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Novel quantitative trait loci for weed competitive ability traits using the early generation of backcross rice populations

Niña Gracel B. Dimaano[1#], Jauhar Ali[2,#,*], Mahender Anumalla[2,#], Pompe C. Sta. Cruz[1], Aurora M. Baltazar[1], Maria Genaleen Q. Diaz[1], Yun Long Pang[3], Bart L. Acero Jr.[2], and Zhikang Li[3]

[1] University of the Philippines Los Baños, Los Baños, Laguna 4031, Philippines; ninadimaano@gmail.com (N.G.D); pcstacruz@up.edu.ph (P.C.C); amb@mudspring.uplb.edu.ph (A.M.B); genaleen@yahoo.com (M.G.D)

[2] Rice Breeding Platform, International Rice Research Institute (IRRI), Los Baños, Laguna 4031, Philippines; m.anumalla@irri.org (M.A); J.Ali@irri.org (J.A); bartzjr16@gmail.com (B.A.J)

[3] Academy of Agricultural Sciences, No.12 Zhongguancun South St., Haidian District, Beijing, P.R. China; pangyunlong8804@126.com (Y.P); zhkli1953@126.com (Z.L),

[#] Authors contributed equally

[*] Correspondence: J.Ali@irri.org; Tel.: +63-2580-5600 (ext. 2541)

Abstract: Weed competitive ability (WCA) is a desirable key trait for the improvement of grain yield under direct-seeded rice (DSR) and the aerobic rice ecosystem. The present study targeted screening of 167 introgression lines (ILs) of a Green Super Rice (GSR) IR2-6 population derived from a cross between Weed Tolerant Rice 1 (WTR1) as the recipient parent and Y134 as the donor parent developed at IRRI for weed competitiveness in screen house conditions (SHC). The ILs were phenotyped for WCA traits such as early seed germination (ESG) and early seedling vigor (ESV) in Petri dishes and pot experiment conditions. The results of phenotypic variance revealed ESG-related traits, especially first germination count (1st GC) that positively correlated with second germination count (2nd GC), germination percentage (GP), total dry weight (TDW), total fresh weight (TFW), and vigor index (VI-1), whereas, in ESV, all the traits were positively correlated with each other except for three traits: root dry weight (RDW), 1st GC, and GP-2. The ESG and ESV traits are vital for weed competitiveness. A 6K SNP array was used to study the genetic association for the WCA traits. Forty-four QTLs for WCA traits were mapped on all chromosomes (except on chromosomes 4 and 8) through single marker analysis (SMA). Out of 44 QTLs, 29 were associated with ESG traits and 15 with ESV traits, with LOD scores of 2.93 to 8.03 and 2.93 to 5.04 and explained phenotypic variance ranging from 7.85% to 19.9% and from 7.85% to 13.2%, respectively. However, 31 QTLs were contributed by a negative additive allele from Y134, whereas a positive additive allele was contributed by WTR1 in 13 QTLs. Among them, two QTL hotspot regions were mapped on chromosome 11 (24.7-27.9 Mb) and chromosome 12 (14.8-17.4 Mb). The majority of the QTLs related to WCA traits were grouped into two QTL hotspots: QTL hotspot-I (*qAFW*$_{11.1}$, *qFC*$_{11.1}$, *qFC*$_{11.2}$, *qSC*$_{11.1}$, *qGP-1*$_{11.1}$, *qGP-1*$_{11.2}$, *qTFGS*$_{11.1}$, *qVI-1*$_{11.1}$, and *qVI-1*$_{11.2}$) and QTL hotspot-II (*qFC*$_{12.1}$, *qFC*$_{12.2}$, *qSC*$_{12.1}$, *qFC*$_{12.2}$, *qGP-1*$_{12.1}$, *qGP-1*$_{12.2}$, *qTFGS*$_{12.1}$, *qTFGS*$_{12.2}$, *qVI-1*$_{12.1}$, *qIV*$_{12.2}$, *qFC*$_{12.1}$, and *qGC*$_{12.2}$), and a few of them were co-localized on chromosomes 11 and 12. Further, we fine-tuned in the genomic regions of QTL hotspots and identified a total of 13 putative candidate genes on chromosomes 11 and 12 collectively. The present study is the first report on the genetic basis of WCA-related traits and the co-localized QTLs, which could be highly valuable in future breeding programs aiming to improve WCA in rice.

Keywords: Weed competitive ability, early seed germination and seedling vigor traits, quantitative trait loci (QTLs), single nucleotide polymorphism, direct seeded rice.

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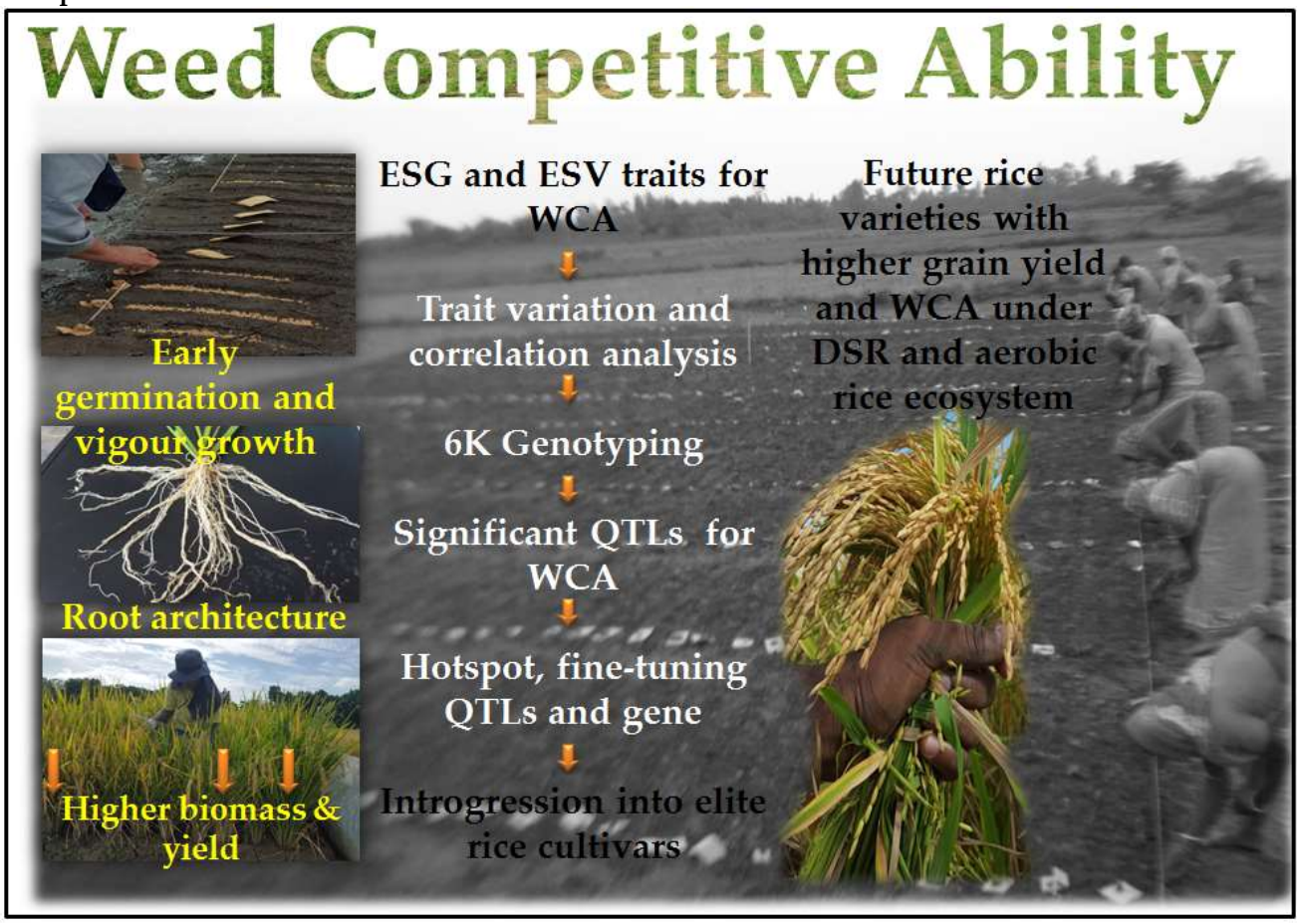
Graphical abstract



1. Introduction

Rice is a major food crop for half of the global population. By 2050, 42% more rice yield will be needed to feed the rapidly growing global population [1,2]. Out of 193 countries, rice is cultivated in 114 countries worldwide on six continents (Asia, Australia, Africa, South America, North America, and Europe) [3]. However, more than 90% of global rice is produced and consumed by Asia alone. About 55% of world rice is grown under the irrigated rice ecosystem, which contributes nearly 75% of global rice production [4] (Papademetriou et al., 2000). However, unfortunately, the enhancement of global rice grain yield has been constrained by various biotic and abiotic stresses in the diverse rice ecosystems [5,6,7,8]. Especially in Asian countries, the irrigated rice ecosystem will be drastically threatened by water shortage by 2025 [9]. The future threat to natural resources, rising labor shortages, decline in arable lands, increasing prices of fertilizer inputs and energy scarcity, and changing climatic conditions are the major factors contributing to the decrease in rice production [10,11].

To overcome these situations, shifting from the conventional puddled transplanted rice system to direct-seeded rice (DSR) is a most prominent strategy for sustainable global rice production [12,13,14,15,16]. DSR is an emerging and widely applied technology, which has several advantages such as minimizing 35% to 57% of water use, labor efficiency, reducing crop duration, lowering methane emissions, and reducing the cost of cultivation [17,18,19,20]. However, vigorous growth of weeds is one of the significant biological constraints to attaining optimal grain yield in DSR, and this also adversely affects grain quality [21,22,23,24]. Many options exist to control weeds, such as a hand or mechanical weeding operations (HWO/MWO) and using herbicides promptly. Both of these approaches are laborious and need multiple applications during the cropping season, with an increasing financial burden on farmers [25,26]. From 2002 to 2011, herbicide applications in cultivated regions increased more than 39% [27,28]. However, the rigorous application of herbicides in the field leads to

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environmental contamination, despite this being a cost-effective method compared with other strategies [26,29]. Appleby et al. [30] have estimated that more than USD 100 billion is lost annually by the economy due to weed-controlling activities globally. Therefore, urgent attention is required to develop alternative sustainable weed management technologies.

Breeding approaches are vital strategies to reduce HWO/MWO and herbicide inputs. To date, very little information is available for WCA in various crops such as maize [31], sorghum [32], soybean [33], wheat [34,35], and rice [20,36,37,38,39]. Although the diversity pattern among genotypes has a different ability to compete with weeds by improving WCA traits, only limited information is available on WCA cultivars [40,41,42,43]. The tolerance of WC rice cultivars can suppress the growth of weeds, without a yield penalty under weedy conditions [20]. Therefore, screening of WC rice genotypes could provide valuable information on tolerant genotypes, which could be useful for integrated weed management strategies. However, to date, only a few promising rice cultivars have been identified from diverse genotypes and backcross inbred lines for the weed competitiveness trait [20,44].

