Haplogenic quantitative effects regulate flooded germination, subsequent water deficit stress, and recovery in direct seeded rice

Parameswaran Chidambaranathan[1], S. Sabarinathan[1], Priyadarsini Sanghamitra[1], Goutam Kumar Dash[1], Devraj Lenka[2], Cayalvizhi Balasubramania[1], Kishor Jeughale P[1], Raj Kishore Sahoo[1], Sangamitra Samantaray[1], Prabhukarthikeyan SR[1], Devanna BN[1], Jawahar Lal K[1], Ramlakhan Verma[1], Rameswar Prasad Sah[1], and Patra B C[1]

[1]ICAR - National Rice Research Institute
[2]Odisha University of Agriculture and Technology

September 30, 2022

Abstract

Rice genes namely TPP7 (*Trehalose Phosphate Phosphatase 7*), Sub1A (*Submergence 1a*) and their interactions regulate tolerance to submergence at germination and seedling stage, respectively. Sequential stress of initial submergence during the germination and subsequent seedling stage water deficit stresses are not properly addressed in direct seeded rice cultivation. In our initial linkage disequilibrium and meta-QTL analysis, Sub1 locus and TPP7 genes co-segregated for tolerance to submergence at germination and seedling stage and were in linkage disequilibrium only in aus subpopulations of rice. Phenotypically, root traits were positively correlated (correlation coefficient = >0.8) with seedling growth in germination under submergence and also in subsequent water deficit stresses in recombinant inbred lines developed from N22 and Bhalum 2 cross. Further, favourable alleles of Sub1 in N22 and 3'-UTR allele of TPP7 in Bhalum 2 enhanced the root traits (>20 per cent) and seedling survival (>25 per cent), respectively in germination under submergence stress. Additionally, the interaction of N22 alleles of Sub1 and TPP7 significantly enhanced the culm diameter and root dry weight in submergence at germination stress. Interestingly, two QTLs with high additive effects associated with sixteen different traits for submerged germination and water deficit tolerance traits were identified within the genomic regions spanning Sub1 and TPP7 genes (~4 Mbp) in Chr09 indicative of genomic region effects on the trait response rather than Sub1 and TPP7. Further, favourable haplotype within the Sub1 and TPP7 genomic region had an epistatic effect on trait responses and enhanced the crown root number, root dry weight, and specific root area by 11.45%, 15.69%, and 33.15% respectively in flooded germination condition, indicative of haplo-allelic contribution in trait response. A wall-associated kinase 79 (*WAK79*) and malectin-like receptor-like kinase 59 (*MRLK59*) were identified as candidate genes, that through regulation of cell wall elongation might coordinate the 'haplogenic model' of quantitative trait response under flooded germination, recovery, and subsequent water deficit conditions and thus, favourable haplotypes could be employed in direct seeded rice improvement.

Sabarinathan Selvaraj[1,2], Priyadarsini Sanghamitra[1], Goutam Kumar Dash[1,3], Parameswaran Chidambaranathan[1*], Devraj Lenka[2], Cayalvizhi Balasubramaniasai[1], Kishor P Jeughale[1], Raj Kishore Sahoo[1], Sangamitra Samantaray[1], Prabhukarthikeyan SR[4], Devanna BN[1], Jawahar Lal K[1], Ramlakhan Verma[1], Rameswar Prasad Sah[1] and B C Patra[1]

[1]*Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India*

[2]*Department of Seed Science and Technology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India*

1

[3]*Department of Biochemistry and Crop Physiology, Centurion University of Technology and Management, Paralakhemundi, Odisha, India*

[4]*Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India*

*Corresponding author

Parameswaran C, PhD

Scientist

Crop Improvement Division

ICAR-National Rice Research Institute (ICAR-NRRI)

Bidyadharpur, Cuttack-753006, Odisha, India

E-mail: *parameswaran.c@icar.gov.in*

Abstract

Rice genes namely TPP7 (*Trehalose Phosphate Phosphatase 7*), Sub1A (*Submergence 1a*) and their interactions regulate tolerance to submergence at germination and seedling stage, respectively. Sequential stress of initial submergence during the germination and subsequent seedling stage water deficit stresses are not properly addressed in direct seeded rice cultivation. In our initial linkage disequilibrium and meta-QTL analysis, Sub1 locus and TPP7 genes co-segregated for tolerance to submergence at germination and seedling stage and were in linkage disequilibrium only in aus subpopulations of rice. Phenotypically, root traits were positively correlated (correlation coefficient = >0.8) with seedling growth in germination under submergence and also in subsequent water deficit stresses in recombinant inbred lines developed from N22 and Bhalum 2 cross. Further, favourable alleles of Sub1 in N22 and 3'-UTR allele of TPP7 in Bhalum 2 enhanced the root traits (>20 per cent) and seedling survival (>25 per cent), respectively in germination under submergence stress. Additionally, the interaction of N22 alleles of Sub1 and TPP7 significantly enhanced the culm diameter and root dry weight in submergence at germination stress. Interestingly, two QTLs with high additive effects associated with sixteen different traits for submerged germination and water deficit tolerance traits were identified within the genomic regions spanning Sub1 and TPP7 genes (~4 Mbp) in Chr09 indicative of genomic region effects on the trait response rather than Sub1 and TPP7. Further, favourable haplotype within the Sub1 and TPP7 genomic region had an epistatic effect on trait responses and enhanced the crown root number, root dry weight, and specific root area by 11.45%, 15.69%, and 33.15% respectively in flooded germination condition, indicative of haplo-allelic contribution in trait response. A wall-associated kinase 79 (*WAK79*) and malectin-like receptor-like kinase 59 (*MRLK59*) were identified as candidate genes, that through regulation of cell wall elongation might coordinate the 'haplogenic model' of quantitative trait response under flooded germination, recovery, and subsequent water deficit conditions and thus, favourable haplotypes could be employed in direct seeded rice improvement.

Keywords

Germination under submergence, water deficit, Sub1, TPP7, favourable alleles, additive effects

1. INTRODUCTION

Global food security is challenged by the increasing effects of climate change (Furuya and Kobayashi, 2010). Cereal grains make up more than half of the world's daily caloric intake and are essential for food security throughout the world. Rice is the world's second-largest cereal crop that consumes more water to complete its life cycle than any other cereal crops. In rice cultivation, puddling and field preparation for transplanting and maintenance of standing water in the field up to physiological maturity are the major reasons for the high water requirement (Renault, 2004). Rice is primarily grown in transplanted conditions wherein seeds are sown in nurseries and seedlings are transplanted in the puddled fields. Flooding and water deficit stress severely affect the optimum water requirements of paddy cultivation and significantly affect the yield. Additionally,

2

progressive water scarcity for agricultural activity due to high demand for various anthropogenic use, labour scarcity, and high production cost are the major concerns for the profitability of puddled transplanted rice (Matloob et al., 2014; Turral et al., 2011). Alternatively, direct seeded rice (DSR) cultivation is a viable and climate-resilient rice cultivation method (Sudhir-Yadav et al., 2011). DSR occupies about 27-29 Mha of the rice area in South Asia, South East Asia, and East Asia, accounting for ˜ 20% of the total cultivated rice area (Pandey and Velasco, 2002). Also, direct seeded rice (DSR) cultivation reduces up to 60% of the water requirement in rice cultivation due to the absence of nursery preparation and field puddling practices (Pathak et al., 2011). Since rice consumes one-fifth of fresh water in the world for irrigation (Kumar and Ladha, 2011), DSR has become an effective climate-resilient strategy for meeting the demand of the urban freshwater requirement and sustainable rice production (Jat et al., 2022). Besides, DSR as compared to PTR also contributes to lowering greenhouse gas emissions (Bhullar et al., 2016).

Seedling establishment and weed infestation are the significant problems commonly encountered by farmers in DSR cultivation (Jabran and Chauhan, 2015; Rao et al., 2007). The simultaneous emergence of weeds along with rice seedlings at the early stages of crop growth in the DSR system (Kumar et al., 2013) causes yield losses of up to 40% and the absence of adequate weed control strategies would result in 90% loss (Mahajan and Chauhan, 2013). Poor crop establishment was another major problem in DSR due to flood/waterlogging caused by unanticipated rainfall and poor field levelling (Ismail et al., 2012). Interestingly, waterlogging in DSR during germination is quite helpful for the farmers to reduce weed emergence provided the rice varieties are capable of germination and seedling growth under prolonged flooded conditions for 2-3 weeks. However, floods or submergence after seed sowing in DSR severely affect the crop stand in direct seeded rice; adding to that a 90% reduction in seed establishment was observed in the popular variety, IR64 (Chamara et al., 2018). Thus, rice varieties with anaerobic germination potential and better early seedling growth can provide an effective weed management strategy in DSR under prolonged flooded conditions during germination (Mahajan and Chauhan, 2013).

The ability of rice seeds to germinate under submergence and maintenance of seedling vigour after emergence is advantageous in DSR for proper seedling establishment and weed competitiveness (Miro and Ismail, 2013). Rice plants grow in waterlogged and flooded soils, unlike other cereal crops because of their ability to develop aerenchyma tissues that facilitate aeration of roots and rhizosphere (Zhang et al., 2017). However, rice seeds either failed to germinate or shows retarded growth due to hypoxia or anoxia under submerged germination conditions. In severe cases, seed embryos become non-functional due to poor breakdown and mobilisation of stored carbohydrate (Mondal et al., 2020a). In general, seeds cope up with oxygen deficiency by rapid emergence and elongation of coleoptiles to reach above the water-air interface for gaseous exchange with the submerged parts (Ismail et al., 2009). Alternatively, shifting to anaerobic metabolism under submerged germination conditions provides the necessary energy for coleoptile development (Lal et al., 2018). In such conditions, starch catabolic enzymes such as α-amylase release glucose from starch to support the elongation of coleoptiles through fermentative metabolism (Ismail et al., 2009; Magneschi and Perata, 2009). In rice, a major QTL namely qAG-9-2 providing significant seedling survival under anaerobic germination stress was identified in a mapping population between IR64 (Susceptible) and Kho Hlan On (Tolerant to anaerobic germination) (Angaji et al., 2010). Later, the trehalose phosphate phosphatase 7 (TPP7) gene was characterised as a causal gene for anaerobic germination tolerance (Kretzschmar et al., 2015). Further, introgression of AG2 showed significantly higher seedling emergence than intolerant checks (Septiningsih et al., 2013). Besides, 23 QTLs were identified for anaerobic germination tolerance in rice using linkage disequilibrium and bi-parental mapping strategies (Angaji, 2008; Angaji et al., 2010; Baltazar et al., 2019; Saimohan et al., 2021). However, most of these QTLs were identified for germination tolerance, survival percentage, and coleoptile length in rice. The influence of roots in germination under submergence was not been adequately understood so far.

