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Research Article

Identification of superior haplotypes for *CCD8* regulating tiller number and grain yield in rice

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Abstract
Rice is one of the major food crops of the world. In the present day of increasing population there is an urgent need to increase the rice grain production. Yield is a complex trait and is mainly orchestrated by plant architecture. In the present study, a diverse set of 100 accessions from the 3K RG panel were evaluated for *Carotenoid cleavage dioxygenase 8* (*CCD8*) loci which plays a role in altering tiller number which ultimately governs the crop yield. A normal phenotypic distribution for total tiller numbers and number of productive tillers was observed for the set of 100 accessions used in the study. Five non synonymous SNPs were present for *CCD8* loci for the 100 rice 3K accessions in this study. Haplotype analysis was carried out using five non synonymous SNPs to understand the genetic diversity of the population and of which two significant SNPs in the positions 31223371 and 31223383 grouped the accessions into two haplotype H1 and H2 with the allelic combination of GC and AT respectively. Haplotype group H1 with 89 accessions and H2 with 8 accessions were formed. Among the two haplotype groups, H2 had the maximum mean value for both tiller number and productive tiller number indicating the superiority of the H2 allelic combination over H1. Hence H2 is considered as a superior haplotype that can be potentially explored for allele mining and can be used in future crop improvement programs.

Keywords:*CCD8*, tiller number, genetic diversity, haplotype.

INTRODUCTION

Rice (*Oryza sativa*) is the most important staple food crop of the world that feeds nearly half of the global population. Increase in the global population and a drastic decrease in aerable land, makes upsurging of grain yield a priority. Rice productivity has attained plateau almost for a decade. Hence, enhancement of grain yield has been the foremost objective in rice-breeding programs. The two ways by which yield can be enhanced are either by increasing the yield potential of the crop or by decreasing the yield gap (Bidhan Roy *et al.,* 2021). Green Revolution has led to development of high yielding varieties in rice by inducing novel variability through Wide Hybridization and Mutagenesis. Initially, the crop varieties have been developed by basic breeding techniques and nowadays marker assisted selection and various genomic techniques are used for accelerating crop improvement. Yield is a complex trait in rice which is determined by plant height, tiller number, panicle length, number of grains and grain size/weight (Wang *et al.,* 2017). There are several proofs explaining alteration in gene expression of yield contributing traits increased panicle length, tiller number, number of grains which as a whole increases plant yield (Li *et al.,* 2016; Zeng *et al.,* 2020). In the aspect of rice breeding, tiller number is an important trait for improving yield. Tiller number and development are controlled by a complicated network of genetic pathway, the density of the plants, plant hormones, and nutrient input (Wang *et al.,* 2017; Umehara *et al.,* 2008; Xing and

Zhang, 2010). The gibberellins and strigolactones are two main phytohormones influencing plant tillering and they both have similar effects on plant tillering but reverse effect on plant height (Liao *et al.,* 2019). Strigolactones are a novel class of plant hormones that play an essential role in determining plant architecture, number of tillers and regulating the growth of primary/lateral roots. *Carotenoid cleavage dioxygenase 7 (ccd7)* and *ccd8* mutants from *Arabidopsis thaliana*, pea (*Pisum sativum*), petunia (*Petunia hybrida*), and rice showed increased branching or tillering characteristics (Ruyter *et al*., 2013). Butt *et al.* (2018) reported that mutants of *CCD7* gene produced a novel phenotype of increased tillers and consequently grain number with yield increase.

In the view of mining superior and novel alleles for *CCD8* gene, 100 3K rice germplasm lines were evaluated for total tiller number and productive tillers and haplotype analysis was done for the same (Bevan *et al.,* 2017). In the present study, haplotype analysis and the level of significance of the haplotypes was carried out for 100 diverse accessions from the rice 3K-RG panel for the potential target gene *CCD8*.

MATERIALS AND METHODS

A diverse set of 100 lines from 3K RG panel were sown in *Kharif* 2020 in Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. Plants were maintained with standard agronomic practices and fertilizer dose of 150:50:50 (N:P:K) kg/ha. Data were recorded from five random plants for the traits, number of tillers and number of productive tillers. To understand the level of phenotypic variation among the population for the target traits total tiller number and number of productive tillers, descriptive statistics and frequency distribution was performed using the statistical software Minitab (Version 19.1) (Allen, 2019).

