International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 9, Issue 10, 2017, pp.-309-313.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

MAPPING QUANTITATIVE TRAIT LOCI FOR TILLERS NUMBER, PLANT HEIGHT AND THEIR CORRELATION IN RICE [*Oryza sativa* L.]

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Received: November 01, 2017; Revised: November 19, 2017; Accepted: November 20, 2017; Published: November 28, 2017

Abstract- Rice (*Oryza sativa* L.) is a staple food for most of the world's people. About, 122 RILs population derived from a cross Danteshwari × Dagad deshi was used to identify QTL for tillers number per plant and plant height. The normal frequency distribution was followed for both the traits tillers per plant and plant height. Correlation between tiller number and plant height was evaluated and shown significant negative correlation, which means that dwarf plant having more tiller as compare to tall plant. A total of four QTLs were identified for tillers per plant and two for plant height using QTL cartographer 2.5 on chromosomes 1 and 3, respectively. The *"qTN1.1"* and "*qTN3.1"* for tiller number per plant on chromosomes 1 and 3, respectively. The both QTLs for tillers number per plant "*qTN1.1"* and *"qTN3.1"* showed positive additive effect, means that alleles from the parent Danteshwari acted to increase the measured trait tiller number per plant. Two significant major QTLs, *"qPH1.1"* and *"qPH1.2"* also mapped for plant height on chromosomes 1, with very high phenotypic variance of 53.97 and 46.29%, respectively. The QTL, *"qPH1.1"* for plant height found between marker RM3825 and HvSSR1-87 exactly co-localized the "*qTN1.1"* of tillers number per plant on chromosome 1. Both the negatively correlated traits tightly linked and present on same loci, showing linkage drag. The "*qTN1.1"* and *"qTN3.1"* could be useful for the improvement of plant type by pyramiding *via*. marker-assisted selection as tiller number a key component of grain yield.

Keywords- Rice, Tillers per plant, Plant height, QTL analysis, RILs.

Citation: Mandal Lincoln, et al, (2017) Mapping Quantitative Trait Loci for Tillers Number, Plant Height and Their Correlation in Rice [*Oryza sativa* L.]. International Journal of Genetics, ISSN: 0975- 2862 & E-ISSN: 0975-9158, Volume 9, Issue 10, pp.-309-313.

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Introduction

Rice (*Oryza sativa* L.) is the staple food of half of the world's human population [1]. Tillering in rice is one of the most important agronomic traits for grain production because tillers number per plant determines panicle number, a key component of grain yield [2-7]. Tillers number per plant is a quantitative trait with a relatively low heritability of 29.8-49.6% [8]. Many rice researchers have attempted to dissect the genetic basis of tiller number [9]. The *Monoculm1* (*MOC1*) is important in the control of rice tillering in rice [10]. The *OsMAX1a* and *OsMAX1e* are involved in the biosynthesis of strigolactones and regulated rice tillering [11]. Although, a large number of QTLs controlled tillering in rice were reported by different researchers [4, 12-24].

Plant height in rice is generally considered to be controlled by both qualitative and quantitative genes [25]. The high-yielding rice varieties of reduced plant height are important as high lodging resistance and high-harvest index [26]. At least 60 dwarfing genes, designated *d*-1 to *d*-60, have been identified in rice by classic genetic analysis [27]. Molecular mapping of Quantitative trait loci (QTL) for plant height has been reported by various researchers in rice [12, 15, 25, 28-36]. The objectives of this study were (a) mapping of QTLs for tiller number per plant as it main components of yield and plant height (b) find out the correlation of tiller number with plant height.

Materials and Methods

Planting material

The planting materials used in present study was 122 F_{14} recombinant inbred line (RIL) mapping population derived from the parent Danteshwari × Dagad deshi. The mapping population were developed and kindly provided by Dr S. B. Verulkar, Professor and Head, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur. The parent Danteshwari is a high tillers *indica* rice cultivar while Dagad deshi, an *indica* rice cultivar that has low tillers. Similarly, Dagad deshi is tall and Danteshwari is dwarf. The characteristic features of parents given in [Table-1].