Understanding WCA and dissecting the agro-morphological traits associated with it is important for the development of varieties with WCA, especially in DSR conditions. The molecular genetics of WCA is a complex/polygenic trait, which is governed by several agronomic and morphological traits related to ESG and ESV and significantly involved in DSR and aerobic rice cultivation [19,35,38,45,46,47,48]. Thus, it is imperative to dissect these complex traits for greater understanding of the molecular aspects in crop growth and weed interactions, associated with genomic regions and key traits, as a vital strategy to control weeds under favorable environments [36]. Therefore, it has become a foremost task for breeders and scientists to develop WCA in rice.

However, few reports exist on crop growth pattern and rapid, uniform germination traits [20,44,49]. The progress of rice cultivars for WCA has not been significant so far because of the limited knowledge of molecular genetics [20]. Therefore, a better understanding of the molecular genetics of WCA in rice and molecular profiling of genotypes could significantly overcome these limitations because of their efficiency and effectiveness [50]. Genetic mapping can provide a feasible solution to speed up a WCA breeding program when compared with a conventional breeding program. DSR and aerobic-related traits in rice have been identified from different genetic backgrounds of mapping populations such as RILs, BILs, DHs, F_2-F_3, and BC_3F_3 by employing molecular markers such as RFLP, AFLP, SSR, and SNPs [49,51,52,53,54,55, 56,57,58,59,60,61,62]. However, developing genotypic information from a large number of genetic resources of rice using molecular markers is a very laborious and time-consuming process. Therefore, accessibility to the high-quality reference genome of rice sequences has led to the discovery of single nucleotide polymorphisms (SNPs) [63,64]. Recently, the availability of 3K genome sequences in the public domain should allow the discovery of and more access to SNP markers [65]. To date, there is not much molecular breeding information related to WCA QTLs, whereas very few reports have been directed to DSR and aerobic rice. Based on this information, the present study conducted a weed competitive experiment to assess the phenotypic variance and molecular genetics of WCA, performed using a 6K SNP array.

2. Materials and methods

2.1 Plant material

A total of 167 ILs of the BC_1F_5 generation of a GSR IR2-6 population were derived from a cross between Weed Tolerant Rice 1 (WTR1) as a Xian (*indica*) recipient parent and Y134 as a Xian donor parent developed at IRRI, Los Baños, Philippines [20]. To create the weed competitiveness, we used jungle rice [*Echinochloa colona* (L.)] as one of the most dominant grass weed species in the pot experiment.

2.2 Experiment 1: Estimation of pre-germination counting for 10 traits

The phenotypic experiments were conducted in an IRRI (14◦11′N, 121◦15′E) screen house facility during the dry season of 2014. Initially, seed dormancy was broken by subjecting the seeds to 50 °C temperature for 4 days [66]. Two replications of 25 seeds from each of the 167 ILs were germinated in a 9-cm-diameter Petri dish lined with filter paper and placed in a germination chamber for 14 days. Seeds showing 2 mm radicle length were considered germinated. Seed germination was counted at two different intervals: after 48 hours was

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considered as first germination count (1st GC) and after 7 days as second germination count (2nd GC). After 14 days, seedling traits were recorded for shoot length (SL), root length (RL-1), total fresh weight of germinated seeds (TFGS), total dry weight of germinated seeds (TDGS), germination percentage (GP-1), average fresh weight (AFW), average dry weight (ADW), and vigor index (VI-1). The pre-germination traits were calculated from five normal seedlings randomly selected from each replication (Table 1, Fig. 1). SL was measured from the collar region to the tip of the topmost leaf and expressed in centimeters, whereas RL was measured from the collar region down to the tip of the longest root. The total weight of seedlings in milligrams divided by the number of seedlings gave the average weight of each seedling. Vigor index (VI) was calculated by multiplying the rate of germination by dry seedling weight.

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Table 1 Rice agro-morphological characters for WCA traits in rice

S. No.		Trait observations	Description of trait information
Early seed germination (ESG) traits			
1	1st GC	1st germination count	No. of germinated seeds after 48 hours
2	2nd GC	2nd germination count	No. of germinated seeds after 7 days
3	GP-1	Germination percentage	Ratio of the first count to the final count of germination
4	SL	Shoot length (cm)	Measured from the collar region to the tip of topmost leaf
5	RL	Root length (cm)	Measured from collar region down to the tip of the longest root
6	TFGS	Total fresh weight of germinated seeds	Total fresh weight of all seeds that germinated
7	TDGS	Total dry weight of germinated seeds	Total dry weight of all seeds that germinated
8	AFW	Average fresh weight	Fresh weight/no. of seeds that germinated
9	ADW	Average dry weight	Dry weight/no. of seeds that germinated
10	VI-1	Vigor index	Measured by computing % germination x seedling dry weight
Early seedling vigor (ESV) traits			
1	GC	Germination count	Number of seedlings germinated
2	GP-2	Germination percentage	Ratio of the first count to the final count of germination
3	PH	Seedling plant height (cm)	Plant height at 7, 14, 21, and 28 DAS
4	NLs	No. of leaves	Number of leaves at 7, 14, 21, and 28 DAS
5	NT	No. of tillers	Number of tillers per plant
6	LCC	Leaf chlorophyll content	Reading based on SPAD meter
7	VI-2	Vigor index (VI)	Measured by computing % germination x seedling dry weight
8	LFW	Leaf fresh weight (g)	Fresh weight of leaves
9	LDW	Leaf dry weight (g)	Dry weight of leaves after drying at 65 ^{o}C for 3 days
10	RFW	Root fresh weight (g)	Fresh weight of roots
11	RDW	Root dry weight (g)	Dry weight of roots after drying at 65 ^{o}C for 3 days
12	TFW-2	Total fresh weight (g)	Measured by computing leaf fresh weight + root fresh weight
13	TDW-2	Total dry weight (g)	Measured by computing leaf dry weight + root dry weight
14	RL-2	Root length (cm)	Measured from collar region down to the tip of the longest root
15	WFW	Weed fresh weight	Fresh weight of weeds
16	WD	Weed density	Density of weeds
17	WDW	Weed dry weight	Dry weight of weeds after drying at 65 ^{o}C for 3 days

2.3 Experiment 2: Pot experiment for seedling vigor traits: 18 traits

The soil type used in the experimental pots is Maahas clay loam (iso-hyperthermic mixed Typic-Tropudalf). Two replications of 5 seeds of 167 ILs along with parents WTR1 and Y134 were used. The pre-germinated seeds were sown in plastic pots filled with soil, thinned to 1 seedling pot^{-1}, and maintained until 28 days to investigate ESV-related traits. One hundred seeds of jungle rice [*Echinochloa colona* (L.) Link] were sown in each pot to homogenize weed emergence. Germination count (GC), germination percentage (GP-2), seedling plant height (PH), and number of leaves (NL) were measured at 7, 14, 21, and 28 days after sowing (DAS), whereas other traits such as number of tillers (NT), leaf chlorophyll content (LCC), and vigor index (VI-2) were recorded after 28 days. Further, the seedlings were pulled out carefully, shoots and roots were separated, and individual observations were recorded for leaf fresh weight (LFW), leaf dry weight (LDW), root length (RL),

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root fresh weight (RFW), root dry weight (RDW), total fresh weight (TFW-2), and total dry weight (TDW-2). Weed biomass was clipped at the soil surface from each pot and measured for fresh weight and dried in an oven at 70 °C for 5 days for observation of dry weight. Each of these traits was measured with three replications of each IL. Lastly, weed density (WD), weed fresh weight (WFW), and weed dry weight (WDW) were gathered to correlate with seedling vigor performance as related to weed competitiveness (Table 1, Fig. 1).

3. Genotyping using a 6K SNP array

3.1 DNA extraction and genotyping

A total of 167 ILs and parents of plant leaf samples were collected at 21 DAS. The genomic DNA was extracted and purified following a modified CTAB method [67] and quantified by a NanoDrop 8000 spectrophotometer (Thermo Scientific, USA). The concentration of DNA sample was adjusted to 50 ng/µl and used in the SNP array. Further, DNA quantification, incubation, and hybridization of bead chip staining and image scanning according to the manufacturer's instructions for the Illumina Infinium assay were conducted in the Genotyping Services Laboratory of the International Rice Research Institute. The resulting intensity data were processed by using genotyping module V2011.1 of Genome Studio software (Illumina Inc., San Diego, CA, USA) for SNP calling.

3.2 Statistical and QTL mapping analysis

The association between marker and trait was determined by SMA. The phenotypic data from 167 ILs and the corresponding marker data were used in the analysis. The average phenotypic trait values of the ILs were used as inputs to detect main-effect QTLs using the software IciMapping4.0 [68]. The permutation test method was used to obtain the empirical thresholds of the ESG and ESV experiments by 1000 runs of randomly shuffling the trait values [69]. LOD values of 2.5 were used for identifying a significant QTL in both experiments. QTLs with a phenotypic variance of greater than 10% were considered major QTLs. For the additive effect, a positive value means that the desirable allele is from the recurrent parent (WTR1), whereas a negative value means that the desirable allele is from the donor parent (Y134). A linkage map was constructed by using MapChart software [70]. The descriptive statistics, correlation analysis, analysis of variance, and frequency distribution were obtained for all phenotypic traits using PB tools Version 1.4 (http://bbi.irri.org/products) and R software package [71]. The summary of the genotypic data indicated that 677 high-quality SNP markers were used in the construction of high-density linkage maps for WCA QTLs related to ESG and ESV, and their corresponding genome size and physical distance per centimorgan (cM) are presented in Table 2.

