Original Research Article

Exploiting the allelic variation and superior haplotypes for *OsMIT3*regulating tiller number in rice

ABSTRACT

Rice (*Oryza sativa* L.) is the staple food for more than 60 % of the population globally and it isconsumed ~~sss~~in various forms. Increased crop yield under variable climatic circumstances is necessary in light of the world population's rapid growth, yield plateaus, resource depletion, and climatechange. In order to overcome these obstacles, novel genes and alleles in the rice gene pool mustbe found, and unique features like C4 photosynthesis must be modified. The negative effects ofclimate change, stagnated yields, and diminishing agricultural resources are major obstacles. Tillering is one of the important traits to be considered forincreased rice crop production and productivity. The target gene *OsMIT3*phenotypically shows higher tillering and is caused by strigolactone deficiency that directly linked to carotenoidbiosynthesis pathway, signaling strigolactone genestowards rice tillering. In this study, 100 diverse accessions from Rice 3K-RG panel were selected and analyzed, which shows three significantSNPs and grouped into fourhaplotypes group with allelic combinations of TAT(H1), ACA(H2), TAA(H3) and ACT(H4). Among the four haplo-group, H3 shows higher mean value for both total number of tillers and productive tillers, which can be furtherconsidered as a source of breeding strategy for crop yield.

KEY WORDS

Allelic diversity, OsMIT3, strigolactones, superior haplotypes, haplotype analysis.

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary and essential cereal crop, catering to the nutritional needs of nearly half of the global population (Gross and Zhao, 2014). In order to meet the growing food demands of our ever-expanding population, it is projected that rice production needs to triple by the year 2050 (Gross and Zhao, 2014; Mishra *et al*., 2018). However, to achieve sustainable food production for ever growing global population several challenges such as a production plateau, diminishing resources, and abiotic/biotic stress resulting from climate change, are major obstacles to achieve the target (Pandey *et al*., 2017). To unlock rice's potential for increased yield, traditional methods like hybridization and selection, F_1 hybrid breeding, as well as innovative approaches such as targeted mutagenesis viz., ZFNs, TALENs, CRISPR-Cas9 method of genome editing are used.

Yield is a complex trait and extensive functional assessments have been carried out on various significant genes associated with rice grain production and related traits. Over the years, several high-throughput omics platforms have been developed to elucidate the roles of crucial genes influencing important aspects of rice (Li *et al*., 2018). Several yield-related genes have been identified, cloned, tested, and validated, with their functions predominantly influenced by factors such as plant height, tiller number, grain number per panicle, number of panicles per plant, and grain weight (Wang *et al*., 2017).

Numerous genes in rice have been targeted to enhance yield and productivity, including *DEP1, Gn1a, GRAIN SIZE3 (GS3), GRAIN WIDTH5 (GW5), CCD7, CCD8, MIT3, TB1, and GHD7*. Among these traits, tiller number plays a pivotal role in augmenting rice yield during breeding. The two primary phytohormones influencing plant tillering are strigolactones and gibberellins, both of which have similar

effects on tillering but reverse effects on plant height (Liao *et al*., 2019). Strigolactones (SL), a novel class of plant phytohormones, are terpenoid lactones synthesized from the carotenoid pathway. They play an essential role in establishing plant architecture, regulating shoot branching (tillers), and promoting root growth (Conn *et al*., 2015; Raez *et al*., 2009). Mutations in *ccd7* and *ccd8* in various plants, including *Arabidopsis thaliana*, pea (*Pisum sativum*), petunia (*Petunia hybrida*), and rice, have shown increased branching or tillering characteristics (Ruyter-Spira *et al*., 2013 and Al-Babili*et al*., 2015).
MIT3 gene encodes a carotenoid isomerase, which is a critical enzyme responsible for converting prolycopene to all-trans-lycopene in carotenoid biosynthesis (Liu *et al*., 2018). Through double mutant analysis of *MIT3* mutants with the mutant *d17* (mutant of gene involved in strigolactone pathway), it was determined that *MIT3* regulates tiller development upstream of the SLs biosynthesis pathway. Deletions or novel alleles in these genes have been found to increase tiller numbers, thereby improving rice yield.
Haplotype analysis plays a vital role in disease gene mapping and gaining insights into populations. Computational algorithms are used to estimate frequencies and predict phases in genetics research (Xu *et al*., 2002). Understanding haplotypes assists in identifying and selecting donors carrying desired traits through the examination of allelic variants and haplotype variations, thereby contributing to crop improvement (Varshney *et al*., 2018).

