight breeding lines identified with at least two bacterial blight resistance genes (*xa13* + *Xa21*/*xa13* + *Xa4*/*Xa4* + *Xa21*/*Xa4* + *xa5*). The breeding line NVSR 2107 carries 11 QTL followed by 9 QTL in PAU 7180-9-17-0-0-0, PAU 7180-113-14-0-0-0, CR 4116-3-2-1-1-1-, and PAU 9562-1-1.

Figure 4. The heatmap indicating the favorable alleles frequency associated with various biotic/abiotic resistance/tolerance traits, seedling establishment, root traits improving the nutrient uptake, grain yield, and yield-related traits in the advanced breeding lines panel. The numerical code represents the breeding panel constituting the advanced breeding lines/donor check/control checks in the breeding panel. The detailed information on the numerical codes has been provided in Table 1. The numerical codes highlighted in the red color indicate the selected promising breeding lines.

3.4. Selection of Promising Breeding Lines from the Breeding Panel

The genotypes PAU 6456-8-2-1-1-1, PAU 5187-RIL1649-F8, PAU 6456-8-1-1-1-3, PAU 6456-8-2-1-1-2, NVSR 2107, and PAU 6778-12-1-4-1-1 are the genotypes which were performing best under DSR and TPR (Table 5). The genotype PAU 5187-RIL1649-F8 had comparable yield under both conditions having 6.29% reduction under DSR. The selected genotypes showed early maturity and were semi-dwarf in height. In contrast, the genotype NVSR 2107 was taller as compared to other genotypes screened in the present study. The higher yield of these genotypes could be attributed to their better seedling vigor, good tillering ability, spikelet fertility %, thousand grain weight, and higher SPAD value (indicates better photosynthetic ability). The root characteristics like root shoot ratio, root length, and root volume were desirable for contributing to efficient nutrient uptake. They had good fertility percentage and tillering ability but low milling quality. Molecular characterization revealed that most of these better performing genotypes had grain yield under direct seeded rice, grain yield under drought, and root hair density had QTL. The highest number of QTL combination (11 QTL) was observed in selected promising breeding lines NVSR 2107. The best performing genotypes PAU 6456-8-2-1-1-1, PAU 5187-RIL1649-F8, PAU 6456-8-1-1-1-3, PAU 6456-8-2-1-1-2, NVSR 2107, and PAU 6778-12-1-4-1-1 had the QTL associated with early uniform emergence, biotic stress resistance/tolerance, root traits, and grain yield.

Table 5. The performance of selected advanced breeding lines in terms of morpho-physiological traits, grain yield, and yield-related traits, and root architecture traits under direct seeded and transplanted puddled system of rice cultivation.