Allelic variation present in the accessions used in the study for *CCD8* loci was retrieved from the Rice SNP seek database to perform haplotype analysis. The 3K SNP dataset was retrieved from the SNP seek database. Allele mining was carried out by selecting only the non-synonymous SNPs. This dataset was consequently converted into haplo-view file set using gPLINK (version 1.07) (Purcell *et al.,* 2007). HaploView (version 4.1) was used to analyze the number of haplotypes based on the strength of linkage disequilibrium (LD) and to determine the significant SNPs using a cutoff value of 0.001 (Barrett *et al*., 2005). Phenotypic difference between haplotypes and their level of significance were analysed using the Minitab (Version 19.1) by performing Hsu simultaneous multiple comparison test.

RESULTS AND DISCUSSION

Tiller number is an important trait to be considered in breeding programme for determining yield of rice. The *CCD 8* is one of the gene involved in plant growth architecture, determining the number of shoot branches/ tillers and regulating the growth of primary and lateral roots. *Carotenoid cleavage dioxygenase 7 (CCD 7)* and *Carotenoid cleavage dioxygenase 8 (CCD 8)* mutants from *Arabidopsis thaliana*, pea (*Pisum sativum*), petunia (*Petunia hybrida*), and rice showed increased branching or tillering characteristics (Ruyter *et al.,* 2013). A point mutation (C-T) caused in *OsCCD7* in the Nanjing 6 background and the mutant *htd1* (high-tillering and dwarf 1) reported in rice showed a dwarf phenotype and increased number of tillers (Zhang *et al*., 2011).

A subset of 100 accessions from 3K-RG panel were evaluated for total number of tillers and productive tillers. These data were collected at the time of harvest from the selected germplasm accessions raised in the field. Histogram and box plot were generated using Minitab 19 statistical software for total number of tillers and number of productive tillers. The histogram revealed a normal frequency distribution of the traits among the selected accession for the target traits *viz.,* number of total tiller (**Fig 1**) and number of productive tiller (**Fig 2**). Descriptive

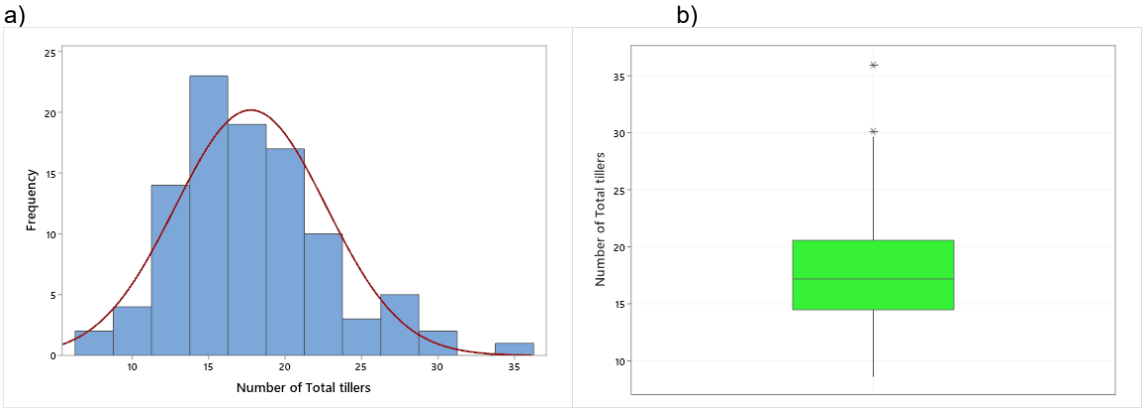

Fig. 1. Frequency distribution explaining the phenotype variation a) Frequency distribution for total tillers, b) Box plot explaining variation in total tiller number.