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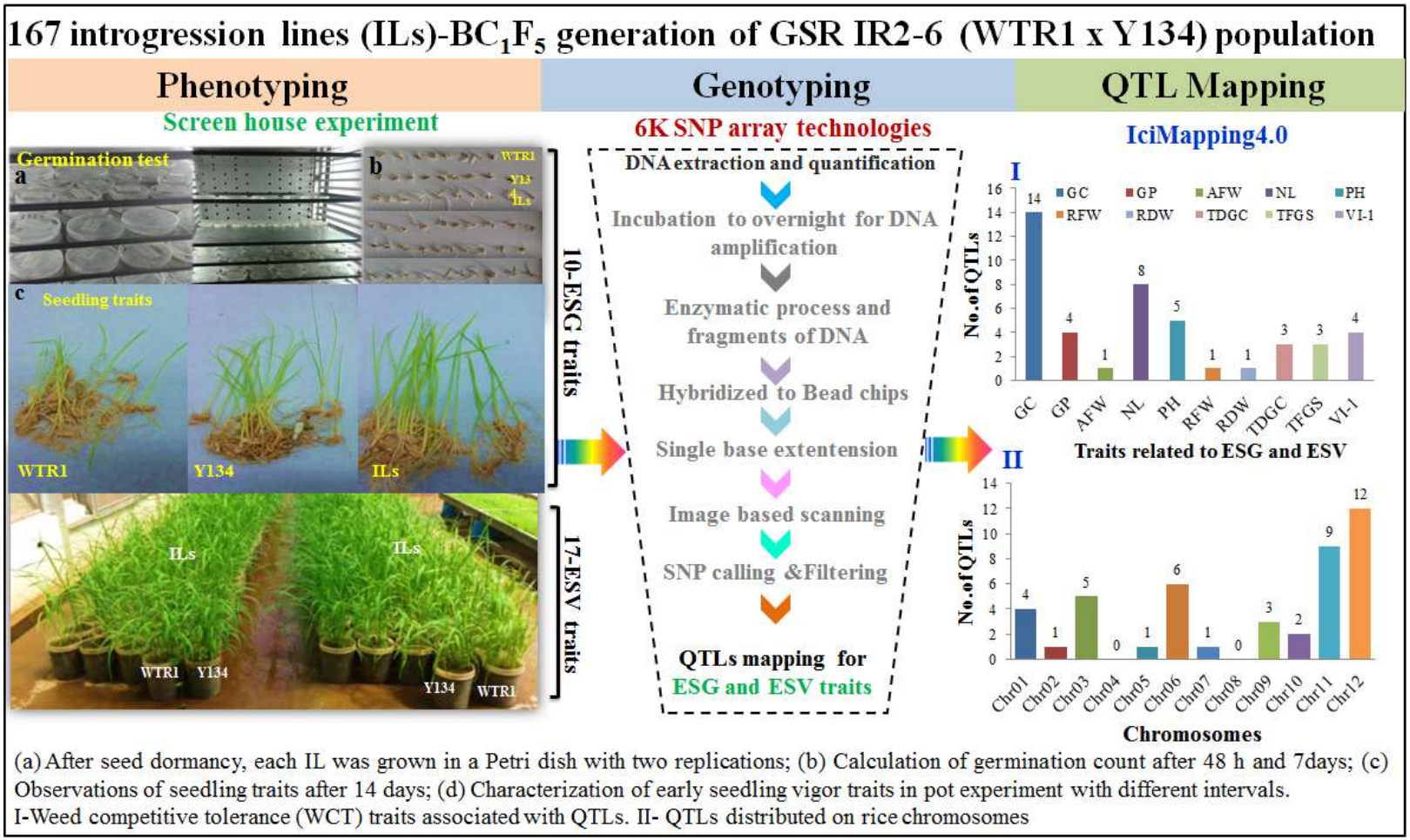

(a) After seed dormancy, each IL was grown in a Petri dish with two replications; (b) Calculation of germination count after 48 h and 7days; (c) Observations of seedling traits after 14 days; (d) Characterization of early seedling vigor traits in pot experiment with different intervals. I-Weed competitive tolerance (WCT) traits associated with QTLs. II- QTLs distributed on rice chromosomes

Figure 1. Phenotypic and genotypic evaluation of weed competitive tolerance traits and associated QTLs in rice.

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Table 2 Summary of markers used in genotyping 167 lines of Green Super Rice (GSR) IR2-6 population.

S. no.	Chr	No. of markers	Average dis (Kb)	GSC by SNPs (Kb)	TGS (Gramene)	PC	GD (cM)	PD (kb/cM)
1	1	77	549.3	42297.1	43270.9	97.7	181.8	238.0
2	2	53	651.2	34511.0	35937.3	96.0	157.9	227.6
3	3	61	573.3	34970.6	36413.8	96.0	166.4	218.8
4	4	35	939.8	32891.7	35502.7	92.6	129.6	273.9
5	5	55	513.4	28236.0	29958.4	94.3	122.3	245.0
6	6	74	392.5	29043.4	31248.8	92.9	124.4	251.2
7	7	76	378.4	28758.3	29697.6	96.8	118.6	250.4
8	8	56	492.1	27559.2	28443.0	96.9	121.1	234.9
9	9	47	466.3	21918.2	23012.7	95.2	93.5	246.1
10	10	35	505.7	17699.3	23207.3	76.3	83.8	276.9
11	11	58	488.1	28309.3	29021.1	97.5	117.9	246.2
12	12	50	506.7	25333.7	27531.9	92.0	109.5	251.4
Total		677	6456.8	351527.6	373245.5	1124.5	1526.8	2960.5

Chr-Chromosome; **GSC**-Genome size covered; **TGS**-Total genome size; **GD**-Genetic distance; **PC**-Percentage of coverage; **PD**-Physical distance per centiMorgan

4. RESULTS

4.1 Phenotypic variation for ESG-related traits

A total of ten ESG traits (1st GC, 2nd GC, GP-1, SL, RL-1, TFGS, TDGS, AFW, ADW, and VI-1) were investigated from 167 ILs and the results showed significant phenotypic variation among the ILs. The mean GP of WTR1 and Y134 was 68% and 94%, respectively, with an overall average of all ILs of 63.9%. The phenotypic variation (PV) of ESG traits ranged from 2.72 to 9.57 cm (SL), 2.5 to 9.97 cm (RL), 0.12 to 1.19 g (TFW), 0.01 to 0.45 g (TDW), 0.016 to 0.132 g (AFW), 0.0050 to 0.0547 g (ADW), and 0.01 to 37.35 (VI-1), with a wide range for each trait in the ILs (Supplementary Table 1). However, one of the major traits (GP) showed more than 90% germination in 11 ILs, including the donor parent Y134, and, in the same set of ILs, we found the highest values for VI in the range of 27.6 to 37.35. Out of the ten ESG traits, the highest coefficient of variation (*CV)* values were identified in AFW (48.25%) and in 1st GC after 48 h (44.05%), whereas the lowest *CV* was observed in TDW (17.83%) and SL (18.58%). Analysis of variance (ANOVA) of these traits revealed a significant difference ($P < 0.001$) in ten traits, except RL ($P = 0.0834$) and SL ($P = 0.0137$) (Table 3). This implies that shoot and root length were not significantly different. In the pair-wise correlation coefficient of ESG traits, 1st GC positively and significantly correlated with 2nd GC, GP-1, TDW, TFW, and VI-1 ($r = 0.58$, $r = 0.57$, $r = 0.49$, $r = 0.30$, $r = 0.58$, $P < 0.001$), whereas a significant negative correlation was observed with RFW, RDW ($r = -0.40$ and -0.21, $P < 0.001$), RL ($r = 0.15$, $P < 0.001$), and SL ($r = -0.13$, $P < 0.01$). The highest positive significant *CV* was recorded between the traits 2nd GC and VI-1 ($r = 0.94$, $P < 0.001$), GP and VI-1 ($r = 0.94$, $P < 0.001$), and TDW and VI-1 ($r = 0.86$, $P < 0.001$), whereas a negative significant correlation was observed between 1st GC with RFW ($r = -0.40$) and RDW ($r = -0.21$), 2nd GC with RFW ($r = -0.66$) and RDW ($r = -0.45$), GP-1 with RFW ($r = -0.63$) and RDW ($r = -0.41$), TDW with RFW ($r = -0.40$), RFW and VI-1 ($r = -0.51$), and RDW with VI-1 ($r = -0.26$) at $P < 0.001$ respectively (Supplementary Table 2). Moreover, a genetic non-significant positive correlation was observed between TDW with RL and SL. Similarly, TDW and RDW had a non-significant positive correlation with each other. Therefore, the significant correlation of ESG traits indicated that 2nd GC, GP, and TDW were strongly correlated with VI-1.

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Table 3 ANOVA for the testing of significance of genotype effect per trait in ESG test

S. No.	Trait	Sum Sq	Mean Sq	F value	Pr (>F)
1	1st GC	8383.0	50.5	2.88	0.0000***
2	2nd GC	7918.8	47.7	3.88	0.0000***
3	GP-1 (%)	128918.7	776.6	3.79	0.0000***
4	SL (cm)	33068.2	199.2	1.41	0.0137*
5	RL (cm)	54759.7	329.9	1.24	0.0834
6	TFW (g)	8.2	0.0493	2.96	0.0000***
7	TDW (g)	1.8	0.0107	4.06	0.0000***
8	AFW (g)	0.1	0.0007	1.44	0.0099**
9	ADW (g)	0.01	0.0001	1.83	0.0001***
10	VI-1	23039.1	138. 8	3.59	0.0000***

Significance codes: P >0.05; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** P <0.001

Table 4 ANOVA for the testing of significance of genotype effect per trait for ESV test

S. No.	Trait	Sum Sq	Mean Sq	F value	Pr (>F)
1	GC	546.9	3.3	1.44	0.0093**
2	GP-2	218771.3	1317.9	1.44	0.0093**
3	PH at 7 DAS (cm)	6096.1	36.7	1.16	0.1742
4	PH at 14 DAS (cm)	17092.2	102.1	1.49	0.0050**
5	PH at 21 DAS (cm)	39066.5	235.3	1.31	0.0409*
6	PH at 28 DAS (cm)	38934.8	234.6	2.19	0.0000***
7	NL at 7 DAS	130.8	0.8	1.64	0.0008***
8	NL at 14 DAS	379.6	2.3	1.62	0.0010***
9	NL at 21 DAS	2206.2	13.3	1.71	0.0003***
10	NL at 28 DAS	4559.5	27.5	2.10	0.0000***
11	NT	259.3	1.6	2.64	0.0000***
12	LCC	17313.0	104.3	1.48	0.0061**
13	RL (cm)	5372.4	32.4	1.67	0.0005***
14	LFW (g plant^{-1})	1801.3	10.9	1.49	0.0055**
15	LDW (g plant^{-1})	21.3	0.1	1.56	0.0023**
16	RFW (g plant^{-1})	63.4	0.4	2.24	0.0000***
17	RDW (g plant^{-1})	1.3	0.0	1.97	0.0000***
18	TFW (g plant^{-1})	2213.2	13.3	1.61	0.0012**
19	TDW (g plant^{-1})	30.1	0.2	1.66	0.0006***
20	VI-2	375273.9	2260.7	1.56	0.0023**

Significance codes: P >0.05; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** P <0.001

4.2 Phenotypic variation for ESV-related traits

A significant phenotypic variance was observed in 17 ESV-related traits. The average of phenotypic traits ranged from 1 to 5 (GC), 10% to 100% (GP-2), 8.5 to 26.2 mm (PH.7 DAS), 20.3 to 56.5 mm (PH.14 DAS), 35 to 58.4 mm (PH.21 DAS), 2 to 3 (NL.7 DAS), 2 to 6 (NL.14 DAS), 8 to 35 (NL.28 DAS), 2 to 6 (TN), 8.8 to 45.2 mm (RL), 32.5 to 43.0 (LCC), 1.73 to 24.11 g (LFW), 0.30 to 1.83 g (LDW), 0.19 to 2.45 g (RFW), 0.05 to 0.48 g (RDW), 2.01 to 24.47 g (TFW), 0.38 to 1.93 (TDW), and 0.0 to 154.5 (VI-2) (Supplementary Table 1). Nine out of 167 ILs showed a VI-2 value of more than 130 and also had maximum GP-2, NLs, PH, LCC, RFW, SFW, SDW, and RDW. Five ESV traits (GC, GP-2, LDW, RDW, and TDW) had a significant *CV* of 45.42%, 45.42%, 45.26%, 40.52%, and 50.08%, respectively, observed between the ILs, whereas the lowest *CV* values (<30%) were observed in PH.28 DAS, NT, LCC, and 28 DAS.NLs . The highest *CV* suggests that the selected ILs exhibited higher genetic variability. The summary of ANOVA (Table 4) shows significant genotypic effects on all the traits

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(P ≤0.0001) at the 1% level, except PH at 7 DAS (P = 0.174). ESV traits have exhibited highly significant variation, including PH at 28 DAS, NL at 7, 14, 21, and 28 DAS, NTs, RL, RFW, RDW, and TDW (P < 0.001). PH at 21 DAS showed significance only at the 5% level, which indicates that very few QTLs, can be located for this trait. The ESV correlation trait analysis in Supplementary Table 3 shows that all the traits are significantly and positively correlated with one another except for RDW with GC and GP-2 (P >0.05; r = 0.07), RL with WD and WDW (P >0.05; r = 0.11), RFW with WD (P >0.05; r = 0.11), and RDW with WDW (P >0.05; r = 0.1). Among these, only eight traits had a high positive correlation observed between the traits such as TFW and LFW (r = 0.99), TDW and LDW (r = 0.98), TN and NL at 28 DAS (r = 0.88), PH.21 DAS and NL.14 DAS (r = 0.87), PH.14 DAS and NL.14 DAS (r = 0.86), TDW and TN (r = 0.80), VI-2 with GC and GP-2 (r = 0.88), PH.7 DAS with PH.14 DAS (r = 80), PH.21 DAS (r = 84), NL.7 DAS (r = 80), and NL.14 DAS (r = 0.80). Weed competitive traits such as WD, WFW, and WDW are negatively correlated with each other, except in RL, RFW, and RDW.