Table-1 *Characteristic features of parents*

S. No.	Parent	Pedigree	Salient Features
1.	Danteshwari	Shamridhi ×IR 8608-298	High yielding, Dwarf, Early and high tillering, Resistant to gall midge, Early maturity 105 days, Long slender grain, Moderately susceptible to water stress
2.	Dagad deshi	Land race	Strong culm, Tall, Shy tillering, Broad leaves, Bold seeded, Early maturity 100 days, Tolerance to water stress

Field trial and phenotyping for tillers number per plant and plant height

The trial was conducted during wet season 2013 in RCBD with three replications, each genotype having 2 rows of 1.5m length at research cum instructional farm of IGKV, Raipur (C.G.) (21^0 16' N and 81^0 36' E at altitude of 289.6 meter above sea

level). The mapping populations along with their parents were evaluated for tillers per plant and plant height. The nursery of rice seedlings was prepared before 30 days and transplant with one plant per hill. The labelled field prepared as clean weeds, well plough, paddled and given basal dose of fertilizers for requirement of Nitrogen, Phosphorus and Potash etc. The plant-to-plant and row-to-row spacing was taken as 15 and 20cm, respectively. The plants made clean from weeds and provided top dress urea time to time. From 50 days after transplanting, tiller number per hill and plant height was evaluated from two hill of each replication based on SES [37]. Plant height was recorded as the distance in cm from the soil surface to the tip of the tallest panicle at maturity.

Phenotypic analysis

The phenotypic data of each RIL and parents were analysed. The Mean and SD for Tillers number per plant and plant height were calculated in [Table-2].

Table-2 *Statistics analysis of Tillers number per plant, plant height in parents and RIL population*

Traits	Parents				RIL population	
	Danteshwari		Dagad deshi			
	Mean	SD	Mean	SD	Mean	SD
Tillers per plant	10.50	2.35	4.17	1.47	6.29	1.72
Plant height	83.5	6.28	151.08	13.80	108.59	18.98

DNA isolation and PCR amplification

The genomic DNA isolated from leaves of single tagged plant using MiniPrep method [38]. The detail of DNA isolation method used as around 0.1 g of leaf sample was grinded in a 2 ml eppendorf tube contained 0.4 ml of extraction buffer with the help of MoBIO tissue lyzer. Then 0.4 ml of chioroform-isoamyl alcohol (24:1) mixture was added. Mixed well by vortexing. Centrifuged at 13000 rpm for 30 sec. Supernatant was collected and transfered to a new Eppendorf tube. Then 0.8 ml of absolute ethanol was added and mixed properly by tube inversion. Centrifugation was done at 13000 rpm for 2 min. Supernatant was discarded and pellets were washed with 70 % ethanol. Dried the pallets for 15-20 minutes. Pellets were dissolved in 50-100 µl (based on the size of pellet) TE buffer. The optimized PCR protocol was used for identify the informative SSR markers on the basis of parental polymorphism. Polymerase chain reaction (PCR) amplification for SSR was performed in a total volume of 20 µl and the reaction mixture contained 10 X Assay buffer, 1 mM dNTP mix, 5 pM forward and reverse primers, 40 ηg of template DNA and 1 unit Taq polymerase in 96 well veriti Applied Biosystems thermal cycler, USA. After an initial denaturation step of 95^{0}C for 5 min, the amplification was carried out for 34 cycles comprising 1 min each of 94^{0}C (denaturation), 55^{0}C (annealing) and 72^{0}C (extension). The final elongation step was extended to 7 min at 72^{0}C followed by 4^{0}C. After the PCR reaction was completed, 5 µl of 6 X loading dye was added to PCR amplicons and 7 µl (PCR product with dye) was loaded on 5 % PAGE in a vertical electrophoresis system (CBS scientific, model MGV-202-33, USA) with 180V for 1.5 hours. DNA fragments were then stained with ethidium bromide and visualized with a UV transilluminator Bio-rad XR+ manufactured from USA.