The Rice 3K RG panel contains novel alleles that harness haplotype diversity and can be utilized to improve major traits, including grain yield, quality and tolerance to biotic/ abiotic stresses. This study aimed to explore the genetic diversity and variability of *OsMIT3* genes in a set of rice germplasm lines. The discovery of superior and unique alleles of *OsMIT3* genes within the 3K RG panel subgroup could have significant implications for rice breeding efforts. The identified lines in superior haplotype can be used as potential candidates for yield improvement in breeding programs.

MATERIALS AND METHODOLOGY

A sub-set of 100 diverse rice genotypes were raised in augmented design at the Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore (11◦00´N, 76◦54´E, 426 MSL), India during Kharif 2022. Twenty-five days old rice seedlings were transplanted in the field with a spacing of 20 x 20 cm and fertilizer application of 150:50:50 kg/ha of N, P, and K respectively, and the regular agronomic practices were followed. Agronomic data for all the 100 diverse rice genotypes were recorded and used in this study. Descriptive statistical analysis (mean, median, range, and coefficient of variation (CV) and frequency distribution graph was made using Minitab 19 Statistical Software (Allen, 2019). Using Rice SNP seek database the non-synonymous SNPs and INDELs were retrieved for the gene *OsMIT3* (LOC_Os11g36440IN) which is located the chromosome 3.

At first the allelic variations of the candidate gene were downloaded in PLINK format from the Rice-SNP seek database and later translated into haploview format using PLINK software to perform the haplotype analysis (Jonathan, 2010). According to the degree of linkage disequilibrium (LD), the number of haplotypes was analyzed by using HaploView (version 4.1), and relevant SNPs were determined with a minimal frequency of at least 0.001 (Barrett *et al*., 2005). Using the Minitab (Version 19.1), the Hsu simultaneous multiple comparison test was done which helps to analyze the phenotypic differences between haplotypes and compared to their level of significance.

RESULTS AND DISCUSSION

The deficiency of specific carotenoids and SLs in mit3 mutants has a direct impact on the tillering phenotype. This research provides valuable vision at evaluating the allelic diversity of MIT3 genewell known for its rolein increasing tillernumber. This study could aid in the development of high-yielding rice cultivars.

A subgroup of 100 accessions from the Rice 3K-RG panel were evaluated for total number of tillers and the number of productive tillers in order to identify high-performing lines to be exploit into rice breeding and crop improvement. The phenotypic data was collected from the field evaluation at the time of harvest. The descriptive statistics explains mean, standard deviation, range and coefficient of variance. A precise test of the Hardy-Weinberg equilibrium is conducted. Histograms for both number of tillers and the number of productive tillers were constructed using the statistical programme Minitab 19 (Allen, 2019).

The histograms explain the normal frequency distribution for both total tiller number (**Figure1)** and productive tiller number (**Figure 2)** for the 100 accessions.

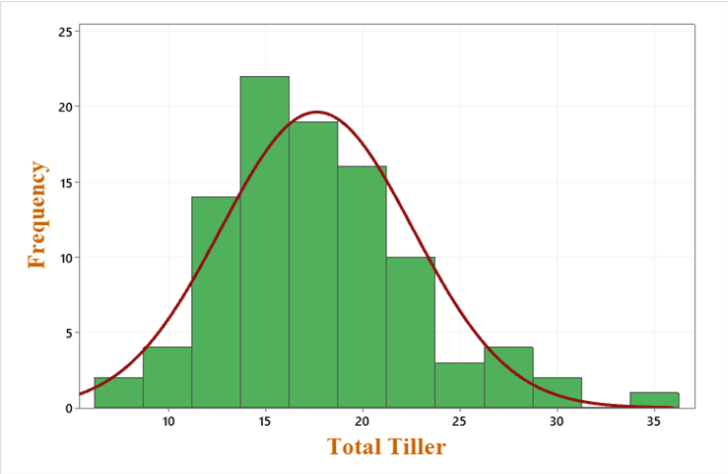

Fig1. Frequency distribution elucidating the phenotype differencefor total tiller number.