DSR																					
Advanced Breeding Line	**QTL/Gene Combination**	**DTF**	**TN**	**PH**	**YLD**	**SPD**	**TGW**	**TRR**	**MRR**	**HRR**	**FER %**	**RL**	**AD**	**RV**	**SA**	**Tips**	**Forks**	**Crossings**	**RSR L**	**RSR B**	**SV**
PAU 6456-8-2-1-1-1	*Gm4* + $qGY_{10.1}$ + $qEUE_{11.1}$ + $qRHD_{5.1}$ + $qDTY_{1.1}$	98	260	100	6503.83	37	25.78	79.90	69.98	53.93	93	1763.64	0.32	0.95	122.14	21,608	37,783	13,468	0.42	0.42	3
PAU 5187-RIL1649-F8	*Xa4* + $qGY_{10.1}$ + $qRHD_{5.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$	99	228	97	6296.48	40	22.27	78.17	68.73	44.39	89	1889.21	0.33	0.78	133.21	18,822	32,339	15,620	0.41	0.40	3
PAU 6456-8-1-1-1-3	*xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$	97	250	104	6077.37	38	25.18	80.86	69.46	51.05	92	1921.01	0.32	1.11	144.47	20,345	42,305	15,822	0.37	0.51	3
PAU 6456-8-2-1-1-2	*xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$	100	256	99	5758.21	40	26.50	80.62	69.14	45.66	93	1784.71	0.31	0.78	111.86	22,394	38,319	14,359	0.36	0.50	3
NVSR 2107	*Xa4* + *Gm4* + $qGY_{10.1}$ + $qNR_{5.1}$ + $qAG_{9.1}$ + $qRHD_{8.1}$ + $qRHD_{5.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$ + $qEUE_{11.1}$	95	220	115	5498.57	38	31.15	80.85	71.13	34.45	90	2090.10	0.33	0.86	124.54	27,465	49,785	22,970	0.59	0.55	3
PAU 6778-12-1-4-1-1	*Xa4* + $qGY_{10.1}$ + $qDTY_{2.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$	94	270	111	5422.08	35	19.43	80.95	68.17	48.94	88	1821.69	0.32	0.94	127.63	22,086	42,351	16,854	0.36	0.39	3
PR 126	-	88	286	97	6312.15	33	21.03	79.67	69.47	56.71	92	1903.20	0.32	0.78	112.43	22,168	39,862	15,974	0.38	0.35	3
PR 121	-	107	308	85	5638.23	40	22.37	81.81	71.38	64.25	91	1656.70	0.34	0.82	110.52	17,015	33,187	10,950	0.36	0.38	3
Trial mean		100	210	99	4610.00	36	23.07	80.00	68.31	43.48	86	1542.90	0.34	0.78	102.63	14,566	30,777	11,100	0.23	0.37	3
LSD		2.184	18	3	299.8	1.99	1.22	0.73	0.86	0.85	1.82	200.11	0.22	0.26	10.11	2998	5442	3345	0.11	0.15	0.12
TPR																					
Advanced Breeding Line	**QTL/Gene Combination**	**DTF**	**TN**	**PH**	**YLD**	**SPD**	**TGW**	**TRR**	**MRR**	**HRR**	**FER %**	**RL**	**AD**	**RV**	**S A**	**Tips**	**Forks**	**Crossings**	**RSR L**	**RSR B**	**SV**
PAU 6456-8-2-1-1-1	*Gm4* + $qGY_{10.1}$ + $qEUE_{11.1}$ + $qRHD_{5.1}$ + $qDTY_{1.1}$	101	293	106	7070.07	40	27.85	79.08	53.47	58.37	92	1611.31	0.38	1.66	128.62	22,048	38,719	28,197	0.40	0.42	3
PAU 5187-RIL1649-F8	*Xa4* + $qGY_{10.1}$ + $qRHD_{5.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$	105	277	100	6718.90	41	25.13	77.46	51.30	62.63	87	1556.65	0.35	1.45	133.2	15,279	29,982	22,312	0.37	0.30	3
PAU 6456-8-1-1-1-3	*xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$	102	256	106	7047.72	40	28.60	81.45	55.02	58.48	90	1714.64	0.37	1.89	145.37	20,450	41,369	30,733	0.39	0.32	3
PAU 6456-8-2-1-1-2	*Xa13* + *Gm4* + $qGY_{10.1}$ + $qAG_{9.1}$ + $qEUE_{11.1}$	101	264	109	7003.56	38	25.72	80.03	52.87	56.70	91	1605.19	0.34	1.45	130.57	22,527	38,111	28,346	0.29	0.34	1
NVSR 2107	*Xa4* + *Gm4* + $qGY_{10.1}$ + $qNR_{5.1}$ + $qAG_{9.1}$ + $qRHD_{8.1}$ + $qRHD_{5.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$ + $qEUE_{11.1}$	94	272	121	7182.57	39	32.68	80.58	56.63	38.52	85	1922.61	0.40	2.04	151.48	27,829	51,423	40,003	0.34	0.41	3
PAU 6778-12-1-4-1-1	*Xa4* + $qGY_{10.1}$ + $qDTY_{2.1}$ + $qRHD_{1.1}$ + *BPH3* + $qLDG_{3.1}$	102	274	115	6622.74	39	26.38	80.91	53.65	59.81	90	1717.46	0.39	1.67	134.27	22,531	41,784	32,315	0.37	0.32	3
PR 126	-	93	272	97	7333.84	39	22.22	79.29	50.75	62.03	89	1669.51	0.37	1.48	146.33	22,975	40,585	30,558	0.33	0.21	3
PR 121	-	107	322	94	6890.17	40	25.98	81.11	53.55	65.24	89	1444.68	0.42	1.61	129.43	17,413	34,817	25,394	0.37	0.31	3
Trial mean		102	233	104.5	6095	38	23.33	74.44	50.44	57.42	88	1386.97	0.32	1.33	112.3	12,334	24,432	20,212	0.30	0.28	3
LSD		1.85	18	2.21	219.0	1.89	1.07	2.11	1.11	1.01	1.2	168.8	0.17	0.11	8.8	566.7	2247	1887	0.08	0.10	0.12