4.3 Promising G-6 ILs with weed competitive ability

Selection of WCA traits in rice cultivars is becoming imperative, especially in DSR breeding programs. The present study revealed that GP and VI are the critical regulators for ESG and ESV traits in WCA in rice. However, in both experiments of ESG and ESV trait analysis, VI and GP were significantly correlated with each other. Based on the VI (>120) and GP (>90) values, 17 ILs and 10 ILs were identified as promising ILs in ESV and ESG conditions, respectively. Five ILs (*G-6-Y4-WL-3, G-6-Y15-WL-3, G-6-L5-WU-2, G-6-L9-WU-1* and *G-6-L12-WU-2*) with ESG and 15 ILs (*G-6-RF7-WL-3, G-6-Y14-WU-1, G-6-L2-WL-2, G-6-Y14-WL-3, G-6-Y4-WL-3, G-6-Y14-WL-1, G-6-L5-WU-2, G-6-Y9-WU-2, G-6-RF13-WL-1, G-6-L12-WL-2, G-6-Y15-WL-2, G-6-L12-WU-2, G-6-Y5-WU-3, G-6-Y5-WU-3* and *G-6-L3-WL-3*) with ESV were shown to possess higher GP and VI than their parents. Interestingly, two ILs (*G-6-Y3-WL-1* and *G-6-Y4-WL-3*) were commonly identified in both experiments of ESG and ESV. Therefore, these promising ILs can be useful in breeding programs for the development of rice cultivars with WCA.

5. Genotypic analysis

5.1 QTLs for WCA traits

A total of 677 SNP markers were used to generate a linkage map with a total distance coverage length of 1526.8 cM and an average distribution of each SNP marker of 9.53 cM. The number of SNP markers on different chromosomes ranged from 35 on chromosomes 4 and 10 to 77 on chromosome 1. The number of SNP markers per chromosome, length coverage of each chromosome distance, and average distribution of SNP marker information appear in Table 2.

A total of 44 QTLs for ESG and ESV traits were mapped on all 12 chromosomes, except on chromosomes 4 and 8, and explained phenotypic variation that ranged from 7.85% to 19.9%, with an LOD score of 2.93 to 8.03, respectively (Fig. 2). Of these 44 QTLs, 29 were associated with seven ESG-related traits (1st GC, 2nd GC, TFGS, TDGS, AFW, GP-1, and VI-1), while the remaining 15 QTLs were associated with four ESV-related traits (NL, PH, RDW, and RFW) (Tables 5 and 6). More than 10% of PV of each QTL was considered as a major QTL, with an LOD score of >4.0, and the remaining QTLs were considered as minor-effect QTLs with less than <10% of PV. Out of 44 QTLs, 16 major QTLs were located on seven chromosomes (1, 3, 5, 6, 9, 11, and 12), whereas 28 QTLs showed minor effects with less than <10% of PV, located on nine chromosomes (1, 2, 3, 6, 7, 9, 10, 11, and 12). For ESG and ESV traits, the WTR-1 allele contributed to 13 QTLs related to 1st GC, 2nd GC, GP-2, VI-2, TDGS, and two ESV traits (NL at 7 and 14 DAS), whereas, for 31 QTLs, the Y134 allele contributed the effect, especially for seven ESG traits (1st GC, 2nd GC, GP-1, TFGS, TDGS, AFW, and VI-1) and eight ESV traits (PH.7 DAS, PH.14 DAS, PH.21 DAS, PH.28 DAS, NL.7 DAS, NL.21 DAS, RDW, and RFW).

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Table 5 QTLs identified for early seed germination (ESG) traits related to WCA

S. No.	QTL	Trait name	Chr	Position (bp)	Peak marker	LOD	PV (%)	Additive effect	Parent*
1	q1st GC2.1	1st GC	2	4930742	SNP_2_4930742	3	8.1	-0.3	Y134
2	qTDGS3.1	TDGS	3	33713486	SNP_3_33713486	3.91	10.2	0	Y134
3	qTFGS3.1	TFGS	3	33713486	SNP_3_33713486	3.56	9.4	-0.1	Y134
4	q1st GC5.1	1st GC	5	29061672	SNP_5_29061672	5.61	14.3	2.52	WTR1
5	q1st GC6.1	1st GC	6	2871279	SNP_6_2871279	3.46	9.1	-1.7	Y134
6	q1st GC6.2	1st GC	6	4641044	SNP_6_4641044	3.13	8.3	-1.6	Y134
7	q1st GC10.1	1st GC	10	20723502	SNP_10_20723502	3.57	9.4	1.74	WTR1
8	qTDGS10.1	TDGS	10	20723502	SNP_10_20723502	3.47	9.1	0.03	WTR1
9	q1st GC11.1	1st GC	11	22546707	SNP_11_22546707	6.24	15.8	2.3	WTR1
10	q1st GC11.2	1st GC	11	27994133	SNP_11_27994133	8.03	19.9	2.65	WTR1
11	q2nd GC11.1	2nd GC	11	24779246	SNP_11_24779246	4.34	11.3	1.93	WTR1
12	qAFW11.1	AFW	11	24728131	SNP_11_24728131	4.88	12.6	0	Y134
13	qGP-111.1	GP-1	11	22044151	SNP_11_22044151	3.09	8.2	7.24	WTR1
14	qGP-111.2	GP-1	11	24779246	SNP_11_24779246	4.34	11.3	7.73	WTR1
15	qTDGS11.1	TDGS	11	27994133	SNP_11_27994133	5.54	14.2	0.03	WTR1
16	qVI-111.1	VI-1	11	22546707	SNP_11_22546707	2.98	7.9	2.73	WTR1
17	qVI-111.2	VI-1	11	27994133	SNP_11_27994133	5.21	13.4	3.66	WTR1
18	q1st GC12.1	1st GC	12	14835375	SNP_12_14835375	3.08	8.2	-1.6	Y134
19	q1st GC12.2	1st GC	12	17788006	SNP_12_17788006	3.15	8.3	-1.6	Y134
20	q1st GC12.1	1st GC	12	14936674	SNP_12_14936674	3.09	8.3	-0.4	Y134
21	q1st GC12.2	1st GC	12	17443323	SNP_12_17443323	3.09	8.3	-0.4	Y134
22	q2nd GC12.1	2nd GC	12	16286946	SNP_12_16286946	3.28	8.7	-1.6	Y134
23	q2nd GC12.2	2nd GC	12	17902839	SNP_12_17902839	3.27	8.6	-1.6	Y134
24	qGP-112.1	GP-1	12	16286946	SNP_12_16286946	3.28	8.7	-6.4	Y134
25	qGP-112.2	GP-1	12	17902839	SNP_12_17902839	3.27	8.6	-6.3	Y134
26	qTFGS12.1	TFGS	12	19786034	SNP_12_19786034	4.26	11.1	0	Y134
27	qTFGS12.2	TFGS	12	25792416	SNP_12_25792416	4.01	10.5	0	Y134
28	qVI-112.1	VI-1	12	16287347	SNP_12_16287347	4.16	10.8	-3.1	Y134
29	qVI12.2	VI-1	12	25792416	SNP_12_25792416	3.9	10.2	-3	Y134

***Parent**- contributing desirable allele; **Chr**- chromosome; For the additive effect, a positive value means that the desirable allele is from the recurrent parent (WTR1),
while a negative value means that the desirable allele is from the donor parent (Y134)

5.2 QTLs associated with ESG traits

As shown in Fig. 2 and Table 5, a total of 29 QTLs associated with ESG traits were mapped on seven chromosomes. Of these, 12 QTLs were located on chromosome 12, nine on chromosome 11, two on chromosomes 3, 6, and 10, and one on chromosomes 2 and 5, which explained their PV ranging from 7.9% to 19.9%, respectively. Eleven QTLs had a positive additive allele of WTR1, which explained the PV of five ESG traits (1st GC, 2nd GC, GP-1, TDGC, and VI-1) ranging from 7.9% (LOD score 2.91) to 19.9% (LOD score 8.03). Moreover, 18 QTLs had a negative additive allele contributed by Y134, and are significantly associated with seven ESG traits (1st GC, 2nd GC, AFW, GP-1, TDGC, TFGC, and VI-1). However, the largest expression of PV was observed in the 1st germination count on chromosome 5 (*q1st GC5.1*), which explained 14.3%. Similarly, three QTLs for 1st GC and total dry weight of germinated seeds on chromosome 11 (*q1st GC11.1*, *q1st GC11.2*, and *qTDGS11.1*) were explained by R^2 values of 15.8%, 19.9%, and 14.2%, respectively (total variance is 64.2%). The high ranges of PV of the QTLs were controlled by a positive allele from WTR1. Of these 29 QTLs, 14 (11 QTLs for 1st GC and three for 2nd GC) for 1st GC and 2nd GC on chromosomes 2, 5, 6, 10, 11, and 12, four QTLs for GP-1 on chromosomes 11 and 12, seven QTLs for TFGS, TDGS, and AFW on chromosomes 3, 10, 11, and 12, and

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four QTLs for VI on chromosomes 11 and 12 were explained by the significant PV (Table 5). Among these additive effects of QTLs, one major QTL (*q1st GC11.2)* on chromosome 11 was marked by SNP_11_27994133 and exhibited the highest PV (19.9%), with a LOD score of 8.3, which is influenced by the effect of the WTR1 allele. Interestingly, to date, there have been no reports on AFW- and TFGS-associated QTLs for ESG and ESV. A single major QTL (*qAFW11.1*) for AFW was mapped on chromosome 11, which is marked by SNP_11_24728131 at the position of 24.7 Mb, with an R^2 value of 12.6%, in the current study. The novel QTL *qAFW11.1* was contributed by a desirable allele coming from donor parent Y134. For TFGS, two major QTLs (*qTFGS12.1*, *qTFGS12.2*) on chromosome 12 and one minor QTL (*qTFGS3.1*) on chromosome 3 were detected with a negative additive allele contributed by donor parent Y134 (-0.03 and -0.06). Together, the TFGS QTLs explained 11.2% of PV. Three major QTLs on chromosomes 11 and 12 that controlled the ESG-related trait VI were *qVI-111.2*, *qVI-112.1*, and *qIV12.2*, with R^2 of 13.4%, 10.8%, and 10.2% and an LOD score of 5.21, 4.16, and 3.9, respectively. The fourth QTL (*qVI-111.1*) explained PV of 7.9% and had an LOD score of 2.98. The additive effect of the QTLs on chromosome 11 (*qIV11.1* and *qIV11.2*) was positive, implying that the desirable alleles were contributed by WTR1, whereas the additive effect of the QTLs on chromosome 12 (*qIV12.1* and *qIV12.2*) was negative, and was contributed by Y134.