Genotyping of RIL and construction of linkage map

The polymorphism survey was conducted between the parents Danteshwari and Dagad deshi by using 830 SSR markers randomly distributed on all 12 rice chromosomes. Only 162 SSR (RM and HvSSR) [39-40] markers found polymorphic. The genotypes data was prepared for each line based on the banding patterns. All of 162 clearly polymorphic markers were used in segregation analysis of the 122 RILs. The linkage map was constructed using MapMaker/exp ver. 3.0 program [41]. All pairs of linked markers were identified using the "group" command with an LOD value of 3.0. The marker order was determined using the "orders" and the "compare" commands and verified using the "ripple" command. The frequency of recombination between two markers was converted to genetic distance using Kosambi map function [42]. Assignment of linkage groups to the respective chromosomes was based on genetic maps developed by [39] and Gramene Annotated Nipponbare Sequence map [43].

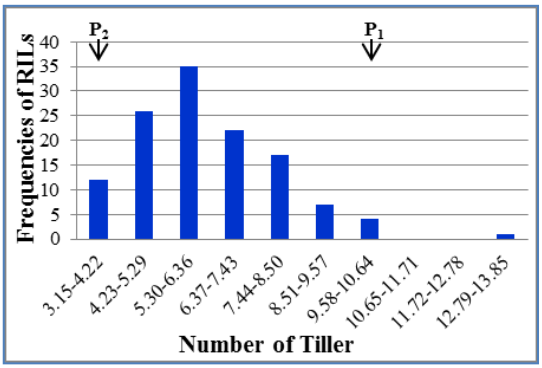
QTL mapping

The mean phenotypic data of three replications for each line and parents was used as the raw value for QTL analysis. The composite interval mapping (CIM) was performed by using QTL cartographer [44]. The threshold log likelihood ratio (LOD) score was estimated empirically with 1000 permutations [45]. The presence of putative QTLs declared if the LOD threshold was larger than 3 for the traits. The proportion of phenotypic variation explained by each QTL was calculated on the basis of R^2 value.

Results

Phenotypic distribution of tillers number and plant height in RILs population

The parents along with RILs exhibited marked variation for tillers number per plant and plant height. The parent Danteshwari showed high tiller number per plant than Dagad deshi. Similarly, Dagad deshi is tall and Danteshwari is dwarf. The frequency distribution indicated that normal distribution was followed for both traits, tiller number per plant and plant height given in [Fig-1].



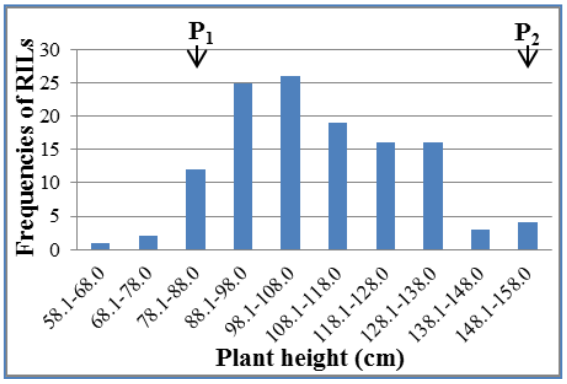


Fig-1 Frequency distribution of tillers per plant and plant height across RILs

Phenotypic correlation between tillers number per plant and plant height

The correlation between tillers per plant and plant height was evaluated at P ≤ 0.05 and P ≤ 0.01 in [Table-3]. In this experiment both the traits showed highly significant negative correlation.