DTF: days to 50% flowering (days), TN: tiller number (m^{-2}), PH: plant height (cm), YLD: yield, SPAD: Soil Plant Analysis Development Meter Value (nmol cm^{-1}), TGW: thousand grain weight (g), TRR: total rice recovery (%), MRR: milled rice recovery (%), HRR: head rice recovery (%), FER %: Spikelet fertility (%), RL: root length (cm), AD: average root diameter (mm), RV: root volume (cm^3), RSR L: root shoot ratio (length), RSR B: root shoot ratio (biomass), SV: seedling vigor.

4. Discussion

Direct seeded rice is a promising technology with water- and labor-saving possibilities [24]. However, the varieties being used have been basically developed for the puddled transplanted conditions and do not possess several attributes needed for adaptation to direct seeding. Serious problems inherent to the 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 spots, bacterial blight, blast and gall midge, nematode infestation, iron deficiency under light soils, and poor milling quality pose a challenge to wider adoption and success of DSR. An ideal plant type for DSR should have the ability to germinate under anaerobic conditions coupled with tolerance of early submergence, good seedling vigor, root traits improving nutrient uptake, and resistance to biotic stresses. There is a strong need to develop high yielding varieties for direct seeded cultivation conditions which possess a favorable allele combination for good establishment, germination, early vigor, quality, yield, and high root density, lodging resistance along with tolerance to various biotic and abiotic stresses. Therefore, it has become necessary to direct concerted breeding efforts towards development of high yielding DSR-adapted genotypes. The present study was conducted to evaluate a set of advance breeding lines of rice for yield and quality traits under DSR and TPR conditions. Based on the comprehensive information on the DSR-related traits, associated QTL were also investigated in advanced breeding lines using KASP assay.

A genotype possessing early and improved seedling vigor has significantly affected the weed competitiveness and water utilization efficiency to maintain the sustainable rice production in rainfed and direct seeded rice conditions [25]. Panda et al. [26] reported that the root traits such as the root length, number of crown roots and adventitious roots, and root volume are desirable for developing hybrid varieties and resources efficient for direct seeded genotypes with wide adaptability. Identifying the ideal root architecture and breeding new varieties with efficient root architecture has great potential to improve resource-use efficiency and grain yield, especially under DSR [26]. The days to 50% flowering have great effect on the plant height and on the yield of the rice plant. Short to medium duration varieties are mostly preferred over the long duration varieties as this helps in saving irrigation water and other resources. Moreover, medium and early maturity varieties vacate the field timely for the sowing of the wheat crop. In the present study, the mean days to 50% flowering were lower under DSR compared to TPR conditions. Similarly, Sandhu et al. [11] observed that direct seeded rice genotypes were early in flowering by 5–7 days than transplanted rice. Genotypes such as PAU 7180-5-14-0-0-0, PAU 5187-RIL1649-F8, PAU 5567-32-3-1-5, PAU 5729-60-5-4-1, RP 6273-HHZ4-DT3-LI1-LI1, RP 6314-GSR IR 1-DQ 150-R5-Y1, NVSR 2107, PAU6778-12-1-4-1-1, PAU6456-8-1-1-1-3, PAU6456-8-2-1-1-1, PAU5533-56-3-1-3-1-1-1, CR 4116-3-2-1-1-1, and PAU 9562-3-1 are early to medium maturity genotypes, and are preferred under Punjab conditions as they mature early and the rice-wheat cropping system is followed. The genotype NVSR 2107 was the early flowering variety. Plant height was less in direct seeded rice. The plant height was less under DSR compared to the TPR conditions. At present, the semi-dwarf plant type has been a major focus in the rice breeding program. Bhadru et al. [27] reported that plant height is highly correlated with lodging and ease of harvest and the plant height is one of the most important characters influencing the acceptability of the variety by the farmer.