Table 6 QTLs identified for early seedling vigor (ESV) traits related to WCA

S. No.	QTL	Trait name	Chr	Position (bp)	Peak marker	LOD	PV (%)	Additive effect	Parent*
1	qNL-7$_{1.1}$	NL.7 DAS	1	1022215	SNP_1_1022215	4.3	11.4	0.14	WTR1
2	qPH-14$_{1.1}$	PH.14 DAS	1	42397588	SNP_1_42397588	3.29	8.7	-2.02	Y134
3	qPH-21$_{1.1}$	PH.21 DAS	1	13777831	SNP_1_13777831	3.06	8.1	-1.71	Y134
4	qPH-28$_{1.2}$	PH.28 DAS	1	24722142	SNP_1_24722142	3.3	8.8	-1.62	Y134
5	qNL-7$_{3.1}$	NL.7 DAS	3	34568654	SNP_3_34568654	3.43	9.2	-0.15	Y134
6	qPH-7$_{3.1}$	PH.7 DAS	3	2230854	SNP_3_2230854	3.18	8.5	-1.01	Y134
7	qRDW$_{3.1}$	RDW	3	34568654	SNP_3_34568654	2.93	7.85	-0.03	Y134
8	qNL-7$_{6.1}$	NL.7 DAS	6	12183428	SNP_6_12183428	3.12	8.4	-0.15	Y134
9	qNL-7$_{6.2}$	NL.7 DAS	6	17750942	SNP_6_17750942	3.14	8.4	-0.16	Y134
10	qNL-7$_{6.3}$	NL.7 DAS	6	25277863	SNP_6_25277863	3.78	10.1	-0.14	Y134
11	qRFW$_{6.1}$	RFW	6	25277863	SNP_6_25277863	3.65	9.7	-0.16	Y134
12	qNL-21$_{7.1}$	NL.21 DAS	7	15241178	SNP_7_15241178	3.14	8.4	0.49	WTR1
14	qNL-21$_{9.1}$	NL.21 DAS	9	21885499	SNP_9_21885499	3.25	8.7	-0.61	Y134
13	qNL-7$_{9.1}$	NL.7 DAS	9	21896910	SNP_9_21896910	5.04	13.2	-0.17	Y134
15	qPH-14$_{9.1}$	PH.14 DAS	9	9834510	SNP_9_9834510	3.08	8.1	-1.69	Y134

*Parent- contributing desirable allele; DAS- days after sowing

5.3 QTLs associated with ESV traits

Fifteen QTLs associated with ESV-related traits PH.7, 14, 21, and 28 DAS, NL.7 and 21 DAS, RFW, and RDW were mapped on five different chromosomes (1, 3, 6, 7, and 9). Five QTLs associated with PH.7, 14, 21, and 28 DAS were located on chromosomes 1, 3, and 9 (Table 6 and Fig. 2) and explained 42.2% of PV. On chromosomes 1 and 9, two QTLs for PH.14 DAS (*qPH-141.1*, R^2 = 8.7%, and *qPH-149.1*, R^2 = 8.1%) were marked by SNP_9_21885499 and SNP_9_9834510. The other three remaining QTLs (*qPH-73.1*, *qPH-211.1*, and *qPH-281.2*) were linked with PH.7, 21, and 28 DAS, with LOD scores of 3.18 (R^2 = 8.5%), 3.06 (R^2 = 8.1%), and 3.30 (R^2 = 8.8%), respectively. The additive effect indicated that Y134 contributed the desirable alleles.

Among these QTLs, three are major and are associated with NLs on chromosome 1 (*qNL-71.1*, R^2 = 11.4%), chromosome 6 (*qNL6.3*, R^2 = 10.1%), and chromosome 9 (*qNL-79.1*, R^2 = 13.2%), with LOD scores of 4.30, 3.78, and 5.04, respectively. Of these three QTLs, two (*qNL-71.1* and *qNL-79.1*) had a positive allele contributed by WTR1, and the other QTL had a negative allele contributed by Y134. Among these additive effects of the QTLs, one major QTL (*qNL-71.1)* on chromosome 7 was marked by SNP_1_1022215 and exhibited the highest PV (11.4%), with a LOD score of 4.3. To date, there have been no reports on QTLs associated with a number of leaves at different growth stages. Among the eight novel QTLs, one major QTL (*qNL-71.1*) and another minor QTL (*qNL-*

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21$_{7.1}$) were contributed by WTR 1 alleles and the remaining six QTLs were influenced by a negative allele coming from donor parent Y134. However, the overall WCA QTLs were contributed by both parents. Out of 44 QTLs, 31 were contributed by a negative additive allele of Y134, whereas 13 were contributed by a positive additive allele of WTR1. Therefore, the frequency of ESG and ESV traits associated with QTLs showed continuous segregation, and it is controlled by multiple QTLs and genes in rice

6. Hotspots and co-localized QTLs for ESG and ESV traits

QTLs associated with WCA-related traits were identified in two hotspot regions on chromosomes 11 and 12. Nine QTLs are located on chromosome 11 at the position from 22.4 Mb to 27.9 Mb, and it is labeled as "QTL hotspot I", which covers a total genomic length of 5.5 Mb. Similarly, chromosome 12 contained a total of 12 QTLs that were located at the position from 14.8 Mb to 25.7 Mb, and this is labeled as "QTL hotspot II", with a total coverage length of 10.9 Mb (Fig. 2). QTL hotspot I was associated with six ESV-related traits (GP, 1st GC, VI-1, AFW, 2nd GC, and TDGC) grouped together with an average PV of 12.65%, whereas QTL hotspot II contained five ESV-related traits (1st GC, 2nd GC, GP, VI-1, and TFGC) effectively showing the PV average of 9.19%%. Interestingly, chromosome 11 had a positive additive effect contributed by a WTR1 allele, except for one QTL (*qAFW*$_{11.1}$) associated with an Y134 allele. On the contrary to chromosome 11, the negative additive allele from Y134 contributed to all the QTLs on chromosome 12. Therefore, these hotspot QTLs are largely consistent and are highly correlated with ESG and ESV traits.

Taken together, 58.6% and 26.6% of the ESG and ESV QTLs were co-localized. ESV traits related to two QTLs (*qNL7*$_{6.3}$ and *qRFW-7*$_{6.1}$) on chromosome 6 were marked with SNP_6_25277863 at the position of 25.2 Mb and two QTLs (*qRDW*$_{3.1}$ and *qNL-7*$_{3.1}$) on chromosome 3 were marked by SNP_3_34568654 at the position of 34.5 Mb and were co-localized. However, both of these QTLs were contributed by a donor parent of the Y134 allele. For ESG traits, three QTLs (*q1*st *GC*$_{11.2}$, *qTDGS*$_{11.1}$, and *qVI-1*$_{11.2}$) at 27.9 Mb, two QTLs (*q2*nd *GC*$_{11.1}$, and *qGP-1*$_{11.2}$) at 24.7 Mb, and two QTLs (*q1*st *GC*$_{11.1}$ and *qVI-1*$_{11.1}$) at 22.5 Mb were co-localized on chromosome 11 and two QTLs (*q1st GC*$_{10.1}$ and *qTDGC*$_{10.1}$) at the position of 20.7 Mb were co-localized on chromosome 10. These co-localized QTLs were contributed by a recipient parent of the WTR1 allele. Similarly, on chromosome 12, two QTLs (*q2*nd *GC*$_{12.1}$ and *qGP-1*$_{12.1}$) at 16.2 Mb, two QTLs (*q2*nd *GC*$_{12.2}$ and *qGP-1*$_{12.2}$) at 17.9 Mb, and two other QTLs (*qTFGS*$_{12.2}$ and *qIV*$_{12.2}$) at 25.7 Mb were co-localized. The hotspot co-localized QTLs on chromosome 12 were associated with a negative allele from a donor parent of Y134. Interestingly, studies on the hotspot QTL regions revealed that alleles were associated with both parents and this indicated that a wide range of the molecular and phenotypic diversity of ESG and ESV traits related to WCA existed among the ILs.

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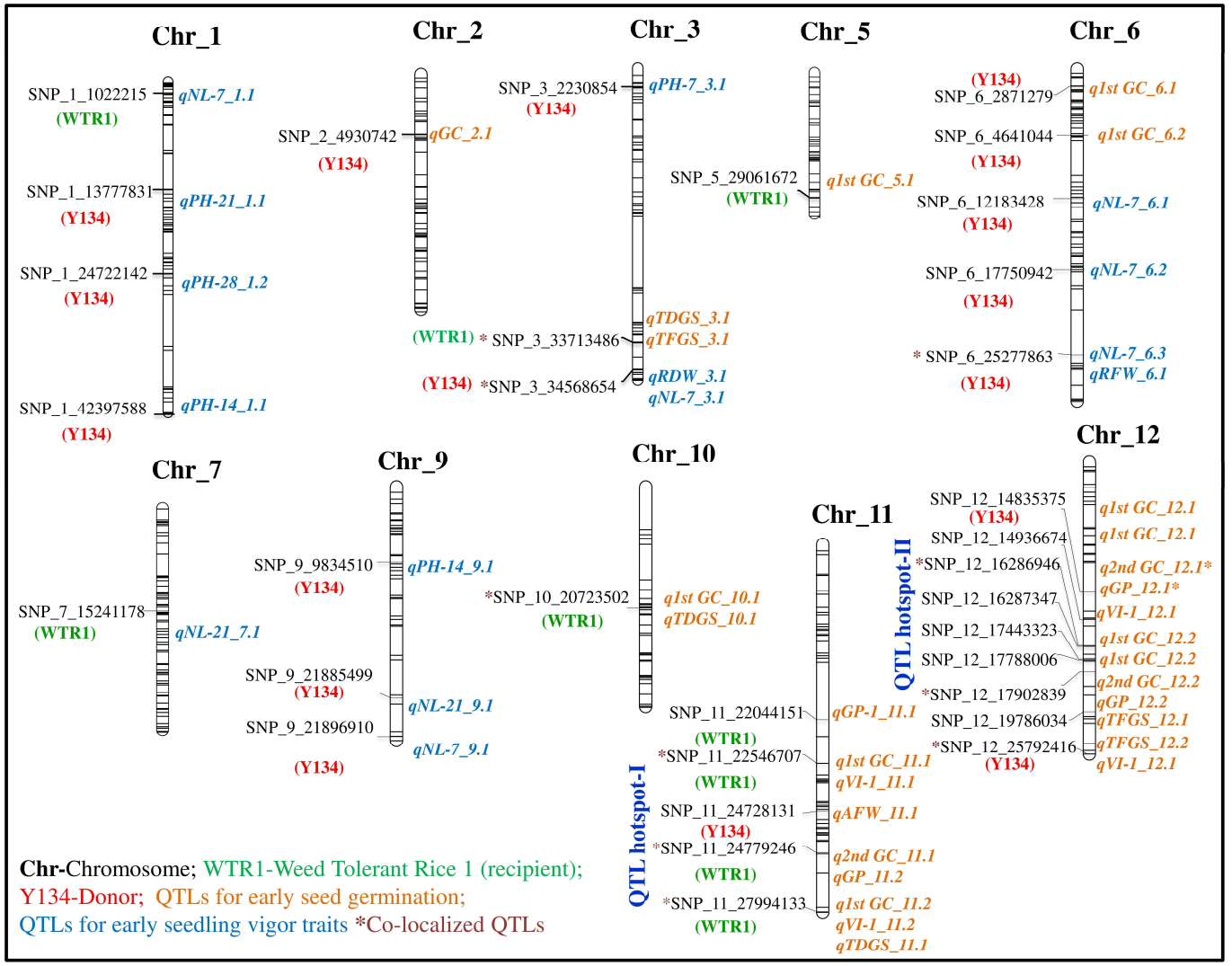