Table-3 *Correlation between tillers per plant and plant height in rice*

Traits	Tillers per plant	Plant height
Tillers per plant	1	
Plant height	-0.44**	1

**= Significant at 1% level

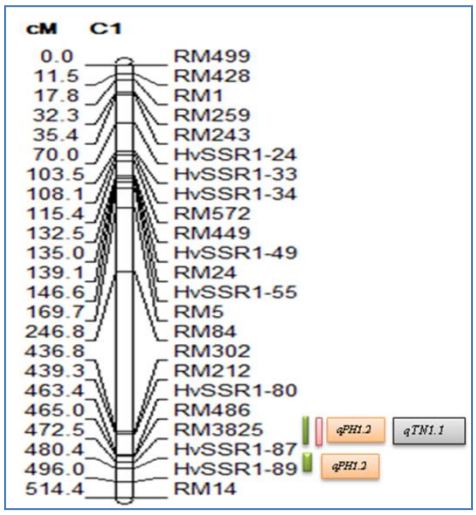
QTL analysis for trait tillers per plant and plant height

A total of four QTLs were identified for tillers per plant and two QTLs identified for plant height using QTL cartographer 2.5. These loci were associated with LOD score above the threshold values i.e. 3 for QTL cartographer 2.5 and determined by permutation test for the traits of the experiment that was 1000. The QTLs were found to be present on chromosomes 1 and 3, respectively. The QTLs along with their position, LOD score, additive effect and R^2 value worked out through composite interval mapping given in [Table-4].

Table-4 *QTLs underlying trait tillers per plant and plant height in rice*

Trait	QTL	Chr.	Closely Linked marker	Marker position (cM)	Marker interval		LOD	Additive effect	R^2
Tillers/ Plant	*qTN1.1*	1	RM3825	472.5	RM3825	HvSSR1-87	3.4682	0.5248	0.0931
	qTN3.1	3	RM231	0.0	RM231	HvSSR3-6	4.4167	0.6877	0.1242
	qTN3.2	3	RM517	215.7	RM517	RM232	3.5701	-0.1547	0.0068
	qTN3.3	3	RM232	229.7	RM232	RM7	3.5583	-0.1500	0.0065
Plant height	*qPH1.1*	1	RM3825	476.5	RM3825	HvSSR1-87	11.7917	-13.3036	0.4905
	qPH1.2	1	HvSSR1-87	484.4	HvSSR1-87	HvSSR1-89	10.7713	-14.5226	0.4629

A QTL, *qTN1.1* for tillers number per plant was mapped between markers RM3825 and HvSSR1-87 on chromosomes 1 with LOD values 3.4682 and explained 9.31% of phenotypic variance by using QTL cartographer 2.5. Another significant QTL such as *qTN3.1* for tillers number mapped on chromosome 3. The *qTN3.1* mapped between marker RM231 and HvSSR3-6 with LOD value 4.4167 and explained 12.42% of phenotypic variation. The QTL, *qTN1.1* and *qTN3.1* both showed positive additive effect. This means that the alleles from the parent Danteshwari acted to increase the measured trait (i.e. tillers number). As per height, parent Dagad deshi is tall and Danteshwari is dwarf. Two QTLs for plant height mapped under same environmental conditions using RILs. The significant major QTLs, *qPH1.1* and *qPH1.2* for plant height mapped between marker RM3825 & HvSSR1-87 and HvSSR1-87 & HvSSR1-89 on chromosomes 1 with LOD values 13.47 and 10.7713; explained high phenotypic variance of 49.05 and 46.29%, respectively. These QTLs showed negative additive effect means that the alleles from the parent Danteshwari acted to increase the plant height. The linkage map depicting locations of QTLs for tillers per plant and plant height showed in [Fig-2 and 3].