Root traits are highly crucial in abiotic stress tolerance in rice. Deep Rooting 1 (DRO1) was reported to provide significant tolerance to water limitation in upland rice cultivation (Uga et al., 2011). DRO1s homolog, namely a significant number of surface roots (qSOR1) provides tolerance to salinity stress (Kitomi et al., 2020). Further, early root growth contributed by *PsTol1* in the seedling stage provides low phosphorus

3

tolerance in rice (Gamuyao et al., 2012). Additionally, Sub1a mediated higher root elongation rate and root tip viability under submergence significantly enhanced the seedling recovery after submergence in rice (Bui et al., 2019). Therefore, root number and root elongation under anaerobic germination/waterlogged condition may be beneficial for the plants, mainly in the rainfed ecologies where the chances of sequential occurrence of submergence during germination and water deficit stress after seedling establishments are relatively high. Under this situation, genotypes with anaerobic germination potential along with promising root architecture will be more adaptable. However, previously it was reported that root growth was highly suppressed in germination under submergence conditions in rice (Miro and Ismail, 2013). Moreover, there was no proper genetic understanding of the role of root traits and seedling survival in submergence stress during germination in rice.

DSR has excellent potential to become an effective climate-resilient strategy for sustainable rice production in rainfed ecologies (Jat et al., 2022). Moreover, the irrigated upland ecosystem might also be subjected to water deficit or drought stress owing to a lack of irrigation or rainfall. Therefore, varieties with the potential of germination under submergence and water deficit tolerance under the vegetative stage are essential in DSR for climate resilience. Thus, the aim of the present work is, 1) to characterise the influence of roots in germination under submergence and seedling survival in rice, 2) to understand the differential root response in aerobic and submerged germination conditions, 3) to assess the role of roots formed during submerged germination condition on subsequent water deficit stress and recovery 4) to identify the favourable alleles/haplotypes of genomic regions between TPP7 and Sub1 influencing root traits in germination under submergence stress and subsequent water deficit stresses.

2. MATERIALS AND METHODS

2.1 Plant materials

Five rice varieties, Bhalum 2 (B2; Rainfed upland ecology), Nagina 22 (N22; aus cultivar), CR Dhan 201 (aerobic ecology), CR Dhan 205 (aerobic ecology), and IR 20 (Irrigated ecology), were used in this study. The parentage, ecology, and varietal traits of five varieties are given in Table S1. The seeds of these varieties were line transplanted and multiplied in the rice farm at ICAR-National Rice Research Institute (NRRI), Cuttack, during the dry season of 2020-21. The matured single panicles of the five varieties were harvested, threshed, oven dried at 40 for five days and used in this study.

2.2 Screening of rice varieties for root and shoot traits and weed control ability in germination under submergence

The screening was done under three conditions with the aforementioned varieties. 1) aerobic condition with manual weed management 2) aerobic condition with no weed management and 3) germination under submergence (GUS) condition. For evaluation, pot based method was designed to study the root and shoot traits in aerobic (AR) conditions and GUS stress conditions. The pot screening method includes the preparation of pots (23 x 27.5 cm) filled with aerobic soil collected from the aerobic farm at ICAR-NRRI, Cuttack. The aerobic soil was finely pulverised, and an equal amount of soil (~7.5 kg) was filled in the pots. The soil was filled up to ~22 cm in the pots for an aerobic condition with and without weed management treatments.

For GUS, the seedling tray based method and field evaluation were used previously for the seedling survival studies in rice (Kretzschmar et al., 2015; Mondal et al., 2020b; Septiningsih et al., 2013). Briefly, tray based evaluation includes direct sowing of dry seeds and maintaining the flooded condition for up to 21 days for measurement of seedling survival percentage. In contrast, pot based method was designed to study seedling survival, and root traits in GUS stress conditions in our experiment (Figure S1 a-c). The pot screening method includes the preparation of pots (23 x 27.5 cm) filled with aerobic soil and the height of the soil layer maintained in the pots was ~ 13 cm (~4 kg). Seeds of five varieties were sown up to 1 cm soil depth (Chamara et al., 2018) in the potting soil and immediately, water was filled up to ~10 cm without disturbing the topsoil layer (small size stones were kept inside a corner of the pot, and slowly water was filled on the stones kept in one corner of pots using the wash bottle) for GUS. All the pots were kept under a shade net house facility

during the experiments. For each variety, 20 seeds were sown with 10 seeds each in two small compartments of a quarter size pot area and maintained as two replications in this experiment. On the 21st day after sowing (21 DAS), seedling survival percentage were evaluated as previously reported by (Kretzschmar et al., 2015; Septiningsih et al., 2013). Briefly, seedling survival is counted as the emergence of seedlings above the 10 cm of water layer under submerged conditions. Besides, the culm diameter of the seedlings was randomly measured for three seedlings per replication using a digital vernier calliper. Further, root and shoot areas were observed using image-based methods as reported by (Anandan et al., 2020). The image of the seedlings was captured using a Nikon D5600 camera, and for each image, a ruler was placed near the plants and labelled properly for further analysis. These images were preprocessed and analysed manually using the open-source ImageJ software (*https://imagej.nih.gov/ij/*). Additionally, the number of crown roots, root length, root dry weight, and specific root area were also measured as reported by (Fernández et al., 2009). For shoot traits, shoot length, number of leaves, relative chlorophyll content (SPAD 502 Plus Chlorophyll Meter), shoot dry weight, and specific shoot area were measured for all three treatments. Three seedlings per replicate and two replications were taken and used in data analysis for all the measurements. In addition, weed density (number of weeds/pot) in all three treatments with two replicates was counted on 21 and 28 DAS.

2.3 Mapping population development

The two parents, N22 and Bhalum 2 which showed varied root traits in our initial experiment, were used for the development of recombinant inbred line (RIL, F_7) mapping population in 2017-18. Genuine F_{1s} identified were advanced through the single seed descent method advancement of filial generation for RIL development (Collard et al., 2005). The seeds of the mapping population comprising 128 RILs and two parents were also multiplied during the dry season of 2020-21; single true-to-type panicles were harvested and used for further study.

2.4 Climate data analysis

The climatology data related to consecutive dry days (CDD) and consecutive wet days (CWD) were retrieved from the climate change knowledge portal (Portal, 2017). These two climate parameters were retrieved for the rice growing countries in the world (*https://www.atlasbig.com/*). The mean ERA5 reanalysis data of CDD and CWD for the rice growing countries was retrieved for 20 years from 1991 to 2020. Additionally, the median percentile of annual and monthly CMIP6 projections data for CDD and CWD were also retrieved for two periods (2020-2039 and 2040-2059) for the future climate scenario of SSP1-1.9 which is the most optimistic scenario projected for understanding the climate change effects. Further, CDD and CWD values for three time periods (1991-2020, 2020-2039 and 2040-2059) were analysed for the months of June, July, and August coinciding with the rice crop's sowing and seedling growth season. Besides, a geographic heat map add-in in MS Excel was used to visualise the effect of climate change on the CDD and CWD in rice growing regions worldwide (Table S2).

2.5 Stress treatments schedule

A total of five treatments were administered to the mapping population and the parents to understand the rice genotypes' response to multiple stresses. Initially, seeds were subjected to GUS for 21 days (21 DAS) and then followed by seven days of germination under submergence stress recovery (GUSR) for up to 28 DAS. This recovery stage was followed by seven days of water deficit stress (WD) up to 35 DAS and continued by severe water deficit stress (SWD) for up to 42 DAS. Furthermore, severe water deficit stress recovery (SWDR) was measured after seven days of the recovery period (49 DAS) (Figure 1a-f). This schedule was followed for all the mapping population lines and parents with two replications per treatment. The basis of this stress schedule is to understand the effect of root development during GUS on GUSR, WD, SWD, and SWDR. In addition to this treatment schedule, individual lines grown under aerobic (AR) conditions (17 DAS) were used as control.

2.6 Germination under submergence stress

A total of 128 RILs and two parents (N22 and B2) were subjected to different stress schedules. The pot based method mentioned above was used to evaluate the RILs. Briefly, each pot was compartmentalised into eight layers using wooden sticks, and one-fourth of the pots were used to evaluate each line. Therefore, four lines were assessed for shoot and root parameters in GUS conditions using a pot based evaluation system. For each line, 20 seeds were sown with 10 seeds each in two small compartments of a quarter size pot area and maintained as replication in this experiment. Germination of seeds under GUS identified with white plumule emergence was observed from the 6th day up to 14th day in GUS. Then, no. of seeds germinated from the 6th to 14th days was used to measure the germination rate (average number of seeds germinated/day). Similarly, seedling survival percentage was also evaluated from the 12th to the 21st days of emergence as previously reported by (Kretzschmar et al., 2015; Septiningsih et al., 2013). However, seedling survival was counted as the emergence of seedlings above the 10 cm of water layer under submerged condition. The number of seedlings surviving from the 12th to 21st days of submerged condition was used to measure the seedling survival rate (number of seedlings surviving per day).

In addition to the aforementioned traits (root and shoot traits), the culm strength of seedlings was also measured for all the lines in the mapping population as well as the two parents. Additionally, on the 21st day of submergence condition, seedlings were gently pushed using the fingertip and then the level of resistance shown by the seedlings was scored as; 0- not survived, 1- Strong (no bending), 3- Moderately strong (few seedlings were bending), 5- Intermediate (half of the seedling bending), 7- Weak (Most of the seedlings fallen on the water surface) and 9- Very weak (completely fallen on the water surface). In total, seventeen traits were measured during GUS (Table S3). After the submerged germination conditions (21 DAS), the water level was reduced to ~ 2-3 cm in the pots and seedling recovery traits namely plant height (cm), the number of leaves (nos.) and a number of tillers (nos.) were measured for mapping population and parents on 28 DAS.

2.7 Water deficit stress

The influence of roots developed during GUS and WD stress response was studied in the pot conditions. Especially, the number of seedlings that survived after GUSR varied within the pots which could influence the WD response. Therefore, an equal number of seedlings were maintained in each pot for the WD experiments. For this experiment, six surviving seedlings per line (three seedlings per replication) in GUSR were used and the remaining seedlings were removed. Altogether, 96 lines that survived under submergence stress and recovery in germination were subjected to WD stress for a week (29-35 DAS) and the following parameters were measured: plant height (cm), leaf length (cm), leaf width (cm), and number of tillers (nos.), SPAD, and canopy temperature (). The WD stress was administered by withholding the irrigation to the pots.

2.8 Severe water deficit stress and recovery

Seedling growth of the surviving seedlings after one week of WD stress varied among the lines in the population. Thus, variation between the pots would impair the recovery analysis and inference. Therefore, a severe water deficit (SWD) stress and recovery (SWDR) experiment were executed to understand the seedling response. The moisture content of <10% in the soil and relative water content of < 30 per cent were considered SWD stress (Olagunju et al., 2022) and the response of the seedlings was measured for each pot under severe stress. Soil moisture content was measured using an HH2 moisture meter, DELTA-T Devices, Cambridge, England. After SWD stress, recovery was studied by irrigating the pots with up to 5 cm of water and recovery was measured after seven days of sufficient irrigation. The traits measured for understanding the variation within the populations for SWDR were number of recovered leaves, number of recovered plants, and seedling recovery percentage.