Figure 2. Linkage map of 44 QTLs associated with ESG- and ESV-related traits on 10 different chromosomes with respective polymorphic markers

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7. Discussion

Several factors such as the decline in water availability, increasing labor cost, severe growth of weeds, and climatic fluctuation are significantly contributing to a decrease in rice productivity and increase in cost of cultivation. DSR is one of the most promising alternatives to transplanted rice, which could save on irrigation water, labor cost, crop duration, and inputs; make planting easier and faster; and lower methane emissions [13,72,73,74,75]. However, compared to the irrigated rice ecosystem, yield has decreased in DSR due to the maintenance of floodwater in early growth stages, which can influence the rapid growth of weeds and can inhibit the growth of rice plants [76]. Therefore, weeds are one of the major biological constraints affecting yield under DSR. Approximately 50% to 90% of yield losses were occurring because of the failure to control weeds [77,78]. There is a possibility to control weeds by adopting weed management strategies such as tillage systems, developing weed-competitive cultivars, and using herbicides in DSR [19,20,79,78,80]. However, these weed management strategies are quite uneconomical, laborious to farmers, and frequently use herbicides, which can lead to contamination of the environment and human health hazards. Among the various methodologies, the development of WCA cultivars is a feasible and safe approach to overcome weed infestations [19,20]. The identification of WCA traits and the associated chromosomal regions of QTLs are vital in a breeding program for the development of rice cultivars with WCA [20]. The knowledge generated in this process will give us more insight into and a better understanding of the genetic and molecular mechanisms of WCA, which can provide rapid early growth toward crop establishment and suppression of weed growth in the different ecosystems of DSR and the aerobic rice ecosystem. Breeding of weed competitive ability in rice is essential for the development of DSR varieties under both dry and wet seed methods. The identification of QTLs for ESG and ESV is essential for DSR rice breeding programs. Therefore, the present study attempted to address WCA traits and their association with chromosomal regions to identify potential QTLs.

7.1 Early seed germination trait and QTLs governing it

ESG traits are key components to improve WCA in rice. The uniformity of seedling growth and germination percentage are significantly associated with strong, vigorous crop growth and better seedling establishment, which can influence the improvement of yield [47,52,60,81]. Germination percentage of the donor and recurrent parents is 94% and 68%, whereas the phenotypic performance of the total ILs showed a significant variation from 2% (*G-6-RF2-WL-1*) to 98% (*G-6-Y15-WL-3*). Among the total of 167 ILs, 11 ILs exhibited the highest GP (>90%), whereas the donor parent had 94%. The extreme phenotypic variation in GP may indicate that transgressive segregation took place in the population and this implies that both parents possess positive QTLs and genes for WCA in rice. ESG traits showed transgressive segregation and also suggested that WCA is controlled by multiple quantitative trait loci and genes in rice.

QTLs for ESG-related traits such as 1st GC, 2nd GC, GP, TDGC, and VI-1 were influenced by WCA in rice. Out of 29 identified QTLs, 11 QTLs for 1st GC ($q1^{st}$ $GC_{2.1}$, $q1^{st}$ $GC_{5.1}$, $q1^{st}$ $GC_{6.1}$, $q1^{st}$ $GC_{6.2}$, $q1^{st}$ $GC_{10.1}$, $q1^{st}$ $GC_{11.1}$, $q1^{st}$ $GC_{11.2}$, $q1^{st}$ $GC_{12.1}$, $q1^{st}$ $GC_{12.2}$, $q1^{st}$ $GC_{12.1}$, and $q1^{st}$ $GC_{12.2}$), six QTLs for TDGC (*qTDGS*$_{3.1}$, *qTDGS*$_{10.1}$, *qTDGS*$_{11.1}$, *qTFGS*$_{3.1}$, *qTFGS*$_{12.1}$, and *qTFGS*$_{12.2}$), four QTLs for GP (*qGP-1*$_{11.1}$, *qGP-1*$_{11.2}$, *qGP-1*$_{12.1}$, and *qGP-1*$_{12.2}$), four QTLs for VI-1 (*qVI-1*$_{11.1}$, *qVI-1*$_{11.2}$, *qVI-1*$_{12.1}$, and *qIV*$_{12.2}$), three QTLs for 2nd GC ($q2^{nd}$ $GC_{11.1}$, $q2^{nd}$ $GC_{12.1}$, and $q2^{nd}$ $GC_{12.2}$), and a single QTL for AFW (*qAFW*$_{11.1}$) are located on seven different chromosomes, 2, 3, 5, 6, 10, 11, and 12. Except for eight GP and VI QTLs mentioned above that were reported earlier [49,57,58,82,83,84], the remaining 21 QTLs were novel. The rate of seedling emergence, germination percentage, and uniformity of seedling growth are the major factors for crop establishment, which provides superior root growth that can help in the absorption of more nutrients [85,86,87,88,89,90,91]. Interestingly, we observed that 8 of the 21 novel QTLs were co-localized on chromosomes 11 and 12 (Fig. 2 and Table 5). Several researchers reported that the speed of germination and seedling vigor had significant positive correlations with field emergence (FE), seedling establishment (SE), GR, PH, and SDW [20,57,92,93,94]. However, Diwan [58] reported that 1st GC, GR, and SDW were significantly correlated with vigor index. Therefore, a significant positive correlation result indicates that traits such as GP-

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1, 2nd GC, and VI exhibited a strong positive relationship with FE and SE may be favorable traits for WCA in rice. Mahender et al. [19] reviewed and mentioned 38 QTLs that were associated with GR, GI, and GP and also germination time in different genetic backgrounds of mapping populations. In the present study, the major QTL *qGP-1*$_{11.2}$ (R^2 = 11.3%) coincided with GP, GR, and GI on chromosome 11 and was also observed in the genetic background of RIL mapping populations of Daguandao (*japonica)* and IR28 *(indica),* respectively [57]. Several QTLs for ESG-related traits (1st GC and 2nd GC) were previously reported: *qLTG-2* on chromosome 2 [95], *qGR3-1* and *qGR3-3* on chromosome 3 [52], *qLTG-4-2*, *qLTG-4-1*, and *qGP-4* chromosome 4 [52,57], *qGR5-1* and *qLTG-5* on chromosome 5 [52,95,96], *qGR6-2* on chromosome 6 [52,57,96], *qGR7-1* on chromosome 7 [52,53], and *qLTG-11, qGR-11,* and *qGI-11* on chromosome 11 [57,95] in the diverse sets of mapping populations as RILs, BILs, and DHs in rice. Therefore, co-localization of these QTLs will provide a genetic basis underlying the correlation among the traits. Chromosomes 11 and 12 contained more than five traits related to ESG, which indicates that they are actively associated with rapid seedling growth in rice. However, in the genetic backgrounds of the rice populations studied, *indica* rice had a higher speed and germination rate than *japonica* rice [53,57].

7.2 Early seedling vigor trait and QTLs governing it

ESV is an essential trait in DSR and the aerobic rice ecosystem. Numerous morphological and physiological key traits are involved in ESV that determines the improvement of seedling growth and grain yield component traits [19,97,98]. Out of the 167 ILs, seven ILs exhibited higher VI-2 values (>130) than the donor parent. Interestingly, the best promising ILs were associated with GP-2, NLs, PH, LCC, FSW, FRW, RDW, and SDW. Among these traits, five ESV-related traits (GC, GP-2, LDW, RDW, and TDW) were significantly associated with the highest coefficient of variation, except for PH at 7 DAS (P = 0.174). Out of 17 ESV-related traits, ten (GC, GP-2, PH at 7, 14, and 21 DAS, NL at 7 and 28 DAS, TN, LFW, and LDW) had the highest positive correlations (>r = 0.80) (Supplementary Table 3). Thus, the identified promising ESV traits were strongly associated with a rapid increase in seedling growth, biomass accumulation, and yield-attributed traits under WCA conditions. ESV traits showed transgressive segregation and also suggested that WCA is controlled by multiple quantitative trait loci and genes in rice. The initial phase of the firm seedling establishment is a key component for the ESV trait, which can determine a massive potential role in uniformity of emerging seedlings, rapid germination, and high vigor in growth against weeds under a wide range of field conditions. There are several ESV-related morphological, physiological, and biochemical traits associated with DSR, aerobic rice, and WCA in rice [19]. However, there is no information regarding WCA traits, which are associated with the genomic segment of QTLs or genes. In the present study, out of the 15 ESV QTLs, seven QTLs for NL.7 DAS (*qNL-7*$_{1.1}$, *qNL-7*$_{3.1}$, *qNL-7*$_{6.1}$, *qNL-7*$_{6.2}$, *qNL-7*$_{6.2}$, *qNL-7*$_{6.3}$, and *qNL-7*$_{9.1}$), two QTLs for NL.21 DAS (*qNL-21*$_{7.1}$, and *qNL-21*$_{9.1}$), two QTLs for PH.14 DAS (*qPH-14*$_{1.1}$, and *qPH-14*$_{9.1}$), and a single QTL for PH.7 DAS (*qPH-7*$_{3.1}$), PH.21 DAS (*qPH-21*$_{1.1}$), PH.28 DAS (*qPH-28*$_{1.2}$), root dry weight (*qRDW*$_{3.1}$), and root fresh weight (*qRFW*$_{6.1}$) were identified on five different chromosomes (1, 3, 6, 7, and 9) and they explained PV ranging from 8.1% to 11.4%, respectively. Among these QTLs, NLs associated with eight QTLs at 7 and 21 DAS located on chromosomes 1, 3, 6, 7, and 9 were novel loci (Table 6). Based on the comprehensive ESV QTL analysis, these five chromosomes were harboring multiple trait-associated QTLs and also were co-localized, promising QTLs for ESV in rice [19]. A total of 15 traits (GR, SDW, SFW, TDW, RSC, SL, SEV, GI, FV, FGC, EGC, RL, TFW, LA, and RA) were found on chromosome 1 [57,59,60,81,99,100,101,102,103]. Seven traits (SL, SDW, GR, RL, SEV, FW, and COL) were found on chromosome 3 [55,59,101,102]. Similarly, chromosome 6 was associated with seven traits (TDW, GR, SDW, RSC, SS, GP, and SL) [57,104], Three traits (GR, GI, and SL) were found on chromosome 7 [60,105], and four traits (RA, SDW, SS, and LA) were found on chromosome 9 [52] that were responsible for more than three ESV traits associated with shoot length, shoot dry weight and leaf area QTLs in different mapping populations. Four QTLs contributing to NL.7 DAS and PH.14, 21, and 28 DAS on chromosome 1 overlapped with earlier reported QTLs related to 15 ESV traits on chromosome 1 at the physical positon from 29.7 cM (RM259) to 146.1 cM (RM315). These ESV traits associated with QTLs were earlier reported by using with diverse genetic resources of rice accessions and biparental mapping populations [59,60,81,99,100,101,103]. However, PH QTLs at 14, 21 and 28 DAS on chromosome 1 shared common genomic regions associated with SL, which was reported by Yan et al. [99], Zhou et al. [101], and Cairns et al. [103]. Similarly, Diwan et al. [81] identified six QTLs for SL