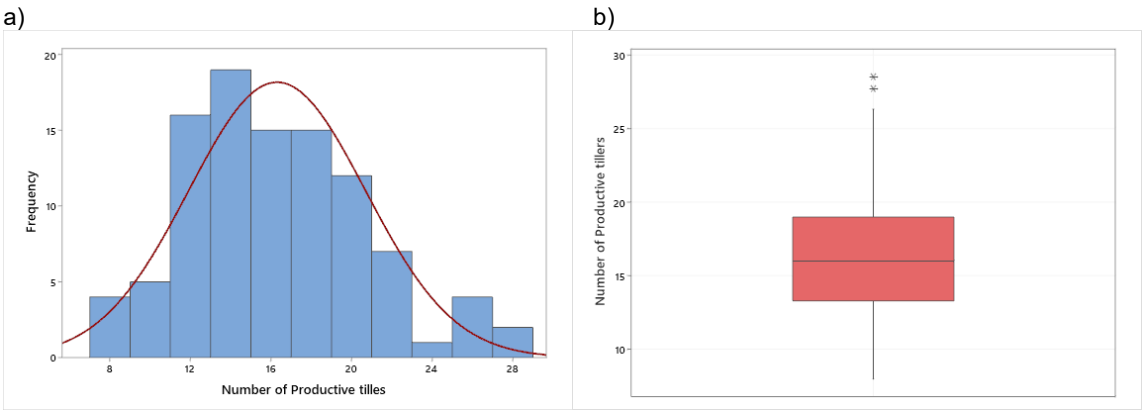

Fig. 2. Frequency distribution explaining the phenotype variation a) Frequency distribution for total tillers, b) Box plot explaining variation in productive tiller number.

statistics performed on these accessions revealed the mean value and coefficient of variation of 17.751, 27.85 and 16.301, 26.83 for total number of tillers and number of productive tillers respectively (**Table 1**)

The diverse accessions for the traits total tiller number and number of productive tillers were grouped into two haplotypes groups H1 and H2 with the allelic combinations GC and AT respectively showing strong Linkage disequilibrium (LD) using Haploview software (**Fig. 3**). Among the 100 accessions GC (H1) allelic combination was found in 89 accessions and AT (H2) allelic combination was found in eight accessions and the remaining two accessions were found to be in heterozygous condition. Of the five SNPs found for the *CCD8* loci two significant SNPs at the positions 31223371

Table 1. **Descriptive Statistics for total tiller and productive tiller**

Statistics	Total tiller	Productive tiller
Mean	17.751	16.301
Median	17.000	15.950
SE Mean	0.494	0.439
StDev	4.943	4.391
Variance	24.433	19.277
CoefVar (%)	27.85	26.93
Minimum	8.700	8.000
Maximum	36.000	28.500
Skewness	0.79	0.49
Kurtosis	1.12	0.04

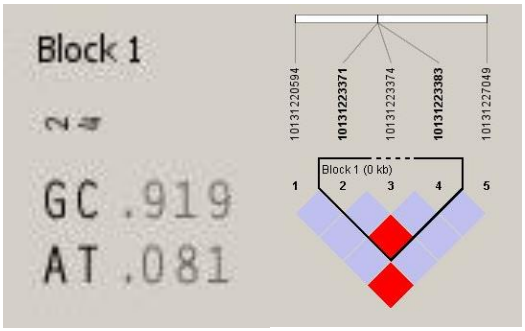

Fig. 3. LD block explaining haplotype formation and Linkage Disequilibrium (LD) for *CCD8* loci.

and 31223383 were responsible for haplotype grouping (**Table 2**). Similarly, Madhuri *et al*. (2021) reported two significant SNPs grouped into three haplotype groups for 150 accessions and Rashid *et al.* (2016) reported seven diverse haplotype groups of 378 rice cultivars for SCM2 gene.

Descriptive statistics on both the haplotype groups showed that Haplotype H2 had the highest overall mean 21.61 and 18.71 for total tiller number and number of productive tillers respectively with a phenotypic range of 8.70-27.3for total tiller and 8-25.2 for productive tiller number (**Table 3 and 4**). The extent of variability in the population was analyzed using CV and H2 was observed to record the highest CV of 38.77 and 29.64 for total tiller number and productive tiller number (**Table 3 and 4**).

The significant difference between the two haplotype groups was analyzed by performing Hsu simultaneous multiple comparison test using Minitab 19 statistical software. The test result revealed a significant difference between H1 and H2 haplotypes for both the traits total tiller and productive tiller (**Fig.4**). The haplotypes H1-H2 showed a p-value than 0.008 for total tiller number and 0.43 for number of productive tillers, indicating a highly significant difference between them (**Table 5 and 6**).