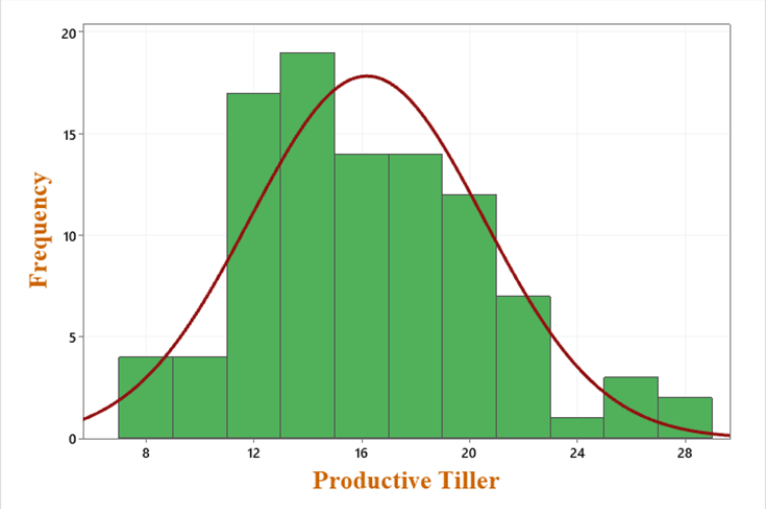

Fig2. Frequency distribution elucidating the phenotypic difference for productive tillers

According to descriptive statistics, the total number of tillers ranged from 9 to 36, with a mean value of 17.658 and a coefficient of variation of 27.90% (Table 1). The average number of productive tillers, which ranges from 8 to 28, is 16.197, with a coefficient of variation of26.78%(Table1).

Haplotype analysis for the gene *MIT3*using the Haploview software distributed the hundred accessions into four haplotype groups, H1,H2, H3 and H4, with the allelic combinationsTAT(35), ACA(42), TAA(2)and ACT(1) respectively, displaying strong Linkage disequilibrium (LD).Haplotype analysis by Mohan *et al*.,(2021) revealed three haplotypes H1, H2 and H3 explaining the allelic variation in *DEP1* gene.Out ofhundredlines,twenty accessions in heterozygous condition for the corresponding allele were removed

and only the remaining eightyaccessions were subjected to haplotype analysis, falling into four different allelic combination. (**Table2).**

Table1. Descriptive statistics of the haplotypes for total tiller number

Variable	Total Tiller number	Productive Tiller number
Mean	17.658±0.5	16.197±0.4
SD	4.93	4.34
Variance	24.27	18.82
CV %	27.90	26.78
Minimum	8.70	8.00
Maximum	36.00	28.50

Table 2- Different allelic combinations produced by 100 accessions.

Factor	N
H1(TAT)	35
H2(ACA)	42
H3(TAA)	2
H4(ACT)	1

Five non synonymous SNPs were foundfor the *MIT3*locus, ofwhich three significant SNPs at the positions 21486097, 21488077, 21488883were only involved in the formation of haplotype groups (**Fig3**).A similarly study byShobicapriya*et al*., (2021) reported seven significant SNPs grouped into four haplotype groups for 150 accessions for AN-1 gene.

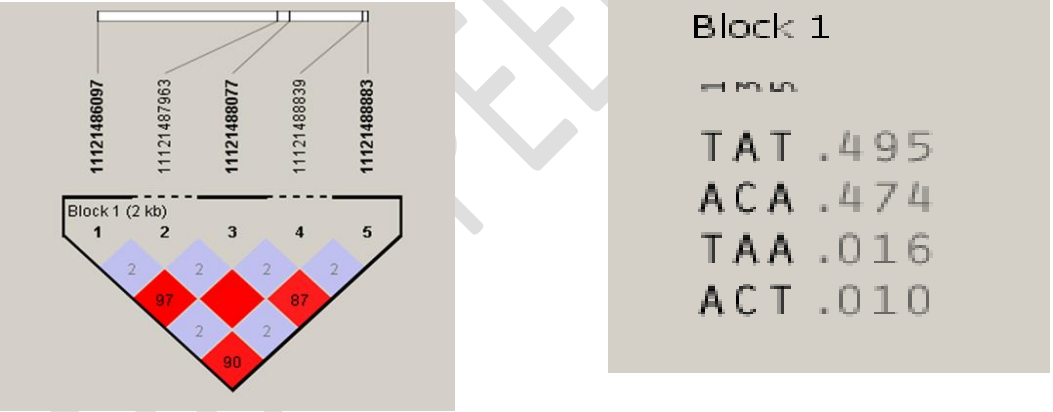

Fig3. LD block demonstrating linkage disequilibrium and haplotype development for the *MIT3* gene

Descriptive statistics on the phenotype of total tillers and number of productive tillerswas carried outto understand the statistical significance between four haplotype groups Haplo-group H3 had the greatest overall mean of 24.85 and 24.15 over H1, H2 and H4 for both total tiller number and productive tiller number, respectively (**Tables 3 and 4**).Hsu simultaneous multiple comparison testwas employed to find thesignificant difference between haplotypes H1, H2,H3and H4 for both the total number of tillers and number of productive tillers attributes, and found to have substantial significant difference between the haplotype groups that clearly explaining the genetic influence on both traits, total tiller number and number of productivetillers (**Table 5 and Table 6)**.