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in the 18.8 to 71.6cM region, which showed 10% to 15% significant PV, and the identical chromosomal segment of the genomic region was controlling other ESV-related traits according to Redoña and Mackill [106] and Zhang et al. [54]. Interestingly, the α-amylase gene amy1B/A is located at 13 cM, which is near the novel QTL *NL-7*$_{1.1}$ on chromosome 1 [107]. This gene may influence higher germination rate and quick seedling growth at the early stage through the degradation of starch energy sources by *α-amylase* in the rice embryo. The same genomic regions were controlling ESV traits and were frequently detected across other mapping populations of *O. rufipogon* and *japonica* cultivar 'Jefferson' [108].

Further, the expression of ESV-related QTLs on chromosome 3 was contributing to three different QTLs (*qNL-7*$_{3.1}$, *qPH-7*$_{3.1,}$ and *qRDW*$_{3.1}$) influencing WCA in rice. The NL.7 DAS and RDW QTL overlapped in the same genomic region marked by SNP_3_34568654, showing the negative allele contributed by Y134 (Fig. 2). Eight ESV-related traits (SL, SDW, GR, RL, SEV, FW, coleorhiza length, and SDW) were significantly expressed on chromosome 3 and were identified from two different RIL populations derived from Lemont/Teqing [55,101,102] and Jiucaiqing/IR26 [59], a doubled-haploid population of CT9993/IR62266 [56], BC_3F_4 lines from Swarna/Moroberekan [84], and a natural diverse germplasm of rice accessions [60]. Further, in support of the findings on chromosome 3, recently Singh et al. [84] reported a QTL hotspot in the chromosome 3 region that had three possible candidate genes (*Os03g0236200, Os03g0324300,* and *Os03g0428700*). These genes were involved in different roles for the development of young seedling, mesocotyl length, coleoptile elongation, and increasing physiological activity via changes in the ethylene signaling mechanism in cell differentiation, elongation, enzyme activities, and expansion genes, which demonstrate early seedling emergence and growth development.

Four QTLs (*qNL-7*$_{6.1}$, *qNL-7*$_{6.2}$, *qNL-7*$_{6.3,}$ and *qRFW*$_{6.1}$) were mapped on chromosome 6, which account for a total PV of 36.6%. Two QTLs (*qNL-7*$_{6.3}$ and *qRFW*$_{6.1}$) overlapped with the same genetic marker of SNP_6_25277863 and were contributed by the donor parent allele. The QTLs located in the region of 48.7 to 101.1 cM were associated with seven ESV-related traits (GR, SDW, TDW, reducing sugar content, seed size, GP, and SL) on two different recombinant populations from ZS97/MH63 [104] and Daguandao/IR28 [57]. The same genetic region is very close to the other QTLs: *qGW-6* for 1000-seed weight [109], *sd6.1* for seed dormancy [110], *qEV*$_{6.1}$ for early vigor, *qEUE*$_{6.1}$ for early uniform emergence, and *qSHL*$_{6.1}$ for shoot length at 21 DAS [84].

On chromosome 7, a single QTL (*qNL-21*$_{7.1}$) was contributed by a positive allele from recipient parent WTR1. The remaining three QTLs (*qNL-7*$_{9.1}$, *qPH-14*$_{9.1}$, and *qNL-21*$_{9.1}$) located on chromosome 9 had a negative additive effect coming from donor parent Y134. The QTL controlling NL at 21 DAS on chromosome 7 was associated with previous reports on six ESV-related traits, SL, TN, weight of mobilized seed reserve (WS), LAI, GR, and GI, in the QTL mapping studies from RILs [55,57,59,105], BC_3F_3 [103], and natural germplasm [60]. The position of the novel QTL located on chromosome 9 at 87.5 and 39.3 cM was close to the genomic region and was associated with four ESV traits (SDW, TDW, root activity, and seed weight) that accounted for a total PV of 27.4% in the genetic background of RILs (Zhenshan 97 and Minghui 63) reported by Cui et al. [52]. The co-localization of all the QTLs related to ESV morphological traits such as seed dormancy, germination-attributed traits, shoot and root length, fresh and dry weight of shoot and root, and mesocotyl length, and physiological traits such as reducing sugar, photosynthetic performance, leaf area, chlorophyll content, amylase activity, nitrate reductase, peroxidase, growth regulation hormones (abscisic acid, auxin, and GA), and antioxidant enzymes (glutamic acid decarboxylase activity) located in the same genetic region provided valuable genomic information for improving WCA in rice.

7.4 The use of WCA traits and novel QTLs in varietal breeding

The Green Super Rice (GSR) breeding strategy [20,111,112,113] helped in developing the mapping population ideally selected for WCA traits in a systematic manner with progeny testing. The study showed promising ILs with WCA traits, with 17 ILs specifically for ESG and 10 ILs for ESV traits. However, 5 ILs in ESG and 15 ILs in ESV showed higher GP and VI than their parents and two ILs (*G-6-Y3-WL-1* and *G-6-Y4-WL-3*) were in common. In support of these findings, several traits such as PH [40,92,114], tiller number [17,115,116,117], early growth rate [46], germination rate and percentage [52,57,104], leaf area index [118,119,120], mesocotyl elongation [121], early crop biomass [122,123], shoot and root dry weight

[48,101,124,125], canopy ground cover [118,126,127], and early vigor [36,37,81] were significantly linked to ESV for tolerance of WC in rice. Anandan et al. [49] suggest that earlier observations of morphological and physiological traits at 14 and 28 DAS were more reliable for estimating for the study of ESV compared with 56 DAS in the analysis of 629 rice genotypes under direct-seeded aerobic conditions.

Using SMA approaches in 167 BC_1F_5 mapping populations, we identified several significant QTLs and validated them with previously reported QTLs [19]. In the present study, we identified 29 novel QTLs that were associated with ESG- and ESV-related WCA in rice. Out of the 29 QTLs, 21 were related to ESG and were located on chromosomes 3, 5, 6 10, 11, and 12, whereas the remaining 8 QTLs were related to ESV and were located on chromosomes 1, 3, 6, and 7 (Tables 5 & 6 and Fig. 2). To date, there is no published evidence on QTLs for WCA traits in rice, especially on periodic germination counts, germinated seedlings with fresh and dry weight, number of leaves at 7 and 21 DAS, and average fresh weight of seedlings. The identified novel QTLs on each chromosome explained a total PV of 11.4%, 8.1%, 28.8%, 14.3%, 44.3%, 8.4%, 21.9%, 18.5%, 73.8%, and 72% on chromosomes 1, 2, 3, 5, 6, 7, 9, 10, 11, and 12, respectively. Among these, the highest numbers of QTLs were on chromosome 3 (*qNL-7*$_{6.1}$, *qNL-7*$_{6.2}$, *qNL-7*$_{6.3}$, *qNL-21*$_{7.1}$, *qNL-21*$_{9.1}$, and *qNL-7*$_{9.1}$) and chromosome 12 (*q1*st *GC*$_{12.1}$, *q1*st *GC*$_{12.2}$, *q1*st *GC*$_{12.1}$, *q1*st *GC*$_{12.2}$, *q2*nd *GC*$_{12.1}$, *q2*nd *GC*$_{12.2}$, *qTFGS*$_{12.1}$, and *qTFGS*$_{12.2}$) from donor parent Y134. The QTLs on chromosome 11 (*q1*st *GC*$_{11.1}$, *q1*st *GC*$_{11.2}$, *q2*nd *GC*$_{11.1}$, *qAFW*$_{11.1}$, and *qTDGS*$_{11.1}$) were contributed by WTR1, the recipient parent. The novel and co-localized QTLs on chromosomes 3, 11, and 12 were associated with multiple traits, such as 1st GC, 2nd GC, VI-1, GP, TDGS, AFW, and TFGC. These QTLs were strongly correlated with ESG and ESV traits. Therefore, further high-resolution mapping studies are required for the validation of expression and pleiotropic effect of these QTLs. However, the majority of early QTLs studies have reported that multiple ESV traits are controlled by the same genomic region of reported chromosomes 1, 3, 5, 6, 9, 11, and 12 [19,52,53,56,57,59,60,81,84,95,96]. The novel QTLs accounting for a higher LOD and PV could be a potential target in future breeding programs and subsequent studies are needed to find the candidate genes and alleles for the strong association and to understand the physiological and molecular mechanism conferring WCA.

7.5 Putative candidate genes and their function

QTL hotspot regions and co-localized QTLs on chromosomes 11 and 12 were used to analyze the candidate genes for WCA in rice. A total of five possible genes on chromosome 11 and eight genes on chromosome 12 were identified (Table 4). Out of 13 putative genes, two were hypothetical, six were putative proteins, and the remaining five genes were well reported to be involved in multiple functions related to biotic and abiotic stress tolerance in rice. On chromosome 11, GP is associated with pentatricopeptide repeat (PPR) domain and three other co-localized QTLs (*q1*st*GC*$_{11.2}$, *qTDGS*$_{11.1}$, and *qVI-1*$_{11.2}$) were associated with tetra-tricopeptide repeat (TPR) domain at 22 Mb and 27.9 Mb positions, respectively. Earlier reports of Gothandam et al. [128], Lin et al. [129], and Yu et al. [130] have suggested that PPR domain located on different chromosomes and their functions are related to chloroplast development, photosynthesis, seedling lethality during early leaf growth stage, embryogenesis, seed development, and cytoplasmic male sterility (CMS) [129,130,131,132,133,134]. In addition to that, TPR-containing protein has been involved in several functions such as cell cycle, regulation of gibberellins (GAs), seed development, hybrid sterility, endosperm development, and seed setting [135,136]. Similarly, other co-localized QTLs (*q1*st*GC*$_{11.1}$ and *qVI-1*$_{11.1}$) at 22.5 Mb on chromosome 11 are found within the range of 4 kbs of *Os11g38010*. This locus has been reported to encode TPX2 homologue, which is considered to be involved in the organization of microtubule spindle formation during cell division [137]. Two hypothetical and putative expressed loci (*Os11g41240* and *Os11g41320*) were found associated with three QTLs (*qAFW*$_{11.1}$, *q2*nd *GC*$_{11.1}$, and *qGP-1*$_{11.2}$).