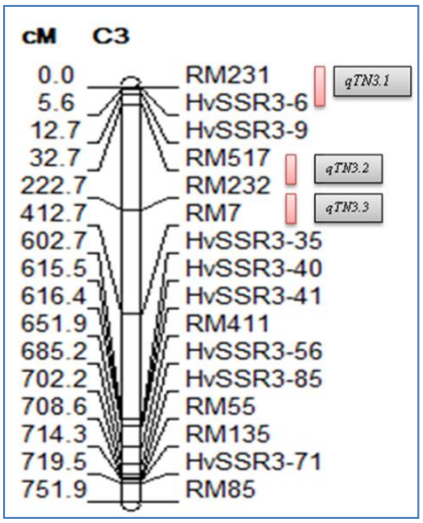
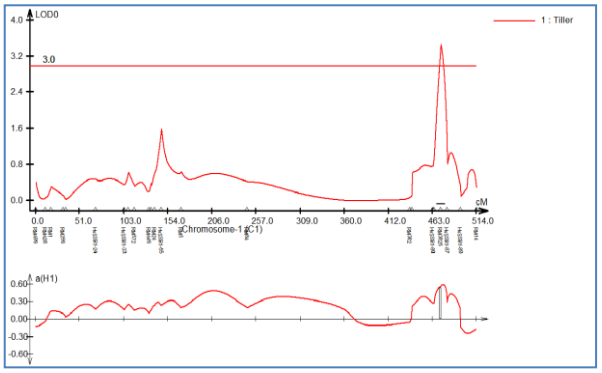
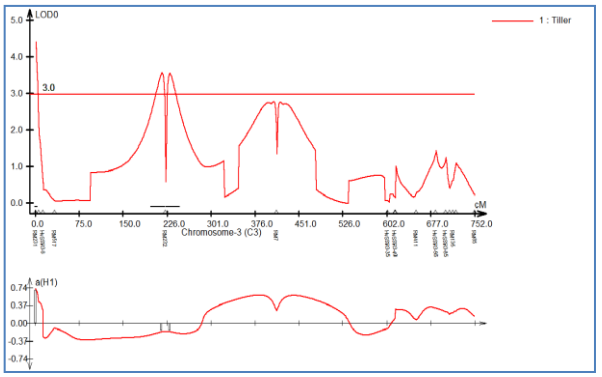
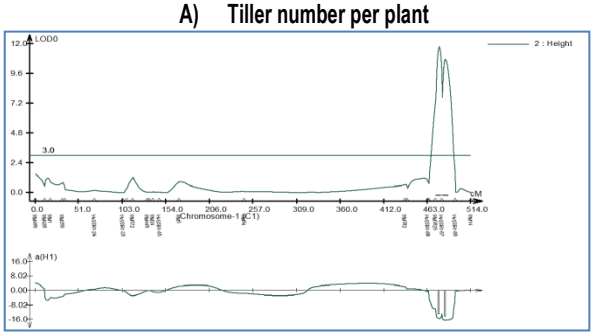


Fig-2 The linkage map depicting locations of QTLs for tillers per plant and plant height





A) Tiller number per plant



B) Plant height

Fig-3 QTLs position for trait tillers number per plant and plant height on chromosome 1 and 3 Note: The bars indicate the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD required for significance.

Discussion

Distribution of RIL population for Tillers number per plant and plant height

The normal frequency distribution of RIL for tillers per plant in this population also found similar as previously. The tillers number of the DH population segregated continuously suggested a normal distribution (2). The phenotypic analysis of the 251 testcross families showed that the frequency distribution of tiller approximately fit normal distribution [13]. The distribution of yield-related trait in the RIL population for trait number of tiller per plant showed a typical normal distribution, indicating that trait was quantitative trait controlled by multiple genes [21]. The tiller number introgression line population segregated continuously [22]. In the RIL population, both tiller number and plant height showed continuous variation [4]. The plant height of the double haploid population segregated continuously and fit

normal distribution for most stages in two locations and was suitable for QTL analysis [29]. The plant height of the double haploid population segregated continuously in 2006 and 2007 [31]. The plant height (ph) a typical quantitative trait with approximately normal distribution in the RIL population was selected as the mapping trait [35]. The phenotypic segregations in the F_2 populations exhibited normal distribution, a typical phenomenon of quantitative trait, which indicates that plant height was regulated by several genes and influenced by the environment [33].