2.9 Seedling and root vigour under aerobic condition

Approximately 50 seeds each of two parents and 128 RIL mapping population developed from a cross between B2 and N22 were sown in aerobic soil, and two replications were maintained for both parents and RILs. The aerobic condition was maintained by irrigating the soil to field capacity and by proper drainage by forming bunds to facilitate excess water flow. The growth of the seedlings was measured on the 17th day after sowing as the initial three days of sowing were considered a germination period. Most of the lines showed

6

uniform germination under aerobic conditions. The selection of the 17th day as the best for phenotyping was considered based on the response of these lines in the GUS. For example, most of the lines showed initial germination only after 6-7 days in the GUS condition. Then, data collection for phenotypic traits was taken only on the 21st day after the initiation of submergence stress from germination. Thus, actual data collected during the submerged germination conditions was seventeen days after germination. The phenotypic traits namely shoot length (cm), root length (cm), number of leaves (nos), number of crown roots (nos), culm diameter (mm), root area, shoot area, dry weight of shoots (g) and roots (g), specific shoot area, and specific root area were collected from three seedlings of each replicates and used for the analysis.

2.10 TPP7-In/Dels development

TPP7 gene was previously characterised for its function in anaerobic germination. However, the TPP7 genomic region was deleted entirely in the susceptible genotype IR64 (Kretzschmar et al., 2015). Thus, TPP7 gene specific In/Dels were designed to understand the presence/absence of TPP7 in released rice varieties. For designing the TPP7 specific In/Dels markers, the locus id of the TPP7 (LOC_Os09g20390, Chr09:12251875-12254087) was searched for the single nucleotide polymorphism, and indels in the SNP seek database against all the subpopulations of the 3k filtered SNPs and Indels database (*https://snp-seek.irri.org/_snp.zul*). Further, the JBrowse option in the SNP seek database was used to map the in/dels with a size of more than 10 bp within the gene region of TPP7. Then, the primer 3plus tool (Untergasser et al., 2007, p. 3) was used to design the primers flanking the in/dels position. The details of primer sequences are given in Table S4, and a schematic diagram of the gene specific primer design is given in Figure S2 a-e.

2.11 Linkage disequilibrium and nucleotide diversity analysis

Single nucleotide polymorphism present in the trehalose phosphate phosphatase 7 (*TPP7* : (LOC_Os09g20390, Chr09:12251875-12254087) and submergence 1 locus (*Sub1B* : LOC_Os09g11480, Chr9: 6406039-6404474 and Sub1C, LOC_Os09g11460, 6389789 - 6387891) was retrieved from SNP seek database using the locus id of the genes as the query against the filtered SNPs in database. The SNPs identified in all three genes were used to find the linkage disequilibrium (LD) between the selected genes. The LD analysis was performed using the TASSEL software, version: 5.2.80 (Bradbury et al., 2007) with the following parameters: sliding window option with a window size of 50 and heterozygous base calls were considered missing values. Further, R^2 and D prime values of the SNPs combinations were visualised using the LD plot option in the TASSEL software. Further, nucleotide diversity indices namely Tajima D, pi per base pair and theta per base pair were analysed in TASSEL software, version: 5.2.80.

2.12 Construction of consensus genetic map and meta QTL analysis for submergence and anaerobic stress tolerance

Six published QTL mapping studies for anaerobic germination and submergence conditions were selected (Table S5) for meta-QTL analysis. A few reported QTLs were removed from the analysis due to missing values or parameters. Further, two types of input data text files were prepared for each study: genetic map file and QTL information file, as mentioned in BioMercator's user guide. The consensus map and meta QTL was constructed using Biomercator v4.2.3 (Veyrieras et al., 2007) available at (*http://bioinformatics.org/mqtl/wiki/*).

2.13 Genotyping, QTL mapping, and candidate gene identification

Total genomic DNA was extracted from young leaves by using a geno/grinder (SPEX 1600 MiniG(r)) followed by the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). Further, DNA quantification, PCR profile, agarose gel, gel documentation and scoring of alleles were followed as reported in our earlier report (Chidambaranathan et al., 2021). The genotypic data were used to construct the linkage maps, and the Kosambi map function was used to calculate the genetic distance (D, 1944). QTLs were detected using the inclusive composite interval mapping (ICIM-ADD) and interval mapping (IM-ADD) function of QTL IciMapping software (Meng et al., 2015). For the identification of candidate genes in the QTL region, mean normalised read counts per million (CPM) data in the gene expression omnibus database of NCBI (Accession number: GE136885) was analysed. The mean CPM values of genes in submergence and

7

anaerobic stress were compared and genes having significant differences in read counts between two different stresses were selected as candidate genes.

2.14 Statistical analysis

Descriptive statistics and linear regression analysis were performed using the data analysis option in MS Excel. Correlation analysis was performed using corrplot (Wei et al., 2017) in R software. Heat map was constructed using the heatmap function in R. The significance of the coefficient of correlation was analysed at 5% and 1% level of significance using SPSS software version 23.0. Analysis of variance (ANOVA) was used to test the significance of different treatments at a 5% level of significance. Z test was used to understand the significant difference between two groups of mean values at a 5% significance level. Haplotype group's mean values were analysed through Fisher's least significant difference (LSD) method in XLSTAT software, version 2014.5.03. Genetic diversity analysis of rice varieties was performed using PowerMarker software version 3.25 (Liu and Muse, 2005).

3. RESULTS

3.1 Shoot and root traits variation within varieties for germination under submergence conditions

Root traits in GUS stress are not properly studied. Thus, five varieties were initially analysed for the trait response in aerobic (with and without weed management) and (GUS) conditions. The analysis of variance showed there was no significant difference between the three treatments (Aerobic with manual weed management; aerobic without weed management; and GUS). The descriptive statistics in three different treatments for 17 different traits among five genotypes were analysed for significance (Table S6). This analysis showed the 14th day number of leaves (P=0.013*), 21st day number of leaves (P=0.042*), and crown root number (P=0.033*) were found to be highly significant between the three treatments.

Out of five genotypes, CR Dhan 201 and CR Dhan 205 didn't germinate in the GUS condition but showed > 80% germination under aerobic conditions. Almost all of the traits showed insignificant variation within the genotypes, however, shoot (P=0.02*) and root dry weight (P=0.042*) showed high significance only in the GUS condition. Besides, shoot length, root length, number of leaves, culm diameter, total number of crown roots, shoot area and root area had no significant variation in all three treatments, although IR 20 (15.95 nos.) and B2 (14.50 nos.) had a higher number of crown roots in aerobic conditions (Figure S3). Similarly, shoot area, root area, specific shoot area and specific root area of B2 and N22 were much higher compared to IR 20 in GUS condition. Furthermore, no weeds were observed in germination under submerged conditions up to 21 DAS and only two weeds were present at 28 DAS. In contrast, > 40 numbers of weeds were observed in aerobic conditions without weed management in 21 DAS, and ˜60 weeds were present in 28 DAS (Figure S4).

Correlation analysis was performed for 17 different traits of three treatments. The highest correlation coefficient (r = 0.97**) was found between the 14th day shoot length and 21st day shoot length. Besides, recovery traits in GUS namely 14th day shoot length, number of leaves, culm diameter, and SPAD were found to have a high correlation coefficient of 0.80**, 0.88**, 0.83**, and 0.89**, respectively with crown root number. Similarly, root length was also highly correlated with recovery traits (0.81 to 0.93) (Figure S5). Thus, root traits were found to be highly important for seedling vigour and recovery in GUS.

3.2 Continuous variation in the mapping population under different stress conditions

One hundred and twenty eight recombinant inbred lines along with two parents were analysed for different stress conditions (Aerobic, GUS, GUSR, WD, and SWDR). The descriptive statistics of 41 different traits analysed are given in Table S7. The kurtosis value of different traits ranged from -1.16 (leaf width under WD stress) to 23.01 (no. of leaves in aerobic condition). Similarly, the skewness value of different traits ranged from -3.14 (no. of leaves in aerobic condition) to 3.31 (specific shoot area in GUS). Among the different traits, shoot (P=0.00**) and root dry weight (P=0.00**) in GUS stress, culm diameter (P=0.02*), shoot (P=0.00**) and root dry weight (P=0.00**) in aerobic condition, and leaf width (P=0.04*) in WD stresses

showed highly significant difference at 95% confidence interval. Further, the mean shoot dry weight was 0.02 g/plant and ranged between 0.00 to 0.09 g/plant. Similarly, the mean root dry weight was 0.004 g/plant and varied between 0.00 to 0.02 g/plant in GUS stress. The culm diameter mean value was found to be 0.87 mm and ranged between 0.00 to 1.30 mm, shoot dry weight 0.03 g/plant varied between 0.00 to 0.07 g/plant and root dry weight 0.01 g/plant ranged between 0.00 to 0.02 g/plant in aerobic conditions. Additionally, the mean value of leaf width was 0.33 cm and ranged between 0.00 to 0.78 cm in WD stress conditions.

3.3 Extent of transgressive segregation for different traits in mapping populations

The extent of transgressive segregation was analysed for different stress treatments using transgressive index (TI) parameters (Table S8a,S8b). Though, the analysis of variance for the transgressive index between different stress treatments was not significant (P= 0.35), the mean transgressive index value for germination under submergence (TI = 37.33) and WD stresses (TI = 35.02) was relatively higher than the other three stresses. Further, both recovery experiments showed the least mean transgressive index values (TI = 3-5). Among all stress treatments, nine traits showed TI of > 25 and specifically six out of nine traits (Germination rate, shoot length, root length, shoot dry weight, root dry weight, and specific root area) were related to germination under submerged conditions. Besides, culm diameter (aerobic condition), no. of tillers and canopy temperature (WD) also showed high TI values. In addition to the transgressive index values, many novel traits were also observed in the mapping population. Out of 128 lines, nine lines (BN55, BN87, BN89, BN100, BN102, BN103, BN106, BN115, and BN10) were germinated only after 29-35 DAS in WD condition (52.2 per cent germination) indicative of secondary dormancy. Further, six lines (BN3, BN4, BN5, BN15, BN59, and BN104) had greater than 90 per cent germination on the 14th day, and three lines (BN19, BN59, and BN104) showed greater than 90 per cent survival on the 21stday, and 16 lines had strong culm strength (BN1, BN3, BN5, BN7, BN34, BN35, BN36, BN40, BN53, BN59, BN64, BN71, BN76, BN97, BN104, and BN114) in GUS conditions (Figure S9c).

3.4 Root traits correlation with different traits associated with germination under submergence and recovery

Correlation analysis was performed for nineteen different traits in GUS in the mapping population comprising 130 lines including two parents. The highest correlation coefficient (r = 0.96**) was found between the seedling survival rate and survival percentage on the 21st day of GUS. Further, the correlation coefficient in GUS was between 0.79 and 0.96 for the traits namely per cent survival on the 21st day, per cent germination on the 14th day, survival rate, and germination rate. Moreover, shoot length on 21st day was found to be highly positively correlated with number of leaves (r = 0.88**), culm diameter (r = 0.84**), number of crown roots (r = 0.82**), and root length (r = 0.75**). Besides, number of crown roots in GUS condition on 21st day was found to be highly positively correlated with culm diameter (r = 0.86**), no. of leaves (r= 0.83**), shoot length (r = 0.82**), and root length (r = 0.81**). Further, root length on the 21st day in submergence under germination was found to be significantly correlated with 28th day plant height (r=0.42**), the number of leaves (r=0.40**), and the number of tillers (0.31**). Similarly, crown root number on the 21st day under submergence was found to be significantly correlated with 28th day plant height (r=0.40**), the number of leaves (r=0.38**), and number of tillers (r=0.33**). Thus, root traits are highly correlated with shoot related traits in the recovery stages after GUS stress (Figure 3a).