On chromosome 12, QTL *q1*st*GC*$_{12.1}$ is associated with SAP domain-containing protein, which is functionally related to stress-associated proteins (SAPs), which are involved in regulating GA and ABA signaling in response to abiotic stresses [138,139,140,141]. Growth hormone regulations are vital to seedling growth and development. The QTL *qTFGS*$_{12}$ is associated with *Os12g32750* at the position of 19.7 Mb. These loci are responsible for flavin monooxygenases (FMO), which have a significant functional role in the tryptophan (Trp)-dependent indole-acetic acid (IAA) synthesis for auxin biosynthetic pathways for the improvement of the quick response of early seedling growth and root tip development [142,143,144]. Interestingly, we identified six

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loci (*Os12g27650, Os12g29950 Os12g41670, Os12g25760, Os12g29370,* and *Os12g29750)* that were associated with unknown and hypothetical proteins in the rice genome databases (Table 4). Therefore, the hotspot regions of QTLs and their associated hypothetical proteins may be showing some potential for the development of novel candidate genes for WCA in rice.

8. Conclusions

WCA is an important target trait that needs to be considered by rice breeders for developing DSR varieties. A systematic GSR breeding strategy involving early backcross breeding with the phenotypic selection and progeny testing for WCA traits led to the development of a population for genetic analysis. This approach led to the identification of donors, QTLs, and genes for many of the WCA traits important to the development of rice varieties for DSR and aerobic systems. We identified seven major ESG traits (1st GC, 2nd GC, GP, TFW, TDW, ADW, VI-1) and ten ESV traits (PH.28 DAS, NL.7, 14, 21, 28 DAS, NT, RL, RFW, RDW, TDW) that were significantly associated with WCA in rice. As many as 29 novel QTLs were identified from a total of 44 QTLs that governed the genetic mechanism of WCA. Among these, the majority of the QTLs were associated with two hotspot QTL regions on chromosome 11 with nine QTLs detected (PV ranging from 7.9% to 19.9%) and on chromosome 12 with 12 QTLs detected (PV ranging from 8.2% to 11.1%), and a few of them were co-localized QTLs. The hotspot and co-localized QTL regions could have a greater potential role toward the improvement of WCA. *In silico* analysis of the QTL hotspots on chromosomes, 11 and 12 regarding their respective genomic positions revealed that two hypothetical and six putative candidate genes were located and further investigation to fine-map and use cloning strategies is required to identify novel candidate genes for WCA in rice. Two promising ILs (*G-6-Y3-WL-1* and *G-6-Y4-WL-3*) identified with desirable ESG and ESV traits, highly suitable for its use in DSR breeding programs. The prominent QTLs from promising ILs for WCA traits can be used in the development of functional markers and their use in QTL pyramiding with desirable genetic backgrounds. These markers could be further employed for the introgression of genes/QTLs into elite rice cultivars through a MAS breeding program for the development of rice varieties with WCA.

Author Contributions: ND conducted the phenotypic experiment. PC, AB, MD, YP, and ND recorded the observations of phenotypic traits and generated genotypic information. ND, YP, and MA performed statistical analysis, and ND and MA interpretation, drafting, and revision of the manuscript. JA and ZL were involved in designing the entire screen house experiment and in the critical revision of the manuscript. Finally, JA conceived the study and contributed to the critical revision of the final manuscript. All authors approved the final version of the manuscript.

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Supplementary table 1 Descriptive statistics of all traits measured for weed competitive ability test.

S. No.	Early seed germination (ESG) traits	Min	Max	Mean	SD	CV (%)
1	1st GC	0	25	9.51	5.87	44.05
2	2nd GC	1	25	15.98	5.49	21.93
3	GP-1 (%)	2	100	63.77	22.25	22.43
4	SL (mm)	27.2	127.40	63.96	13.22	18.58
5	RL (mm)	25.0	133.80	66.71	19.45	24.45
6	TFW (g)	0.12	1.26	0.67	0.18	19.42
7	TDW (g plant^{-1})	0.01	0.57	0.29	0.08	17.83
8	AFW (g plant^{-1})	0.016	0.23	0.05	0.03	48.25
9	ADW (g plant^{-1})	0.005	0.093	0.02	0.01	33.27
10	VI-1	0.01	44	19.71	9.48	31.53
	Early seedling vigor (ESV) traits					
1	GC	1	5	3.33	1.74	45.42
2	GP-2	10	100	66.53	34.89	45.42
3	PH at 7 DAS (mm)	8.5	26.2	15.30	6.02	36.82
4	PH at 14 DAS (mm)	20.3	60.2	26.87	9.70	30.89
5	PH at 21 DAS (mm)	35.0	81.0	44.58	14.94	30.06
6	PH at 28 DAS (mm)	53.7	87.2	68.81	13.42	15.03
7	NL at 7 DAS	2	3	2.03	0.80	34.21
8	NL at 14 DAS	2	6	3.82	1.38	31.09
9	NL at 21 DAS	5	16	8.61	3.31	32.40
10	NL at 28 DAS	8	35	14.04	4.50	25.75
11	NT	2	7	3.46	1.04	22.25
12	LCC	32.5	154.8	36.14	9.38	23.25
13	RL (mm)	8.8	69.1	14.35	5.23	30.67
14	LFW (g plant^{-1})	1.73	41.9	6.80	3.08	39.74
15	LDW (g plant^{-1})	0.30	2.2	0.96	0.33	29.91
16	RFW (g plant^{-1})	0.19	3.6	0.91	0.56	45.26
17	RDW (g plant^{-1})	0.05	0.5	0.16	0.09	40.52
18	TFW (g plant^{-1})	2.01	42.2	7.71	3.31	37.36
19	TDW (g plant^{-1})	0.38	2.6	1.11	0.38	29.69
20	VI-2	0	170.2	76.08	44.82	50.08

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Supplementary table 2 Correlation analysis for all the traits measured for weed competitive ability in ESG test.

Traits	1st GC	2nd GC	GP-1	RL	SL	TFW	TDW	RFW	RDW
1st GC									
2nd GC	0.58***								
GP-1	0.57***	1.00***							
RL	-0.15**	-0.02	-0.02						
SL	-0.13*	-0.06	-0.06	0.37***					
TFW	0.30***	0.57***	0.57***	0.15**	0.11*				
TDW	0.49***	0.74***	0.73***	0.02	0.05	0.72***			
RFW	-0.40***	-0.66***	-0.63***	0.16**	0.13*	-0.04	-0.40***		
RDW	-0.21***	-0.45***	-0.41***	0.05	0.17**	0.04	0.11*	0.61***	
VI-1	0.58***	0.94***	0.94***	-0.02	-0.06	0.68***	0.86***	-0.51***	-0.26***

Significance codes: P >0.05; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** P <0.001

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Supplementary table 3 Correlation analysis of all traits for weed competitive ability in ESV test.

	GC	GP-2	7PH	14PH	21PH	28PH	7LN	14LN	21LN	28LN	TN	RL	LCC	LFW	LDW	RFW	RDW	TFW	TDW	VI-2	WD	WFW	WDW
GC																							
GP-2	1.00***																						
7PH	0.63***	0.63***																					
14PH	0.62***	0.62***	0.80***																				
21PH	0.62***	0.62***	0.84***	0.89***																			
28PH	0.38***	0.38***	0.56***	0.63***	0.62***																		
7LN	0.54***	0.54***	0.80***	0.73***	0.82***	0.46***																	
14LN	0.61***	0.61***	0.80***	0.86***	0.87***	0.58***	0.84***																
21LN	0.53***	0.53***	0.75***	0.77***	0.85***	0.48***	0.84***	0.85***															
28LN	0.11*	0.11*	0.32***	0.34***	0.32***	0.48***	0.46***	0.45***	0.56***														
TN	0.21***	0.21***	0.42***	0.48***	0.44***	0.53***	0.52***	0.55***	0.64***	0.88***													
RL	0.18***	0.18***	0.25***	0.26***	0.27***	0.36***	0.31***	0.36***	0.32***	0.40***	0.48***												
LCC	0.22***	0.22***	0.29***	0.33***	0.33***	0.57***	0.27***	0.33***	0.25***	0.34***	0.43***	0.26***											
LFW	0.14*	0.14*	0.39***	0.36***	0.31***	0.44***	0.35***	0.35***	0.39***	0.54***	0.56***	0.24***	0.29***										
LDW	0.16**	0.16**	0.43***	0.44***	0.41***	0.60***	0.45***	0.46***	0.51***	0.72***	0.78***	0.31***	0.38***	0.64***									
RFW	0.17**	0.17**	0.30***	0.25***	0.27***	0.22***	0.49***	0.42***	0.45***	0.60***	0.60***	0.47***	0.14*	0.34***	0.51***								
RDW	0.07	0.07	0.27***	0.19***	0.20**	0.21***	0.39***	0.32***	0.36***	0.56***	0.60***	0.45***	0.15**	0.30***	0.57***	0.77***							
TFW	0.16**	0.16**	0.41***	0.37***	0.34***	0.44***	0.41***	0.40***	0.44***	0.60***	0.62***	0.30***	0.29***	0.99***	0.69***	0.49***	0.41***						
TDW	0.15**	0.15**	0.43***	0.42***	0.39***	0.56***	0.47***	0.47***	0.52***	0.74***	0.80***	0.36***	0.36***	0.62***	0.98***	0.61***	0.71***	0.68***					
VI-2	0.88***	0.88***	0.62***	0.63***	0.60***	0.42***	0.55***	0.62***	0.59***	0.32***	0.42***	0.21***	0.21***	0.34***	0.49***	0.38***	0.32***	0.38***	0.49***				
WD	-0.27***	-0.27***	-0.16**	-0.20***	-0.18**	-0.15**	-0.1	-0.12*	-0.17**	-0.01	-0.04	0.11	-0.07	-0.14*	-0.1	0.11	0.17**	-0.11*	-0.05	-0.28***			
WFW	-0.33***	-0.33***	-0.27***	-0.29***	-0.28***	-0.24***	-0.16**	-0.24***	-0.24***	-0.04	-0.07	-0.01	-0.12*	-0.16**	-0.1	0.05	0.12*	-0.14**	-0.06	-0.30***	0.66***		
WDW	-0.28***	-0.28***	-0.25***	-0.25***	-0.23***	-0.24***	-0.14**	-0.20***	-0.19***	-0.05	-0.09	0.1	-0.14**	-0.17**	-0.11	0.03	0.1	-0.15**	-0.07	-0.25***	0.56***	0.94***	

Significance codes: P >0.05; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** P <0.001

Note: GC-germination count; GP-2- Germination percentage; 7PH- plant height at 7 DAS; 14PH- plant height at 14 DAS; 21PH- plant height at 21 DAS; 28PH-plant height at 28 DAS; 7LN- no. of leaves at 7DAS; 14LN- no. of leaves at 14 DAS; 21LN- no. of leaves at 21 DAS; 28LN- no. of leaves at 21 DAS; TN- tiller number; RL- root length; LCC- leaf chlorophyll content; LFW- leaf fresh weight; LDW- leaf dry weight; RFW- root dry weight; RDW- root dry weight; TFW- total fresh weight; TDW- total dry weight; VI-2- vigor index; WD- weed density; WFW- weed fresh weight; WDW- weed dry weight