Comparisons QTLs with previous studies

We identified four QTLs for tillers number per plant with significant additive effects in maximum tillering stage on chromosome 1 and 3 using 122 RILs population derived from a cross Danteshwari × Dagad deshi. Recently, several studies have done to identify dynamic QTLs for this quantitative trait, using different population [45-46]. Many of these study, showed their existence on the respective chromosomes. Five QTL were detected on chromosomes 1, 3 and 5 [45]. Although all QTLs had their dynamic curves of main effects during the whole stage, only six of them including QTLs, *Tn1-1, Tn1-2, Tn2, Tn3-1, Tn6-2* and *Tn6-3* were statistically significant at certain stages [17]. Liu [46] was identified three QTLs, *Tn3-1, Tn3-2* and *Tn3-3* on chromosome 3 by using single segment substitution lines between markers RM168–RM571 and RM135–RM55 for both Chenglongshuijingmi as donor and PSM304–RM545 for IR64 as donor, respectively. The QTLs, *qUTn1.1, qUTn1.2, qUTn1.3, qUTn1.4* and *qCTn1.1, qCTn1.2, qCTn1.3,qCTn1.4* by both unconditional and conditional for tiller number around markers RM6887, RM7124, RM7600 and RM1380 of temporal-specific QTLs were identified at two different measuring stages. A QTL, *qCTn3.1* also identified on marker RM5748 on chromosome 3 [22]. Yan *et al*., 1998 [2] was identified 8 QTLs on chromosome 2 and 3, out of these QTL (*tn2-2*) and (*tn3-4*) identified on chromosome 2 and 3, respectively. At these loci, the alleles increasing tiller number of QTL, *tn3-4* from IR64 and *tn2-2* from Azucena. Among QTLs on all chromosome, affecting tiller number at different developmental stages *qTN-1-1, qTN-1-2, qTN-1-3, qTN-1-4, qTN-1-5* and *qTN-3-1, qTN-3-2* were identified on chromosome 1 and 3 respectively [4]. The markers, *qTL*-3, *qTL-6*, *qTL-12*, *qSS-5*, *qSS-9* and *qGY-8* which control the tiller number, seed-setting rate and grain yield per plant, have been detected [21]. Out of detected 49 QTL with phenotypic effect ranging from 3.2 to 46.0% for 14 agronomics traits, identified 10 major-effect QTLs, including *qTA-9, qPH-1, qFLW-4, qGL-3, qGW-5, qAL-1, qAL-3, qPH-2, qHD*-3 and *qCD-2* [47].

As the significant major QTL, *qPH1.1* for plant height mapped between markers RM3825 and HvSSR1-87 on chromosomes 1 with LOD value 11.7917 in this experiment by QTL Cartographer 2.5. Similarly, a QTL for plant height *"qph1"* identified on chromosome 1 between marker RM3825–RM3738 with LOD score 12.8 and 26.4% of phenotypic variance in season 2006 and LOD value 11.9 with 37.3% of phenotypic variation in 2007, respectively. The marker RM3825 was shared commonly in both the studies [34]. The other QTLs also identified on this chromosome by many workers explain its existence. A QTL, *ph1* was detected for plant height in 1994 and this QTL individually explained 14.6 % of the total phenotypic variation [30]. Of the four identified QTLs at the final stage, a QTL, *ph1* identified between markers RZ730 and RZ801 for plant height growth by unconditional mapping on chromosome 1 [29]. A QTL, *qph1* was detected on the long arm of chromosomes 1 close to RM6333 and coincides with the semi-dwarf gene, sd-1 [32]. Another QTL, *qPH1.2* for plant height identified between marker HvSSR1-87 and HvSSR1-89 on the chromosome 1 in this study. The QTL, *Qph1.1* identified between markers RM580-RM246 of LOD 2.64 by Nipponbare × IR1545-339 population with 3.28% phenotypic variation and another one *Qph1.2* mapped on chromosome 1, with flanking markers E60551 and RM1387 in both populations [33]. The QTLs for plant height on the other chromosome also mapped by various researchers using different population. The analyses detected six QTLs for plant height and six QTLs for heading date [30]. A significant QTL for plant height was detected on chromosome 8 (RG20-RZ143) which explained 7.3 % of total phenotypic variation at 2.16 LOD by simple interval mapping. Plant height was associated with two QTLs, which were located on chromosomes 3 and 8 by composite interval mapping [15]. Two QTLs (*ph8-4* and *ph8-5*) were identified to be associated with plant height using both unconditional and conditional mapping methods simultaneously in 2 years [31]. Fifteen M-QTLs were detected by 1 to 31 datasets. Of these, *qph7a* was detected repeatedly by all the 31 ph datasets in 2006 and explained 11.67% to 23.93% of phenotypic variation; *qph3* was detected repeatedly by all the 31 datasets and explained 5.21% to 7.93% and 11.51% to 24.46% of phenotypic variance in 2006 and 2009, respectively [35].