3.5 Effect of climate change on the frequency of germination under submergence and subsequent water deficit stress conditions in rice growing regions of the world

A seed sowing season of rice is majorly June to July, where high consecutive wet days (CWD) might result in GUS, and high consecutive dry days (CDD) may lead to WD conditions. Out of 114 different rice growing countries, one country was predicted to have greater than one week of CWD from June to August of 2020-2059 relative to 1991 to 2020 (Table S9). Further, seven and eight countries had predicted CWD of less than seven days in the months of June, July and August of 2020-2059. Furthermore, consecutive dry days (CDD) of more than one week were predicted in four countries in the months of June and August, and for five countries in July relative to the reference year of 1991-2020. Moreover, the major rice producing countries

9

of India and Pakistan were predicted to have significant WD stress in the months of June and July (Figure 2a-f).

3.6 Root growth in germination under submergence (GUS) correlation with water deficit stress and recovery

Six traits namely plant height, leaf length, leaf width, number of tillers, SPAD, and canopy temperature were correlated with sixteen traits of GUS conditions. The study revealed the existence of a strong correlation between the speed of germination under the submerged condition with the survival percentage under WD conditions. Among these traits, 21st day survival percentage was highly correlated with leaf length (r=0.63**) under WD stress. Further, 21st root length was significantly correlated with plant height (r=0.35**), leaf length(r=0.35**), leaf width(r=0.27**), number of tillers(r=0.35**), and SPAD (r=0.32**) under WD stress. Similarly, crown root number was significantly correlated with plant height (r=0.35**), leaf length(r=0.35**), leaf width(r=0.29**), number of tillers(r=0.35**), SPAD (r=0.33**) and canopy temperature (r=0.23**) under WD stress. Further, root dry weight in GUS conditions was also significantly correlated with all the traits except canopy temperature in WD stresses. Besides, root area in GUS was significantly correlated with all traits except leaf width and canopy temperature in WD stress (Figure 3b).

After one week of WD stress, SWD was imposed for a week and recovery traits namely, no. of recovered leaves, total recovered plants, and recovery percentage also correlated with different traits observed in GUS conditions. A high correlation coefficient was observed between 21st day survival and survival rate (r=0.96**). Further, root length in GUS was significantly correlated with recovery percentage (r=0.32**) and crown root number were also significantly correlated with recovery percentage (r=0.18*). This correlation study concluded that the root growth occurring during the initial GUS might assist plants to withstand WD stress when it occurs immediately after the submergence (Figure 3c).

3.7 Relationship of root growth between aerobic and germination under submergence conditions

Twelve traits in aerobic conditions were correlated with sixteen traits in GUS conditions. Root traits under aerobic conditions showed a significant correlation with root growth in the GUS condition. Specifically, root dry weight under the GUS was significantly correlated with root length (r=0.315**) and root surface area (r=0.309**) under aerobic conditions. Also, root length under GUS was positively correlated with root length under aerobic conditions (r=0.328**). This indicates that genotypes showing higher root length and root dry weight in aerobic conditions can maintain similar growth even in GUS conditions. Additionally, root area under GUS was strongly correlated with root area (r=0.264**), and specific shoot area (r=0.239**) under aerobic conditions (Figure 3d).

3.8 TPP7 and Sub1 nucleotide diversity in rice subpopulations

The nucleotide diversity statistics were compared for all rice populations (indica, japonica, aus, aromatic, and admixtures) and specific for aus sub-populations. There were 70 and 273 single nucleotide polymorphisms (SNPs) present in the TPP7 and Sub1 locus, respectively identified in the 3K filtered database of rice. The nucleotide diversity for the Sub1 locus was relatively higher in aus sub-populations (0.243) than in all rice populations (0.153). In contrast, nucleotide diversity of TPP7 was higher in all rice populations (0.222) than in aus sub-population (0.017). Moreover, a similar pattern was also observed for the theta per base pair diversity value of Sub1 and TPP7 genes. However, Tajima's D value was negative for the TPP7 gene (-1.356) only in aus sub-population as compared to all rice populations. Besides, Tajima's D value for the Sub1 locus was found to be positive in both the analysed populations (Table S10 a,b).

Linkage disequilibrium (LD) analysis was also performed for all rice populations (indica, japonica, aus, aromatic, and admixtures) and specifically for aus subpopulations. The SNPs in the Sub1 locus and TPP7 genes were studied for genetic LD. The LD analysis showed LD decay was relatively steeper in all populations of rice, i.e. R square value reached 0.2 for the nucleotide distance of 100 kb. In contrast, for the similar nucleotide distance between Sub1 and TPP7 genes, the R square value observed was ~ 0.3 in aus sub-

populations of rice (Figure S6 a,b). Additionally, few SNPs in the Sub1 locus (Chr9: 5847273, 5847471, 5847425, 5847410, 5847354, 5847731) was in high LD ($R^2 = 0.433$) with an SNP position in TPP7 gene (Chr9:12253191) only in all the populations of rice but not in aus sub-population. These particular SNPs (C to A) in the TPP7 gene are present in the 5th exon and codes for missense mutation altering serine to arginine amino acids. In addition to the nucleotide diversity analysis using the rice 3k panel, TPP7 gene diversity was also studied in the released rice varieties using gene specific In/Dels. The analysis showed all three In/Dels were polymorphic and the mean gene diversity measured was 0.413. Additionally, the mean major allele frequency and polymorphism information content were 0.69 and 0.32, respectively (Table S11). Further, two clusters were identified in the TPP7 dendrogram in rice varieties. Clutter I is a minor one with only four varieties, and all the remaining varieties were grouped in cluster II. Besides, cluster II was further subdivided into two clusters of which IIB consisted of 24 rice varieties (Figure S7).

3.9 Co-segregation analysis of submergence tolerance at germination and seedling stages in rice

A total of six QTL mapping studies for anaerobic germination and submergence tolerance were used in this study (Table S5) wherein the mapping population size in the selected articles varied from 80 to 423 lines. Also, the number of markers used in these studies ranged between 83 and 680. A total of 30 QTLs associated with anaerobic germination (23) and submergence (7) attributes were reported. These QTLs were distributed on all the chromosomes except chromosomes 5, 6 and 10. Further, chromosome 3 had the highest number of QTLs and chromosome 11 had the lowest number of QTLs. The distribution of QTLs in the consensus map showed that QTLs for anaerobic germination and submergence tolerance were mapped on Chr02, Chr03, Chr09 and Chr12. Especially, Chr03 and Chr09 co-segregated for anaerobic germination and submergence tolerance QTLs (Figure 4a). In Chr03, co-segregated genomic regions showed 12% (LOD:3.10) phenotypic variance for submergence tolerance and 4% (LOD: 2.97) phenotypic variance for anaerobic germination tolerance (RM15576-2499734). Additionally, two additional genomic regions (LOD 5.9 and 5.69) for anaerobic germination tolerance in Chr03 showed a phenotypic variance of 18%, respectively. In Chr09, co-segregated QTL (LOD: 3.6) showed 19% phenotypic variance for submergence tolerance and 24% phenotypic variance (LOD 16.43) for anaerobic germination tolerance (RM23966-id9002721). Moreover, another QTL in the nearby genomic region showed the highest phenotypic variance of 33% (LOD 20.34) for anaerobic tolerance.

3.10 Allelic effect of *TPP7* and *Sub1* genes under aerobic, germination under submergence, water deficit, and recovery conditions

The mean values of the N22 and B2 alleles of TPP7 were statistically compared for different stress treatments. The trait analysis in aerobic conditions showed three traits: shoot length on the 17th day (P=0.00**), shoot area (P=0.016*), and specific shoot area (P=0.021*) were significantly different for the two alleles of TPP7. For all three traits, the N22 allele of TPP7 showed a 6.3 per cent, 7.9 per cent, and 7.1 per cent increase in shoot length, shoot area, and specific shoot area, respectively. However, in GUS conditions, the B2 allele of TPP7 showed significant differences in germination rate (18.2 per cent, P=0.037*) and survival rate (25 per cent, P=0.019*) over the N22 allele of TPP7. Except for these five traits, the mean value of no other traits was statistically significant for the TPP7 alleles between N22 and B2 (Table 1, Figure S9a).

The mean phenotypic value of the Sub1 allele of N22 and B2 also showed significant differences for many traits in this analysis. In GUS conditions, crown root number was found to be significant (P=0.045*) and the N22 allele of Sub1 had 1.27 no.of crown roots higher than the B2 allele. Similarly, root dry weight per plant (P=0.020*) and root area (P=0.029*) were statistically significant and 24, and 22.7 per cent, respectively higher for the N22 allele of Sub1 than the B2 allele. In contrast, the mean value of shoot dry weight was significantly different (P=0.000**) between N22 and B2 Sub1 alleles in aerobic conditions. Similarly, the N22 allele of Sub1 was found to be significant for plant height (P = 0.026*), leaf length (P=0.042*), number of tillers (P=0.05*), SPAD (P=0.00**) and canopy temperature (P=0.00**) under WD conditions. The per cent increase of the N22 allele of the Sub1 allele observed was 12.6, 12.0, 23.9, 7.6 and 18.5 per cent for plant height, leaf length, number of tillers, SPAD and canopy temperature respectively under WD conditions

11

(Table 1, Figure S9b).

3.11 Allelic interaction effects of TPP7 and Sub1 alleles in germination under submergence and aerobic conditions

Four combinations of alleles of TPP7 and Sub1 genes were statistically analysed for their interaction effects. Among the traits studied, two traits, culm diameter (P=0.027*) and root dry weight (P=0.03*) were significant for the allelic combination in flooded germination conditions. N22 combination of TPP7 and Sub1 showed relatively higher culm diameter and root dry weight than other allelic combinations. In contrast, N22-TPP7 and B2-Sub1 lines showed the least culm diameter (less by 32.5%) and root dry weight per plant (less by 62%). Besides, the other two allelic combinations (B2-TPP7: N22-Sub1; B2-TPP7:B2-Sub1) showed relatively lesser trait values for most of the traits than the N22-TPP7 and N22-Sub1 combination including culm diameter and root dry weight per plant but better than N22-TPP7 and B2-Sub1 combinations (Table 2).

3.12 Superior haplotypes analysis for N22 and Bhalum 2 alleles of Sub1 and TPP7

Five polymorphic markers (RM23662, Sub1BC1, RM24093, TPP7_In/Del, and RM24199) were used for the haplotype analysis in the mapping population to understand the superior haplotype for different stress conditions for the genomic regions within Sub1 and TPP7. There were a total of 28 haplotypes identified based on the allelic data of the five markers. A heat map was constructed using the phenotypic values for all the stress treatments. The analysis showed all 28 haplotypes were grouped into three major clusters. Cluster I comprised nine haplotypes (Hap25, Hap1, Hap6, Hap16, Hap13, hap27, Hap20, Hap26, and Hap11) wherein high phenotypic values were observed for nine different traits in the GUS. Further, nine aerobic and five GUS trait values were higher in seven haplotypes (Hap15, Hap28, Hap14, Hap5, Hap24, Hap7, Hap21) of Cluster II. The third haplotype cluster consisted of 11 haplotypes (Hap2, Hap19, Hap17, Hap8, Hap12, Hap18, Hap9, Hap10, Hap4, Hap22, and Hap23) wherein trait values pattern were not found to be unique Figure 4d . Additionally, the N22 allele of RM23622 and Sub1 has been observed in 57% and 94% lines, respectively in cluster I haplotypes. In contrast, 70.37%, 87.08%, and 83.33% of lines in haplotype cluster II consisted of Bhalum 2 allele for Sub1, RM24093 and TPP7, respectively. Besides, the N22 allele of Sub1 and RM24199 was present in 52.38% and 65.21%, respectively of the genotypes grouped under cluster III haplotypes.