Grain yield of the genotypes was higher under TPR compared to DSR as the genotypes were bred for the transplanted conditions. The stable and higher yield of the selected promising breeding lines could be attributed to their better seedling vigor, good tillering ability, spikelet fertility %, thousand grain weight, and higher SPAD value (indicates better photosynthetic ability). The root characteristics like root shoot ratio, root length, and root volume were desirable for contributing to efficient nutrient uptake. The root architecture was reported to play an important role in improving grain yield under DSR [11]. The suitable genotypes for DSR have good crop establishment and efficient use of resources. The

combining of QTL of root and yield into the genotypes will lead to the yielding genotype under DSR. The spikelet fertility percentage was improved under DSR for those genotypes which possess QTL for root, yield, and yield-attributing traits. Rice quality characteristics are a major determinant of market prices and consumer acceptability. There is a need to improve the milling quality character under direct seeded conditions by identifying suitable donors and intensive breeding programs [28].

Identification of promising donors for DSR and utilizing them in the future marker-assisted breeding program may assist to carry precise breeding for introgression of genes/QTL exhibiting better adaptability with improved yield potential under DSR [11]. The genetic loci associated with the mentioned traits have been reported but only a few have been characterized and very few have been assessed for their impact under direct seeded cultivation conditions.

Most of the traits needed to improve rice yield under DSR are extremely complex traits. Unraveling key regulators (QTL/genes) associated with improvement of rice yield and adaptability under DSR cultivation conditions and pyramiding the QTL/genes in the genetic background of high yielding mega rice varieties utilizing the trait-linked markers may ensure food security in the future. The approach of the present study is to bring morpho-physiological and quality traits' evaluation of advanced breeding lines along with molecular profiling of these lines with known markers for direct seeded rice traits. The KASP assay used in the present study may be useful in selecting the favorable alleles in a wide range of genetic backgrounds. The use of the tightly linked set of SNPs such as the KASP assays for gall midge (*Gm4*), bacterial blight (*Xa4*, *xa5*, *xa13*, *Xa21*), anaerobic germination ($qAG_{9.1}$), drought resistance ($qDTY_{3.1}$, $qDTY_{12.1}$), and improved grain yield under DSR ($qGY_{1.1}$) would be very useful in dissecting the "linkage drag". The molecular characterization of lines will be useful in providing more details to rice breeding programs for further improvement in adaptability and yield potential under DSR. The genomic breeding for developing DSR-adapted rice varieties might be further strengthened by combining the superior haplotypes regulating the traits, providing grain yield improvement and adaptability under DSR using haplotype-based breeding [29]. The use of novel approaches, such as forward breeding, haplotype-based breeding and genomic selection in addition to the existing genomic breeding methodologies may accelerate the accuracy and efficiency of genetic gain in rice breeding.

5. Conclusions

The present study was conducted to evaluate a set of advanced breeding lines of rice for seedling establishment, root, yield, yield-related, and quality traits under DSR and TPR conditions. The molecular characterization of lines will be useful in providing more details to rice breeding programs for further improvement in yield potential under DSR. Significant phenotypic variations for root architectural traits, grain yield, and yield-related traits, and the grain quality among genotypes, seasons, treatments, and their interactions (genotype × treatment, genotype × season, treatment × season, and genotype × treatment × season) were observed. The morpho-physiological and quality characteristics play an important role in the success of any variety under direct seeded rice. However, targeted breeding efforts should be diverted towards developing DSR adapted rice varieties with improved grain quality traits under DSR. A total of six advanced breeding lines possessing desirable alleles associated with seedling establishment, root, yield, and yield- related traits with better grain quality have been selected. These promising breeding lines may serve as novel donors to be further used in a genomics-assisted DSR breeding program.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092083/s1, Table S1: Scale for seedling vigor (SES), Table S2: The detailed information on the 106 KASP assays used for molecular characterization of the breeding panel (adapted and modified from Sandhu et al., 2022), Table S3: Mean performance of genotypes under DSR for morpho-physiological and quality traits, Table S4: Mean performance of genotypes under TPR for morpho-physiological and quality traits.