Table 2. List of significant SNPs from CCD8 for the 3K subset panel

Marker No	Position	HWpval	MAF	Alleles
1	31220594	0.0101	0.01	C:G
2	31223371	2.3926E-12	0.081	G:A
3	31223374	0.0109	0.041	C:T
4	31223383	2.8369E-12	0.082	C:T
5	31227049	0.0101	0.01	G:A

HWpval – Hardy Weinberg p value, MAF – Minor Allelic Frequency and Highlighted SNPs were involved in Haplotype formation

Table 3. Descriptive statistics of the haplotypes for total tiller number

Variable	Mean	SE Mean	SD	Variance	CV	Minimum	Maximum	Range
GC (H1)	17.302	0.466	4.397	19.338	25.42	8.7	30.2	21.5
AT (H2)	21.61	2.96	8.38	70.21	38.77	8.7	36.0	27.3

Table 4. Descriptive statistics of the haplotypes for productive tiller number

Variable	Mean	SE Mean	SD	Variance	CV	Minimum	Maximum	Range
GC (H1)	15.971	0.443	4.178	17.453	26.16	8.5	28.5	20.0
AT (H2)	18.71	1.96	5.55	30.77	29.64	8.0	25.2	17.2

Table 5. Hsu simultaneous tests for level mean indicating significant difference between haplotypes in total tillers

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
GC (H1) - AT (H2)	-4.31	1.77	(-7.26, 0.00)	-2.43	0.008
AT (H2) - GC (H1)	4.31	1.77	(0.00, 7.26)	2.43	0.008

Table 6. Hsu simultaneous tests for level mean indicating significant difference between haplotypes in productive tillers

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
GC (H1) - AT (H2)	-2.74	1.58	(-5.37, 0.00)	-1.73	0.043
AT (H2) - GC (H1)	2.74	1.58	(0.00, 5.37)	1.73	0.043

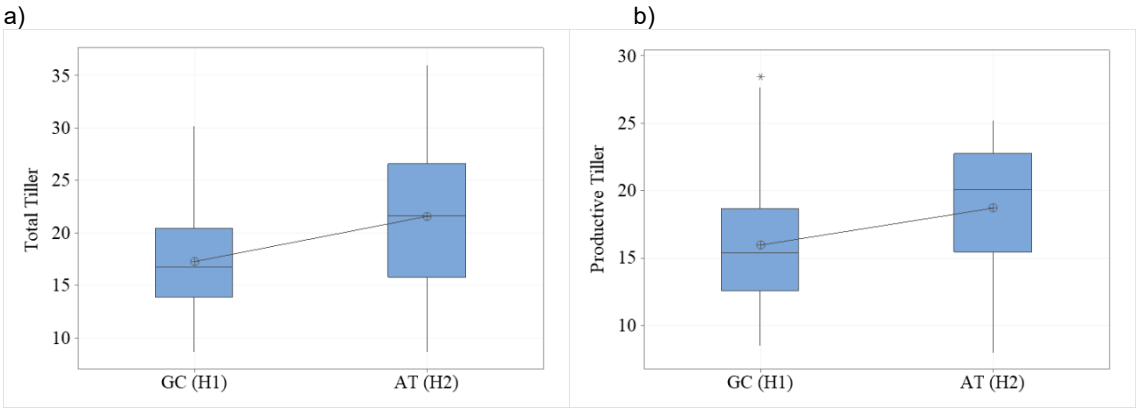

Fig. 4. Box plot of different haplotypes for total tillers (a) and productive tiller (b). Line indicates significant difference between haplotypes based on Hsu simultaneous multiple comparison test

In Haplotype1 GC(H1) with a total of 89 accessions, the genotype ARC 10825 showed the highest number of tillers (30.2) and productive tillers (28.5) whereas, in Haplotype 2 AT(H2) with eight accessions, the genotypes AEB 368 RIP P TYPE ADT and LAWANGAI showed the highest number of tillers (36) and productive tillers (25.2), respectively. The range of the haplo group H1 was 21.5 and 20.0 and for H2 it was 27.3 and 17.2 for total tiller and productive tiller respectively. This clearly explains that the target gene *CCD8* plays an important role in tiller number rather than the productive tiller or number of panicles per plant. Among the two haplotype groups, H2 had high mean value for both tiller number and productive tiller number indicating the superiority of the H2 allele combination over H1. This clearly gives us the strong evidence for considering H2 as a superior haplotype and that can be potentially explored for allele mining and can be used in future crop improvement programs.

In the current scenario of increasing demand for food as a cause of increasing population, there is an urgent need to increase the rice grain production. From that point of increasing the yield in rice, tiller number per plant has a crucial role to play toward increasing the yield per plant. So, it is very important to identify a superior genotype with higher number of tillers that could possibly be used in breeding programmes in the view of increasing crop yield. In the present study, the haplotype group H2 having the allelic combination AT with maximum mean value for both total tiller number and number of productive tillers per plant can be used in identifying the elite or superior donors and to use them in crop breeding programmes.

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