Among the 28 haplotypes, 19 haplotypes were considered minor haplotypes due to their frequency of less than 5 per cent and only the remaining nine haplotypes were taken for further analysis. Further, F-test showed a non-significant variation for different traits between the haplotypes. However, the mean pairwise least significant test (LSD) between haplotypes showed significant differences for different traits in germination under submerged conditions (21st day survival percentage, 14th day germination, survival rate, SPAD, root dry weight), aerobic (SPAD), WD stress (plant height, no. of tillers), and recovery after severe WD stress (no. of recovered leaves, total recovered plants and recovery percentage). For example, 21st day seedling survival was significantly different (P =0.046) between Hap3 and Hap1 and the former haplotype (Hap3) showed 21.3% more survival than Hap1. Similarly, root dry weight in Hap1 under submerged germination condition (0.069 mg per seedling) was 64.7% more than Hap8 (0.0024 mg per seedling) and found to be statistically significant (P =0.047). The list of haplotype comparisons showing significant differences is given in Table 3. Further, considering all the traits (41 nos.) for the mean values, Hap5 was found to be superior followed by Hap 4 and Hap1, Besides, Hap3 were found to be less favourable for different traits. Interestingly, a comparison of Hap4 and Hap3 showed significant insights in terms of trait response and haplogenic constitution (Figure 4e,f). Both these haplotypes were similar for all the markers except RM24199. The B2 allele of RM24199 significantly enhanced the trait values in Hap4 (crown roots:11.45%, root dry weight:15.69%, root area:26.60%, and specific root area:33.18% in GUS condition) relative to the N22 allele.

3.13 QTL mapping and candidate gene identification

The five markers were used to construct the linkage map and QTL identification. A total of eight minor QTLs

12

(qPHR-1-2, qPHWD-1-2, qLLWD-1-2, qSWD-1-3, qGSS-1-2, qGSG-1-1, qGSGR-1-2, and qLWWD-1-2) were identified for multiple correlated traits namely plant height (LOD:9.41) after one week of GUSR condition. Additionally, QTLs for plant height (LOD:13.05), leaf length (LOD:9.50), and SPAD (LOD:19.71) under water deficit conditions were identified between the flanking markers, RM24199 and TPP7_INDEL2. Further, in-silico expression analysis of forty-five genes showed a candidate causal gene namely a wall-associated kinase 79 (WAK79; Os09g0373800) (Table S12a) which was highly expressed in anaerobic condition but significantly downregulated in the genetic background of Sub1 and AG introgressed lines. Furthermore, six minor QTLs (qGSGR-1-1 (LOD:2.53), qPHR-1-1 (LOD:9.27), qPHWD-1-1 (LOD:12.75), qLLWD-1-1 (LOD:9.91), qLWWD-1-1 (LOD:3.12), and qSWD-1-2 (LOD:19.94)) were identified between the RM24093 and TPP7_INDEL2 flanking markers. In this QTL region, a gene namely malectin-like receptor-like kinase 59 (*MRLK59* ; Os09g0355400) (Table S12b) out of 144 genes showed an opposite expression pattern having high expression in Sub1 and AG lines as compared to only AG introgressed lines (Table 4, Figure 4b,c).

4. DISCUSSION

The role of root traits on seedling survival, seedling vigour in GUS conditions and subsequent WD stress was analysed in this present attempt using a mapping population. The strong positive correlation between the root number and shoot traits (length, culm strength, and shoot dry weight) in germination under submerged conditions highlights the importance of roots in rice adaptation over long-term flooding stress during germination. Flooding after seed sowing in direct seeded conditions under rainfed or irrigated ecology significantly affects the seedling establishment and ultimately panicles per unit area due to the inability of seeds to germinate and reduced seedling growth under anaerobic germination (Kretzschmar et al., 2015; Mondal et al., 2020a, 2020b; Septiningsih et al., 2013). The survival percentage, seedling growth, and yield were significantly improved through the introgression of tolerant QTLs (AG1, AG2) in rice (Kim et al., 2019; Mondal et al., 2020a). Though the mechanism of carbohydrate mobilisation through TPP7 involved in the trehalose pathway was characterised for seedling survival under anaerobic germination (Kretzschmar et al., 2015), it was also reported that the interaction of Sub1 and TPP7 combinedly regulate stress tolerance. The findings of the quantitative response of TPP7 and Sub1 alleles for root and shoot traits in different stresses (germination under submergence, water deficit and recovery) in this work support the multi-functional role of these two major genes in regulating the adaptation in rice. Additionally, genomic regions between Sub1 and TPP7 in Chr09 were also found to be regulating the trait responses. Therefore, the introgression of favourable alleles within the genomic regions into rice varieties provides a new avenue in abiotic stress breeding in rice.

4.1 Whether root traits are a valuable factor in sequential stress response in rice?

Roots are generally heterotrophic in nature, and fermentive enzymes related genes are highly expressed for energy management in Arabidopsis under oxygen deficiency (Mustroph et al., 2014). In addition, root coordination with shoots for energy utilisation and developmental decisions are highly imperative for survival in abiotic stresses as reported by (Li et al., 2021). The major findings that optimum crown root number (10-15 nos) positively regulates the seedling vigour in rice show both shoot growth and crown root number are vital for GUS stress tolerance. In contrast, attenuated shoot growth and better root growth are essential for submergence tolerance and recovery under seedling stage submergence in rice (Bui et al., 2019; Jung et al., 2010; Mackill et al., 2012). On the other hand, fewer crown roots at the seedling stage under drought stress in maize were associated with enhanced root depth and better water acquisition (Gao and Lynch, 2016). Thus, crown root influences on plant tolerance are stage and stress specific in plants. In rice, more than fifteen genes and two hormones, auxins and cytokinins, regulate crown root initiation and emergence (Meng et al., 2019). Recently, it was reported in rice that anaerobic germination under darkness enhanced endogenous IAA levels to promote leaf elongation and restrict root growth mediated through the miR167-ARF-GH3 regulatory module. However, this inhibitory effect was relieved by light and oxygen (Lee et al., 2022). Since GUS stress of ~10 cm water level for 21 days was administered at shaded net house condition, it was possible that reduced levels of light penetration might have altered the regulatory modules related to crown root initiation and promoted the crown root development in our experiments. Moreover, light penetration under flooded conditions invariably occurs under natural field conditions and might regulate seedling elongation and

crown root emergence. Thus, the auxin regulatory module and other positive regulatory factors responsible for crown root initiation and emergence in GUS stress need to be further explored.

In GUS stress, total root length and root area were significantly reduced than in aerobic conditions in our study. In support of these findings, Arabidopsis RAP2.12 which is a major anoxic sensor disturbs the overall root growth under anoxic conditions through inhibition of auxin signalling (Shukla et al., 2019). Additionally, low light penetration as observed in submergence stress (Gomez-Alvarez and Pucciariello, 2022) would be applicable also to submergence during germination. In this regard, low light induced shade avoidance response in Arabidopsis involves shoot-formed elongated hypocotyl 5 (HY5) translocation to roots for inhibition of lateral root outgrowths (Li et al., 2021). Thus, reduction in root length and root area in GUSe stress could be regulated by both anoxic and low-light intensity conditions through shoot to root signalling. In addition, not only root length but chlorophyll index was also reduced by 30% in GUS stress than in aerobic conditions. In a similar shoot to root signalling response, mobile signal protein HY5 under normal light promotes nitrogen uptake by regulating the expression of NRT2.1 in roots (Chen et al., 2016). Therefore, identifying the regulators of shoot to root signalling would provide novel strategies for increasing the root length and other traits in rice in GUS conditions. This assumption needs to be validated.

The mean root area and dry weight for the N22 allele of Sub1 were relatively higher (~20%) than the Bhalum 2 allele of Sub1 in GUS stress conditions in rice. Previously, (Bui et al., 2019) reported that FR13A and Swarna-Sub1 showed greater root elongation rate which was correlated with seedling recovery after submergence. Besides, Sub1 also promotes precocious autotrophy of germinated seeds under submergence in rice (Alam et al., 2020). Sub1 is an ethylene response transcription factor (ERF) and cross-talk between ethylene and auxin regulates the root elongation in Arabidopsis and rice (Qin et al., 2019). A recent finding in rice shows inhibition of ethylene signalling using silver nitrate during GUS (~ 4.5 cm water) enhanced the germination percentage of the japonica rice variety (Huang et al., 2019). Further, (Nghi et al., 2021) high auxin levels in rice coleoptiles correlated with long coleoptiles during GUS in darkness. Further, ethylene insensitive mutants (ein2-1) in Arabidopsis showed high root growth under normal conditions and root elongation inhibitory effects of ethylene were specific to epidermal cells and functional EIN2 which creates local auxin maxima to restrict root elongation (Vaseva et al., 2018). Therefore, high auxin and low ethylene signalling could be positively regulating the seedling growth in GUS conditions. In corroborating with our findings, it is proposed that the weak allelic effect of N22-Sub1 might interfere with the local auxin levels in root cells and increase the root length and root dry weight in GUS conditions in rice. Though more experiments are required to validate this hypothesis, the identification of novel allelic effects of Sub1 can be vital in rice breeding strategies for combining submergence and anaerobic stress tolerance.

4.2 Whether nucleotide diversity in Sub1 and TPP7 genes regulate sequential stress response?

Popular irrigated mega rice cultivars like IR64 lack the trehalose phosphate phosphatase 7 (TPP7) gene indicating its function under normal irrigated conditions may be compensated by other TPP homologs in rice. It was identified in this study that TPP7 genes were present in the aerobic, upland, and irrigated rice varieties and its TPP7 alleles in 3' UTR regions between N22 and B2 contributed to the differential response of seedlings in GUS stress. Specifically, as compared to a previous report on the importance of TPP7 in anaerobic germination, which was identified between IR64 (lacks TPP7 gene) and a landrace Khao Hlan On (KHO) donor for anaerobic tolerance, our findings that the B2-TPP7 allele as compared to N22-TPP7 showed a significant difference for germination and survival rate indicates nucleotide variations in TPP7 gene also plays an important role in GUS in rice. Furthermore, only ~1.5% of genotypes within the rice 3k panel comprise this rare 3' UTR allele which indicates recent origin and variation in TPP7 genes in rice. In contrast, different alleles of low phosphorus tolerance (PsTol1) and submergence (Sub1 locus) were not associated with significant differences in phenotypic response (Singh et al., 2020; Vigueira et al., 2016). Further, the characterisation of SNPs and small indels of TPP7 could provide additional insights into the allelic contribution to GUS stress tolerance. Specifically, 3' UTR variations of TPP7 would be necessary for transcript stability or translation efficiency as reported for the other 3' UTRs in plants (Bernardes and Menossi, 2020).