Author Contributions: Conceptualization, N.S. and R.K. (Rupinder Kaur); Phenotyping: H.S.; Genotyping: J.S., H.S. and P.A.A.; writing—original draft preparation, H.S., J.S. and O.P.R.; writing—review and editing, N.S., R.K. (Rupinder Kaur), R.K. (Renu Khanna) and G.S.M.; supervision, N.S.; funding acquisition, N.S. and G.S.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors thank the Department of Biotechnology, India, grant numbers BT/PR31462/ATGC/127/6/2019 and BT/AB/01/IRRI-India/2012, for providing financial support to complete the study.

Data Availability Statement: Not Applicable.

Acknowledgments: We are thankful to Neelam Kumari, Biotechnologist, School of Agricultural Biotechnology, PAU for providing the experimental material (*O. punctata* derived breeding lines) for the present study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. IRRI. *Rice Almanac: Source Book for the Most Important Economic Activity on Earth*; CABI: Wallingford, UK, 2002.
2. FAOSTAT. *FAO Statistical Databases*; Food and Agriculture Organization (FAO) of the United Nations: Rome, Italy, 2020.
3. Farooq, M.; Siddique, K.H.; Rehman, H.; Aziz, T.; Lee, D.J.; Wahid, A. Rice direct seeding, experiences, challenges and opportunities. *Soil Tillage. Res.* **2011**, *111*, 87–98. [CrossRef]
4. Bandillo, N.; Raghavan, C.; Muyco, P.A.; Sevilla, M.A.L.; Lobina, I.T.; Dilla-Ermita, C.J. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: Progress and potential for genetics research and breeding. *Rice* **2013**, *6*, 11. [CrossRef] [PubMed]
5. Koide, Y.; Kobayashi, N.; Xu, D.; Fukuta, Y. Resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). *Jpn. Agric. Res.* **2009**, *43*, 255–280. [CrossRef]
6. Sandhu, N.; Subedi, S.R.; Singh, V.K.; Sinha, P.; Kumar, S.; Singh, S.P. Deciphering the genetic basis of root morphology, nutrient uptake, yield, and yield-related traits in rice under dry direct-seeded cultivation systems. *Sci. Rep.* **2019**, *9*, 9334. [CrossRef] [PubMed]
7. Kumar, V.; Ladha, J.K. Direct seeding of rice: Recent developments and future research needs. *Adv. Agron.* **2011**, *111*, 297–313.
8. Department of Agriculture Chandigarh. 2021. Available online: https://chandigarh.gov.in/dept_agri.htm (accessed on 20 June 2022).
9. Comas, L.; Becker, S.; Cruz, V.M.V.; Byrne, P.F.; Dierig, D.A. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* **2013**, *4*, 442. [CrossRef]
10. Sandhu, N.; Raman, K.A.; Torres, R.O.; Audebert, A.; Dardou, A.; Kumar, A.; Henry, A. Rice root architectural plasticity traits and genetic regions for adaptability to variable cultivation and stress conditions. *Plant Physiol.* **2016**, *171*, 2562–2576. [CrossRef]
11. Sandhu, N.; Rolando, O.T.; Teresa, S.C.; Maturan, P.C.; Jain, R.; Kumar, A.; Henry, A. Traits and QTLs for development of dry direct-seeded rainfed rice varieties. *J. Exp. Bot.* **2015**, *66*, 225–244. [CrossRef]
12. Shahzad, Z.; Amtmann, A. Food for thought: How nutrients regulate root system architecture. *Curr. Opin. Plant Biol.* **2017**, *39*, 80–87. [CrossRef]
13. Dhillon, B.S.; Mangat, G.S. Direct seeded rice in Punjab: Opportunities and challenges. *Technol. Rice Prod.* **2018**, *72*, 535–538.
14. Anonymous. *Package and Practices for Kharif Crops*; Punjab Agricultural University: Ludhiana, India, 2022; pp. 1–17.
15. Uddin, M.R.; Wade, L.J.; Pyon, J.Y.; Mazid, M.A. Rooting behavior of rice cultivars under different planting methods. *J. Crop Sci. Biotechnol.* **2009**, *12*, 17–23. [CrossRef]
16. Yuan, Z.; Cao, Q.; Zhang, K.; Ata-Ul-Karim, S.T.; Tian, Y.; Zhu, Y.; Liu, X. Optimal leaf positions for SPAD meter measurement in rice. *Front. Plant Sci.* **2016**, *7*, 719. [CrossRef] [PubMed]
17. Xu, L.; Li, X.; Wang, X.; Xiong, D.; Wang, F. Comparing the grain yields of direct-seeded and transplanted rice: A meta-analysis. *Agronomy* **2019**, *9*, 767. [CrossRef]
18. Parimala, K.; Raju, C.S.; Kumar, A.H.P.S.S. Evaluation of genotypes for identification of potential restorers and maintainers for development of hybrid rice (*Oryza sativa* L.). *J. Pharmacogn. Phytochem.* **2020**, *9*, 1283–1285.
19. Sharma, N.; Singh, N.; Singh, M.; Bharaj, T.S. Quality characteristics of aromatic fine-grained rice (*Oryza sativa*) genotypes for utilization in basmati improvement. *Ind. J. Agric. Sci.* **2008**, *78*, 42–49.
20. SAS. *The SAS System for Windows*; Version 9.2; SAS Institute Inc.: Cary, NC, USA, 2003.
21. Sandhu, N.; Singh, J.; Singh, G.; Sethi, M.; Singh, M.P.; Pruthi, G.; Kumar, A. Development and validation of a novel core set of KASP markers for the traits improving grain yield and adaptability of rice under direct seeded cultivation conditions. *Genomics* **2022**, *114*, 110269. [CrossRef]
22. Murray, M.; Thopson, W.F. Rapid isolation of molecular weight plant DNA. *Nucleic Acids Res.* **1980**, *8*, 4321–4325. [CrossRef]
23. Perrier, X.; Jacquemoud-Collet, J.P. DARwin Software. 2006. Available online: http://darwin.cirad.fr/ (accessed on 24 June 2022).