Genetic relationship between tillers number per plant and plant height

In this experiment both the traits showed significant negative correlation, which means that dwarf plant having more tillers as compare to tall plant. In comparison with normally-tillered cereals, plants with a single culm are taller [48]. Several lines of evidences have proved that there is a highly negative correlation between tiller number and plant height in rice [49]. The negative correlation between tillers per plant and plant height were also reported [4, 50, 51]. There were significant negative correlations between tiller number and plant height, and between tiller number at maturity and heading date. A large proportion of QTLs and interactions could only be detected in one year, suggesting that QTLs and two-locus interactions for the traits were dependent on the environment [12]. The QTL, *"qPH1.1"* for plant height found between marker RM3825 and HvSSR1-87 exactly co-localized the "*qTN1.1"* of tillers number per plant on chromosome 1 in this study. The major QTLs for tiller number per plant and plant height showed opposite additive effect means allele for tiller number carried from parent Danteshwari and plant height also from Danteshwari similar with correlation result. Both the negatively correlated traits tightly linked and present on same loci, showing linkage drag. Three genomic regions were identified as putative collocated QTL, which showed opposite additive effects on tiller number and plant height [4]. A partial dominant QTL for the four traits was mapped to the same interval flanked by RM310 and RM126 on chromosome 8. The QTL region explained 83.0, 80.2, 94.9 and 93.8% of trait variation of SPP, GPP, HD and PH in the progenies, respectively [52]. All three of the HD loci mapped to approximately the same genomic locations as PH QTLs [28]. A total of 23 QTLs for plant height were located in all 12 rice chromosomes, and eight of these QTLs were shared by at least two populations. Found 13 dwarfing or semi dwarfing genes were in close proximity to the QTLs, providing evidence to support the hypothesis that QTLs and major genes were different alleles of the same loci [25].

Conclusion

The tiller number per plant and plant height has shown normal frequency distribution in rice. The significantly negative correlation shown between tiller number per plant and plant height, which means that dwarf plant having more tiller as compare to tall plant. The QTLs for tillers number per plant showed positive additive effect, means these alleles from the parent Danteshwari. The major QTLs for plant height also mapped on chromosomes 1. A QTL for plant height found exactly co-localized the QTL tillers number per plant on chromosome 1. Both the negatively correlated traits tightly linked and present on same loci, showing linkage drag.

Application of research: The major QTLs "*qTN1.1"* and *"qTN3.1"* could be useful for the improvement of plant type by pyramiding *via*. marker-assisted selection in Rice.

Acknowledgment / Funding resource: We are thankful to College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh 492012, India for providing the necessary facilities.

Author Contributions: All author equally contributed.

Abbreviations: Quantitative trait loci (QTL), *Monoculm1* (*MOC1*), Recombinant inbred line (RIL), Standard deviation (SD), Polymerase chain reaction (PCR), Simple sequence repeat (SSR), Logarithm of odds (LOD), Composite interval mapping (CIM), Plant height (PH)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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