14

The other major findings of this work are the interaction effects of the TPP7 and Sub1 for traits (dry root weight and culm strength) only in germination under submerged condition. Previously, the interaction between IR64 isogenic lines pyramided with Sub1 and TPP7 was reported to affect the growth traits such as elongation and photoautotrophy of germinated seedlings under submergence (Alam et al., 2020). Additionally, our meta-QTL analysis also showed cosegregation of anaerobic tolerance and major submergence tolerance QTLs (Sub1) supporting the direct role of Sub1 in regulating the seedling traits in GUS tolerance and its interaction with TPP7. Besides, these findings were also corroborated with the finding that the Sub1 and TPP7 genomic regions are in linkage disequilibrium though they are localised in a genomic distance of ˜ 4 Mb of genome size. Therefore, the most probable role of Sub1 would be to regulate the root growth of germinated seeds under submergence in addition to photoautotrophy as reported by (Alam et al., 2020). Furthermore, the finding that there is a significant positive correlation between root growth and shoot traits in GUS for 21 days indicates substantial indirect effects of Sub1 in seedling establishment. Thus, targeting the root traits for QTL mapping studies and marker assisted improvement in GUS conditions would provide substantial genetic gain in rice for GUS stress.

The nucleotide diversity studies of TPP7 and Sub1 provided interesting insights into the evolution of these two major loci in rice. The highly negative Tajima D value for TPP7 only in aus ecotypes of rice indicates the presence of rare alleles influencing the trait response in this subpopulation. Further, the TPP7 gene region could have been affected by selective sweeps in aus sub-populations of rice. Similar findings were also reported for rice waxy gene genomic regions with significant negative Tajima D values only in tropical, temperate japonica and aromatic sub-populations resulting in low amylose rice (Olsen et al., 2006). Further, selective sweeps and negative Tajimas D values were also reported in > 28 genomic blocks differentiating rice indica and japonica subpopulations (Yuan et al., 2017). However, Tajimas D value was found to be positive for the combination of Sub1 and TPP7 genomic regions even in aus subpopulations, indicating balancing selection. This finding further corroborates the interaction effect of the TPP7 and Sub1 genes in rice. Besides, rare alleles of the TPP7 gene might be evolutionarily selected in aus subpopulations, and its functional significance would be abiotic stress tolerance, especially GUS and drought tolerance. Another major insight in the nucleotide diversity studies was the relatively lesser rate of reduction in LD values per nucleotide distance in aus sub-population of rice. Previously, LD decay of aus population in *Xa5* locus was found to be ˜ 100kb (Garris et al., 2003) and whole genome studies reported LD decay of ˜123 kb and ˜167 kb in indica and japonica, respectively (Huang et al., 2010). Therefore, nucleotide variations present in the Sub1 and TPP7 locus specifically in aus sub-populations might be associated with phenotypic traits especially in germination under submerged conditions and WD response. These findings further corroborated the interaction effects of TPP7 and Sub1 on culm diameter and root traits in this study.

As novel findings, the culm strength of the seedlings in GUS was combinedly regulated by the alleles of TPP7 and Sub1 interaction. Especially, a combination of the N22 allele of TPP7 and the B2 allele of Sub1 significantly reduced the culm strength of seedlings. The reduced culm strength was associated with the lodging of seedlings after 21 days of submergence stress from seed sowing. Previously it was reported that culm strength in rice was related to culm diameter, wall thickness, few aerenchymatous cells, and structural carbohydrates (Kato et al., 2019). Recently, (Sagare et al., 2020) also highlighted the importance of culm strength in dry direct seeded rice (DDSR) cultivation. The basis of culm strength regulation by TPP7 and Sub1 interaction observed in this study would be a higher accumulation of non-structural carbohydrates and biomass accumulation as observed in FR13A landrace during submergence (Singh et al., 2014). This needs to be analysed for better understanding.

4.3 Whether root traits in GUS are correlated with water deficit stress response?

The next major findings related to the subsequent WD stress to initial GUS stress are that most of the shoot and root traits in submergence under germination were positively and significantly correlated with the seedling recovery and WD related traits. This indicates that the presence of early seedling vigour trait in a genotype has the potential to positively impact the WD tolerance at later stages of crop growth. In support of these findings, several reports on seed priming and cold shock providing WD tolerance in later

stages indicative of the priming mechanism are reported (Agostini et al., 2013; El-Sanatawy et al., 2021). Thus, carbohydrate metabolism and ethylene signalling mediated through TPP7 and Sub1, respectively in GUS, might prime the seedlings for WD tolerance in later stages. Especially, the Sub1 allele rather than the TPP7 allele was associated with the WD stress tolerance in rice. Previously, the Sub1a gene was reported to enhance the recovery after drought stress through upregulation of DREB, LEA, and other genes involved in acclimatation response to drought stress (Fukao et al., 2011). Thus, the introgression of favourable alleles of TPP7 and Sub1 has a multitude of stress response tolerance effects on rice improvement.

Several traits related to GUS and aerobic conditions showed transgressive segregation in the recombinant inbreds suggesting the stability of phenotypic expression and heritability. Previously, transgressive segregation was reported to be frequently observed in the hybrid progenies (Rieseberg et al., 1999) and recombinant inbred lines developed from diverse parents (de los Reyes, 2019). The mechanism of transgressive segregation points to antagonistic QTLs, complementary gene action, and epigenome changes (Dickinson et al., 2003). Recently, genomic regions mapped for transgressive segregation for heading date in rice support the complementary gene action of the minor QTLs (Koide et al., 2019). In rice, several major QTLs namely Sub1, Dro1, PsTol1, qSOR, qDTYs, AG1, and AG2 have been utilised in breeding programs for improvement. The future climate change scenarios entail strategies for trait enhancement in addition to the trait advantage provided by the introgressed major abiotic stress tolerance QTLs for stress tolerance. In this regard, several lines identified in this study showing transgressive segregation could be mapped to identify QTLs responsible for transgressive phenotypes having complementary action with the major QTL/genes. Therefore, tolerance trait enhancement through mapping of transgressive phenotypes for GUS and water deficits would be a better equipped strategy for making the rice crop cope with multiple abiotic stresses in a single growth phase or varied stress occurrences during different growth stages.

4.4 Whether genomic regions spanning between Sub1 and TPP7 also regulate the trait response?

The finding of the meta-QTL analysis for submergence and GUS tolerance also valuable significant insights into the co-segregation of genomic regions in rice for both of these traits. Not only that Sub1 genomic regions (Chr09) are associated with anaerobic tolerance, but genomic regions in Chr02 (RM5789-RM2578), and Chr03 (2610698 - id30101106) were also co-segregated for both GUS and submergence tolerance. This indicates the genetic interaction between these two traits in rice is controlled by the interaction between more than one gene(s) in different genomic regions. This is expected because rice has to maintain a delicate balance to regulate the shoot growth, i.e. attenuation during seedling submergence and vigor in submerged germination conditions (Kuroha and Ashikari, 2020). Therefore, varietal variation for these genomic regions would be modifying the trait response for both the traits which need to be factored in varietal improvement programmes. Further, characterization and validation of the other two genomic regions co-segregating for submergence under germination and seedling stage tolerance would provide additional mechanistic insights for the development of multiple stress tolerance rice.

From this study, the 'haplogenic model' of quantitative trait response is proposed based on the findings that haplotypes within a genomic region showed differences in the magnitude of trait response. The quantitative traits are well understood through 'polygenic models' wherein multiple genes regulate the phenotypic effects and casual genomic regions are present in different linkage groups in the genome (Zhu et al., 2009). Recently, the 'omnigenic model' of inheritance is proposed wherein genes in a pathway affecting phenotypic traits are interconnected, and core genes and peripheral genes act together in the heritability of a trait (Boyle et al., 2017). The genomic regions in the 'omnigenic model' are also distributed in different linkage groups in the genome. In the 'haplogenic model' proposed here, haplotypes of genes within the 'haplogenic regions' would have subtle interaction effects on the trait value and phenotype is regulated by the alleles of a few major genes and other causal genes involved in the similar or related pathways within the haplogenic regions. For example, the region between RM23662 and RM24199 in Chr09 are termed as 'haplogenic regions' and alleles of Sub1 and TPP7 within the haplogenic regions are major genes and alleles of candidate causal genes namely WAK79 and *MRLK59* would be regulating the cell wall dynamics (Yang et al., 2021) and

modulating the tolerance to GUS and WD stress response in rice. Thus, the major difference between the polygenic, omnigenic model of inheritance and with haplogenic model is the casual genomic regions are present within the same linkage groups and not present in different linkage groups. Previously, (Alam et al., 2020) also reported the epistasis interaction of Sub1 and TPP7 was associated with carbon metabolism and cell wall elongation. Thus, further characterization of these two candidate genes (*WAK79* and *MRLK59*) would provide mechanistic insights into the interaction between TPP7 and Sub1 with respect to cell wall elongation and haplogenic regulation of tolerance. In support of the 'haplogenic model', a major drought tolerant QTL in rice, qDTY12.1 also has a set of genes namely NAM and Decussate genes within the QTL region (haplogenic region) regulating the drought stress response in rice (Sanchez et al., 2022). Thus, the 'haplogenic model' and regulation of complex traits could be highly prevalent in rice and other plants.

Conclusion

This study has identified the haplogenic model of trait response for germination under submergence conditions and followed by water deficit stress. Further, Sub1 was identified to influence the root traits in germination under submerged conditions. The most probable mechanism hypothesised would be shoot formed mobile signals to roots, and auxin-ethylene interactions in root plasticity for tolerance in germination under submergence conditions. Further analysis is required for understanding the mechanistic insights of the trait regulation mediated through genes/alleles in the haplogenic regions. In addition, allelic information within the halogenic region can be utilised in the breeding program for trait improvement of direct seeded rice.

Acknowledgements

Author contribution

PC and PS conceptualised the experiment and performed the analysis. BC and RPS provided the materials for the study. PC, GKD and CB wrote the manuscript. SS, KPJ, and RKS did the phenotypic analysis. DL and PKSR did the genotyping analysis. SS, RLV, and JLK did the statistical analysis. SS and DBN did the bioinformatic analysis.

Conflict of interests

We have no conflicts of interest to declare.

Informed consent

All the authors have read and approved the manuscript for submission and publication.

References

Agostini, E.A.T. de, Machado-Neto, N.B., Custodio, C.C., 2013. Induction of water deficit tolerance by cold shock and salicylic acid during germination in the common bean. Acta Sci., Agron. 35, 209–219. https://doi.org/10.4025/actasciagron.v35i2.15967

Alam, R., Hummel, M., Yeung, E., Locke, A.M., Ignacio, J.C.I., Baltazar, M.D., Jia, Z., Ismail, A.M., Septiningsih, E.M., Bailey-Serres, J., 2020. Flood resilience loci SUBMERGENCE 1 and ANAEROBIC GERMINATION 1 interact in seedlings established underwater. Plant Direct 4, e00240. https://doi.org/10.1002/pld3.240

Anandan, A., Mahender, A., Sah, R.P., Bose, L.K., Subudhi, H., Meher, J., Reddy, J.N., Ali, J., 2020. Non-destructive phenotyping for early seedling vigor in direct-seeded rice. Plant Methods 16, 1–18.