24. Kaur, J.; Singh, A. Direct seeded rice: Prospects, problems/constraints and researchable issues in India. *Curr. Agric. Res. J.* **2017**, *5*, 13. [CrossRef]

25. Anandan, A.; Meher, J.; Sah, R.P.; Samantaray, S.; Parameswaran, C.P.; Panneerselvam; Dash, S.K.; Swain, P.; Kartikeyan, P.; Annamalai, M.; et al. Enhancing input use efficiency in direct seeded rice with classical and molecular breeding. In *Rice Research for Enhancing Productivity, Profitability and Climate Resilience*; Pathak, H., Nayak, A.K., Jena, M., Singh, O.N., Samal, P., Sharma, S.G., Eds.; ICAR-National Rice Research Institute: Cuttack, Odisha, India, 2018; pp. 73–89.
26. Panda, S.; Majhi, P.K.; Anandan, A.; Mahender, A.; Veludandi, S.; Bastia, D.; Ali, J. Proofing Direct-Seeded Rice with better root plasticity and architecture. *Int. J. Mol. Sci.* **2021**, *22*, 6058. [CrossRef]
27. Bhadru, D.; Reddy, D.L.; Ramesha, M.S. Correlation and path coefficient analysis of yield and yield contributing traits in rice hybrids and their parental lines. *Electron. J. Plant Breed.* **2011**, *2*, 112–116.
28. Chen, Y.T.; Song, J.Y.; Chuan, Y.; Zhu, G.L.; Hong, X.F. Comparison of yield performance and rice quality between direct-seeded and hand-transplanted rice under different nitrogen rates in Eastern China. *Afr. J. Agric. Res.* **2020**, *16*, 875–883.
29. Sagare, D.B.; Abbai, R.; Jain, A.; Jayadevappa, P.K.; Dixit, S.; Singh, A.K.; Challa, V.; Alam, S.; Singh, U.M.; Yadav, S.; et al. More and more of less and less: Is genomics-based breeding of dry direct-seeded rice (DDSR) varieties the need of hour? *Plant Biotechnol. J.* **2020**, *18*, 2173–2186. [CrossRef] [PubMed]