Angaji, S.A., 2008. Mapping QTLs for submergence tolerance during germination in rice. African Journal of Biotechnology 7. https://doi.org/10.4314/ajb.v7i15.59090

Angaji, S.A., Septiningsih, E.M., Mackill, D.J., Ismail, A.M., 2010. QTLs associated with tolerance of flooding during germination in rice (Oryza sativa L.). Euphytica 172, 159–168. https://doi.org/10.1007/s10681-009-0014-5

Baltazar, M.D., Ignacio, J.C.I., Thomson, M.J., Ismail, A.M., Mendioro, M.S., Septiningsih, E.M., 2019. QTL mapping for tolerance to anaerobic germination in rice from IR64 and the *aus* landrace Kharsu 80A. Breeding Science 69, 227–233. https://doi.org/10.1270/jsbbs.18159

Bernardes, W.S., Menossi, M., 2020. Plant 3' Regulatory Regions From mRNA-Encoding Genes and Their Uses to Modulate Expression. Frontiers in Plant Science 11.

Bhullar, M.S., Kumar, S., Kaur, S., Kaur, T., Singh, J., Yadav, R., Chauhan, B.S., Gill, G., 2016. Management of complex weed flora in dry-seeded rice. Crop Protection 83, 20–26. https://doi.org/10.1016/j.cropro.2016.01.012

Boyle, E.A., Li, Y.I., Pritchard, J.K., 2017. An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell 169, 1177–1186. https://doi.org/10.1016/j.cell.2017.05.038

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y., Buckler, E.S., 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23, 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Bui, L.T., Ella, E.S., Dionisio-Sese, M.L., Ismail, A.M., 2019. Morpho-Physiological Changes in Roots of Rice Seedling upon Submergence. Rice Science 26, 167–177. https://doi.org/10.1016/j.rsci.2019.04.003

Chamara, B.S., Marambe, B., Kumar, V., Ismail, A.M., Septiningsih, E.M., Chauhan, B.S., 2018. Optimizing Sowing and Flooding Depth for Anaerobic Germination-Tolerant Genotypes to Enhance Crop Establishment, Early Growth, and Weed Management in Dry-Seeded Rice (Oryza sativa L.). Frontiers in Plant Science 9.

Chen, X., Yao, Q., Gao, X., Jiang, C., Harberd, N.P., Fu, X., 2016. Shoot-to-Root Mobile Transcription Factor HY5 Coordinates Plant Carbon and Nitrogen Acquisition. Current Biology 26, 640–646. https://doi.org/10.1016/j.cub.2015.12.066

Chidambaranathan, P., Balasubramaniasai, C., Behura, N., Purty, M., Samantaray, S., Subudhi, H., Ngangkham, U., Devanna, B.N., Katara, J.L., Kumar, A., Behera, L., 2021. Effects of high temperature on spikelet sterility in rice (Oryza sativa L.): association between molecular markers and allelic phenotypic effect in field condition. Genet Resour Crop Evol 68, 1923–1935. https://doi.org/10.1007/s10722-021-01106-7

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., Pang, E.C.K., 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142, 169–196. https://doi.org/10.1007/s10681-005-1681-5

D, K.D., 1944. The estimation of map distance. Ann.Eugenics 12, 505–525.

de los Reyes, B.G., 2019. Genomic and epigenomic bases of transgressive segregation – New breeding paradigm for novel plant phenotypes. Plant Science 288, 110213. https://doi.org/10.1016/j.plantsci.2019.110213

Dickinson, H.G., Hiscock, S.J., Crane, P.R., Rieseberg, L.H., Widmer, A., Arntz, A.M., Burke, B., 2003. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 358, 1141–1147. https://doi.org/10.1098/rstb.2003.1283

El-Sanatawy, A.M., Ash-Shormillesy, S.M.A.I., Qabil, N., Awad, M.F., Mansour, E., 2021. Seed Halo-Priming Improves Seedling Vigor, Grain Yield, and Water Use Efficiency of Maize under Varying Irrigation Regimes. Water 13, 2115. https://doi.org/10.3390/w13152115

Fernandez, M.C., Belinque, H., Boem, F.H.G., Rubio, G., 2009. Compared Phosphorus Efficiency in Soybean, Sunflower and Maize. Journal of Plant Nutrition 32, 2027–2043. https://doi.org/10.1080/01904160903308135

Fukao, T., Yeung, E., Bailey-Serres, J., 2011. The Submergence Tolerance Regulator SUB1A Mediates Crosstalk between Submergence and Drought Tolerance in Rice. The Plant Cell 23, 412–427. https://doi.org/10.1105/tpc.110.080325

Furuya, J., Kobayashi, S., 2010. Impact of Global Warming on Agricultural Product Markets: Stochastic World Food Model Analysis, in: Sumi, A., Fukushi, K., Hiramatsu, A. (Eds.), Adaptation and Mitigation Strategies for Climate Change. Springer Japan, Tokyo, pp. 19–35. https://doi.org/10.1007/978-4-431-99798-6_2

Gamuyao, R., Chin, J.H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E.M., Wissuwa, M., Heuer, S., 2012. The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature 488, 535–539. https://doi.org/10.1038/nature11346

Gao, Y., Lynch, J.P., 2016. Reduced crown root number improves water acquisition under water deficit stress in maize (Zea mays L.). Journal of Experimental Botany 67, 4545–4557. https://doi.org/10.1093/jxb/erw243

Garris, A.J., McCouch, S.R., Kresovich, S., 2003. Population Structure and Its Effect on Haplotype Diversity and Linkage Disequilibrium Surrounding the xa5 Locus of Rice (Oryza sativa L.). Genetics 165, 759–769. https://doi.org/10.1093/genetics/165.2.759

Gomez-Alvarez, E.M., Pucciariello, C., 2022. Cereal Germination under Low Oxygen: Molecular Processes. Plants 11, 460. https://doi.org/10.3390/plants11030460

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, Wenjun, Lu, Y., Weng, Q., Liu, K., Huang, T., Zhou, T., Jing, Y., Li, Wei, Lin, Z., Buckler, E.S., Qian, Q., Zhang, Q.-F., Li, J., Han, B., 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42, 961–967. https://doi.org/10.1038/ng.695

Huang, Y.-C., Yeh, T.-H., Yang, C.-Y., 2019. Ethylene signaling involves in seeds germination upon submergence and antioxidant response elicited confers submergence tolerance to rice seedlings. Rice 12, 23. https://doi.org/10.1186/s12284-019-0284-z

Ismail, A.M., Ella, E.S., Vergara, G.V., Mackill, D.J., 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (Oryza sativa). Annals of Botany 103, 197–209. https://doi.org/10.1093/aob/mcn211

Ismail, A.M., Johnson, D.E., Ella, E.S., Vergara, G.V., Baltazar, A.M., 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. AoB PLANTS 2012, pls019. https://doi.org/10.1093/aobpla/pls019

Jabran, K., Chauhan, B.S., 2015. Weed management in aerobic rice systems. Crop Protection 78, 151–163. https://doi.org/10.1016/j.cropro.2015.09.005

Jat, R.K., Meena, V.S., Kumar, M., Jakkula, V.S., Reddy, I.R., Pandey, A.C., 2022. Direct Seeded Rice: Strategies to Improve Crop Resilience and Food Security under Adverse Climatic Conditions. Land 11, 382. https://doi.org/10.3390/land11030382

Jung, K.-H., Seo, Y.-S., Walia, H., Cao, P., Fukao, T., Canlas, P.E., Amonpant, F., Bailey-Serres, J., Ronald, P.C., 2010. The Submergence Tolerance Regulator Sub1A Mediates Stress-Responsive Expression of AP2/ERF Transcription Factors. Plant Physiology 152, 1674–1692. https://doi.org/10.1104/pp.109.152157

Kato, Y., Collard, B.C.Y., Septiningsih, E.M., Ismail, A.M., 2019. Increasing flooding tolerance in rice: combining tolerance of submergence and of stagnant flooding. Annals of Botany 124, 1199–1209. https://doi.org/10.1093/aob/mcz118

19

Kim, S.-M., Kim, C.-S., Jeong, J.-U., Reinke, R.F., Jeong, J.-M., 2019. Marker-assisted breeding for improvement of anaerobic germination in japonica rice (Oryza sativa). Plant Breeding 138, 810–819. https://doi.org/10.1111/pbr.12719

Kitomi, Y., Hanzawa, E., Kuya, N., Inoue, H., Hara, N., Kawai, S., Kanno, N., Endo, M., Sugimoto, K., Yamazaki, T., Sakamoto, S., Sentoku, N., Wu, J., Kanno, H., Mitsuda, N., Toriyama, K., Sato, T., Uga, Y., 2020. Root angle modifications by the DRO1 homolog improve rice yields in saline paddy fields. Proceedings of the National Academy of Sciences 117, 21242–21250. https://doi.org/10.1073/pnas.2005911117

Koide, Y., Sakaguchi, S., Uchiyama, T., Ota, Y., Tezuka, A., Nagano, A.J., Ishiguro, S., Takamure, I., Kishima, Y., 2019. Genetic Properties Responsible for the Transgressive Segregation of Days to Heading in Rice. G3 Genes|Genomes|Genetics 9, 1655–1662. https://doi.org/10.1534/g3.119.201011

Kretzschmar, T., Pelayo, M.A.F., Trijatmiko, K.R., Gabunada, L.F.M., Alam, R., Jimenez, R., Mendioro, M.S., Slamet-Loedin, I.H., Sreenivasulu, N., Bailey-Serres, J., Ismail, A.M., Mackill, D.J., Septiningsih, E.M., 2015. A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nature Plants 1, 1–5. https://doi.org/10.1038/nplants.2015.124

Kumar, V., Ladha, J.K., 2011. Direct Seeding of Rice: Recent Developments and Future Research Needs, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 297–413. https://doi.org/10.1016/B978-0-12-387689-8.00001-1

Kumar, V., Singh, S., Chhokar, R.S., Malik, R.K., Brainard, D.C., Ladha, J.K., 2013. Weed Management Strategies to Reduce Herbicide Use in Zero-Till Rice–Wheat Cropping Systems of the Indo-Gangetic Plains. Weed Technology 27, 241–254. https://doi.org/10.1614/WT-D-12-00069.1

Kuroha, T., Ashikari, M., 2020. Molecular mechanisms and future improvement of submergence tolerance in rice. Mol Breeding 40, 41. https://doi.org/10.1007/s11032-020-01122-y

Lal, B., Gautam, P., Nayak, A.K., Raja, R., Shahid, M., Tripathi, R., Singh, S., Septiningsih, E.M., Ismail, A.M., 2018. Agronomic manipulations can enhance the productivity of anaerobic tolerant rice sown in flooded soils in rainfed areas. Field Crops Research, Rice and Abiotic Stresses: Part II 220, 105–116. https://doi.org/10.1016/j.fcr.2016.08.026

Lee, K.-W., Chen, J.J.W., Wu, C.-S., Chang, Ho-Chun, Chen, H.-Y., Kuo, H.-H., Lee, Y.-S., Chang, Y.-L., Chang, Hung-Chia, Shiuea, S.-Y., Wu, Y.-C., Ho, Y.-C., Chen, P.-W., 2022. Auxin Plays a Role in the Adaptation of Rice to Anaerobic Germination and Seedling Establishment. https://doi.org/10.1101/2022.05.05.490789

Li, H., Testerink, C., Zhang, Y., 2021. How roots and shoots communicate through stressful times. Trends in Plant Science 26, 940–952. https://doi.org/10.1016/j.tplants.2021.03.005

Liu, K., Muse, S.V., 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21, 2128–2129. https://doi.org/10.1093/bioinformatics/bti282

Mackill, D.J., Ismail, A.M., Singh, U.S., Labios, R.V., Paris, T.R., 2012. Development and Rapid Adoption of Submergence-Tolerant (Sub1) Rice Varieties, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 299–352. https://doi.org/10.1016/B978-0-12-394276-0.00006-8

Magneschi, L., Perata, P., 2009. Rice germination and seedling growth in the absence of oxygen. Annals of Botany 103, 181–196. https://doi.org/10.1093/aob/mcn121

Mahajan, G., Chauhan, B.S., 2013. The role of cultivars in managing weeds in dry-seeded rice production systems. Crop Protection 49, 52–57. https://doi.org/10.1016/j.cropro.2013.03.008

Matloob, A., Khaliq, A., Chauhan, B., 2014. Weeds of Direct-Seeded Ricein in Asia: Problems andOpportunities. Adv. Agron.

Meng, F., Xiang, D., Zhu, J., Li, Y., Mao, C., 2019. Molecular Mechanisms of Root Development in Rice. Rice 12, 1. https://doi.org/10.1186/s12284-018-0262-x

Meng, L., Li, H., Zhang, L., Wang, J., 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal, Special Issue: Breeding to Optimize Agriculture in a Changing World 3, 269–283. https://doi.org/10.1016/j.cj.2015.01.001

Miro, B., Ismail, A., 2013. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (Oryza sativa L.). Frontiers in Plant Science 4.

Mondal, S., Khan, M.I.R., Dixit, S., Sta. Cruz, P.C., Septiningsih, E.M., Ismail, A.M., 2020a. Growth, productivity and grain quality of AG1 and AG2 QTLs introgression lines under flooding in direct-seeded rice system. Field Crops Research 248, 107713. https://doi.org/10.1016/j.fcr.2019.107713

Mondal, S., Khan, M.I.R., Entila, F., Dixit, S., Sta. Cruz, P.C., Panna Ali, M., Pittendrigh, B., Septiningsih, E.M., Ismail, A.M., 2020b. Responses of AG1 and AG2 QTL introgression lines and seed pre-treatment on growth and physiological processes during anaerobic germination of rice under flooding. Sci Rep 10, 10214. https://doi.org/10.1038/s41598-020-67240-x

Murray, M.G., Thompson, W.F., 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8, 4321–4326. https://doi.org/10.1093/nar/8.19.4321

Mustroph, A., Barding Jr, G.A., Kaiser, K.A., Larive, C.K., Bailey-Serres, J., 2014. Characterization of distinct root and shoot responses to low-oxygen stress in Arabidopsis with a focus on primary C- and N-metabolism. Plant, Cell & Environment 37, 2366–2380. https://doi.org/10.1111/pce.12282

Nghi, K.N., Tagliani, A., Mariotti, L., Weits, D.A., Perata, P., Pucciariello, C., 2021. Auxin is required for the long coleoptile trait in japonica rice under submergence. New Phytologist 229, 85–93. https://doi.org/10.1111/nph.16781

Olagunju, S.O., Atayese, M.O., Sakariyawo, O.S., Dare, E.O., Tang, C., Olagunju, S.O., Atayese, M.O., Sakariyawo, O.S., Dare, E.O., Tang, C., 2022. Effects of multi-growth stage water deficit and orthosilicic acid fertiliser on lodging resistance of rice cultivars. Crop Pasture Sci. 73, 370–389. https://doi.org/10.1071/CP21563

Olsen, K.M., Caicedo, A.L., Polato, N., McClung, A., McCouch, S., Purugganan, M.D., 2006. Selection Under Domestication: Evidence for a Sweep in the Rice Waxy Genomic Region. Genetics 173, 975–983. https://doi.org/10.1534/genetics.106.056473

Pandey, S., Velasco, L., 2002. Economics of direct seeding in Asia: patterns of adoption and research priorities. Direct seeding: Research strategies and opportunities 3–14.

Pathak, H., Tewari, A.N., Sankhyan, S., Dubey, D.S., Mina, U., Singh, V.K., Jain, N., 2011. Direct-seeded rice: potential, performance and problems-Areview. Current Advances in Agricultural Sciences (An International Journal) 3, 77–88.

Portal, C.C.K., 2017. The World Bank Group.

Qin, H., He, L., Huang, R., 2019. The Coordination of Ethylene and Other Hormones in Primary Root Development. Frontiers in Plant Science 10.

Rao, A.N., Johnson, D.E., Sivaprasad, B., Ladha, J.K., Mortimer, A.M., 2007. Weed Management in Direct-Seeded Rice, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 153–255. https://doi.org/10.1016/S0065-2113(06)93004-1

Renault, D., 2004. Rice and water: a long and diversified story. Proceedings of the International Year of Rice 2004.

Rieseberg, L.H., Archer, M.A., Wayne, R.K., 1999. Transgressive segregation, adaptation and speciation. Heredity 83, 363–372. https://doi.org/10.1038/sj.hdy.6886170

Sagare, D.B., Abbai, R., Jain, A., Jayadevappa, P.K., Dixit, S., Singh, A.K., Challa, V., Alam, S., Singh, U.M., Yadav, S., Sandhu, N., Kabade, P.G., Singh, V.K., Kumar, A., 2020. More and more of less and less: Is genomics-based breeding of dry direct-seeded rice (DDSR) varieties the need of hour? Plant Biotechnology Journal 18, 2173–2186. https://doi.org/10.1111/pbi.13454

Saimohan, B., Chamundeswari, N., Haritha, T., Veronica, N., 2021. Mapping of Qtl for Anaerobic Germination Using the Donor Ac39416a in the Genetic Background of Swarna Sub-1 (Oryza Sativa L.). https://doi.org/10.21203/rs.3.rs-574662/v1

Sanchez, J., Kaur, P.P., Pabuayon, I.C.M., Karampudi, N.B.R., Kitazumi, A., Sandhu, N., Catolos, M., Kumar, A., de los Reyes, B.G., 2022. DECUSSATE network with flowering genes explains the variable effects of qDTY12.1 to rice yield under drought across genetic backgrounds. The Plant Genome 15, e20168. https://doi.org/10.1002/tpg2.20168

Septiningsih, E.M., Ignacio, J.C.I., Sendon, P.M.D., Sanchez, D.L., Ismail, A.M., Mackill, D.J., 2013. QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. Theor Appl Genet 126, 1357–1366. https://doi.org/10.1007/s00122-013-2057-1

Shukla, V., Lombardi, L., Iacopino, S., Pencik, A., Novak, O., Perata, P., Giuntoli, B., Licausi, F., 2019. Endogenous Hypoxia in Lateral Root Primordia Controls Root Architecture by Antagonizing Auxin Signaling in Arabidopsis. Molecular Plant 12, 538–551. https://doi.org/10.1016/j.molp.2019.01.007

Singh, A., Singh, Y., Mahato, A.K., Jayaswal, P.K., Singh, S., Singh, Renu, Yadav, N., Singh, A.K., Singh, P.K., Singh, Rakesh, Kumar, R., Septiningsih, E.M., Balyan, H.S., Singh, N.K., Rai, V., 2020. Allelic sequence variation in the Sub1A, Sub1B and Sub1C genes among diverse rice cultivars and its association with submergence tolerance. Sci Rep 10, 8621. https://doi.org/10.1038/s41598-020-65588-8

Singh, S., Mackill, D.J., Ismail, A.M., 2014. Physiological basis of tolerance to complete submergence in rice involves genetic factors in addition to the SUB1 gene. AoB PLANTS 6, plu060. https://doi.org/10.1093/aobpla/plu060

Sudhir-Yadav, Gill, G., Humphreys, E., Kukal, S.S., Walia, U.S., 2011. Effect of water management on dry seeded and puddled transplanted rice. Part 1: Crop performance. Field Crops Research 120, 112–122. https://doi.org/10.1016/j.fcr.2010.09.002

Turral, H., Burke, J., Faures, J.M., 2011. Climate change, water and food security. Water Reports.

Uga, Y., Okuno, K., Yano, M., 2011. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. Journal of Experimental Botany 62, 2485–2494. https://doi.org/10.1093/jxb/erq429

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., Leunissen, J.A.M., 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 35, W71-74. https://doi.org/10.1093/nar/gkm306

Vaseva, I.I., Qudeimat, E., Potuschak, T., Du, Y., Genschik, P., Vandenbussche, F., Van Der Straeten, D., 2018. The plant hormone ethylene restricts Arabidopsis growth via the epidermis. Proceedings of the National Academy of Sciences 115, E4130–E4139. https://doi.org/10.1073/pnas.1717649115

Veyrieras, J.-B., Goffinet, B., Charcosset, A., 2007. MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. BMC Bioinformatics 8, 49. https://doi.org/10.1186/1471-2105-8-49

Vigueira, C.C., Small, L.L., Olsen, K.M., 2016. Long-term balancing selection at the Phosphorus Starvation Tolerance 1 (PSTOL1) locus in wild, domesticated and weedy rice (Oryza). BMC Plant Biol 16, 101. https://doi.org/10.1186/s12870-016-0783-7

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., Zemla, J., 2017. Package 'corrplot.' Statistician 56, e24.

Yang, H., Wang, D., Guo, L., Pan, H., Yvon, R., Garman, S., Wu, H.-M., Cheung, A.Y., 2021. Malectin/Malectin-like domain-containing proteins: A repertoire of cell surface molecules with broad functional potential. The Cell Surface 7, 100056. https://doi.org/10.1016/j.tcsw.2021.100056

Yuan, Y., Zhang, Q., Zeng, S., Gu, L., Si, W., Zhang, X., Tian, D., Yang, S., Wang, L., 2017. Selective sweep with significant positive selection serves as the driving force for the differentiation of japonica and indica rice cultivars. BMC Genomics 18, 307. https://doi.org/10.1186/s12864-017-3702-x

Zhang, Q., Huber, H., Beljaars, S.J.M., Birnbaum, D., de Best, S., de Kroon, H., Visser, E.J.W., 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. Annals of Botany 120, 171–180. https://doi.org/10.1093/aob/mcx049

Zhu, M., Yu, M., Zhao, S., 2009. Understanding Quantitative Genetics in the Systems Biology Era. Int J Biol Sci 5, 161–170.

Hosted file

`Figures final.doc` available at https://authorea.com/users/511595/articles/588379-haplogenic-quantitative-effects-regulate-flooded-germination-subsequent-water-deficit-stress-and-recovery-in-direct-seeded-rice

Hosted file

`Tables_Final.docx` available at https://authorea.com/users/511595/articles/588379-haplogenic-quantitative-effects-regulate-flooded-germination-subsequent-water-deficit-stress-and-recovery-in-direct-seeded-rice