Table 3. Statistical descriptions of the haplotypes for the total number of tillers

Factor	N	Mean	SD	95% CI
H1(TAT)	35	17.457	4.322	(15.928, 18.987)
H2(ACA)	42	17.298	4.755	(15.901, 18.694)
H3(TAA)	2	24.85	2.62	(18.45, 31.25)
H4(ACT)	1	16.80	*	(7.75, 25.85)

Table 4: Statistical descriptions of the haplotypes for the total number of productivetillers

Factor	N	Mean	SD	95% CI
H1(TAT)	35	16.097	3.944	(14.681, 17.513)
H2(ACA)	42	16.068	4.454	(14.775, 17.361)
H3(TAA)	2	24.15	1.63	(18.23, 30.07)
H4(ACT)	1	11.60	*	(3.22, 19.98)

Table 5. Hsu Simultaneous tests for total tiller number using largest level mean

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
H1(TAT) - H3(TAA)	-7.39	3.30	(-14.51, 0.00)	-2.24	0.041
H2(ACA) - H3(TAA)	-7.55	3.29	(-14.63, 0.00)	-2.30	0.036
H3(TAA) - H1(TAT)	7.39	3.30	(-3.93, 14.51)	2.24	0.041
H4(ACT) - H3(TAA)	-8.05	5.56	(-20.03, 3.93)	-1.45	0.205

Table 6. Hsu Simultaneous tests for productive tillers using largest level mean

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
H1(TAT) - H3(TAA)	-8.05	3.06	(-14.64, 0.00)	-2.63	0.015
H2(ACA) - H3(TAA)	-8.08	3.04	(-14.64, 0.00)	-2.65	0.014
H3(TAA) - H1(TAT)	8.05	3.06	(0.00, 14.64)	2.63	0.015
H4(ACT) - H3(TAA)	-12.55	5.15	(-23.64, 0.00)	-2.44	0.025

One-way an~~n~~ova following Hsu Simultaneous tests clearly explains the statisticalsignificanceof the haplo-group H3against H1, H2 and H4 haplotype groupsboth total tiller number and productive tillernumber (**Fig4,5)**.The p-value for total tiller number explains the significance between the haplotype groupsH1-H3 andH2-H3with 0.041for and 0.036 respectively and similarly the p-value for number of productive tillers between the haplotype groupsH1-H3, H2-H3 and H4-H3 is 0.015, 0.014 and 0.025 respectively at 95% confidence interval. Study by Vrushali *et al*., (2022) used similar statisticalapproach to test the statistically significant difference between the two haplotype groups H1 and H2.

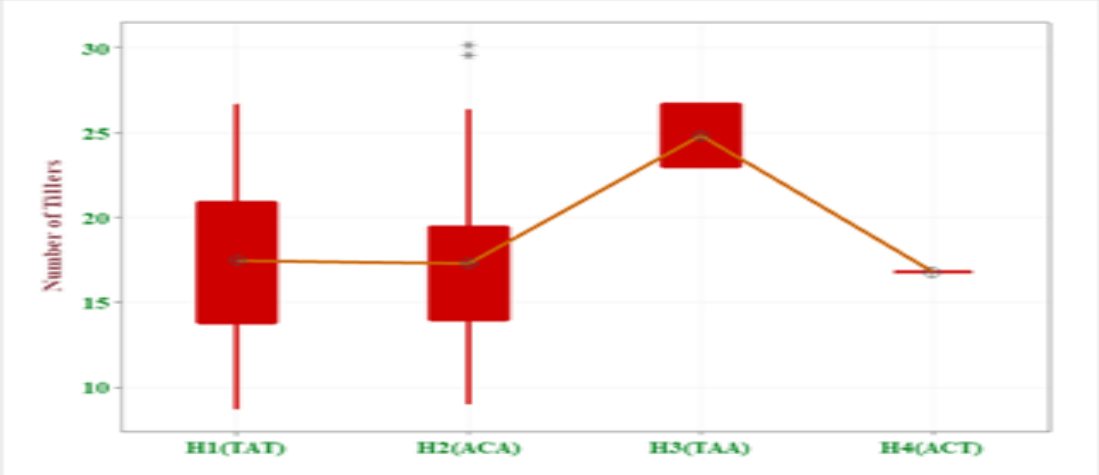

Fig4.Box plot curve for different haplotypesgroups for total number of tillers

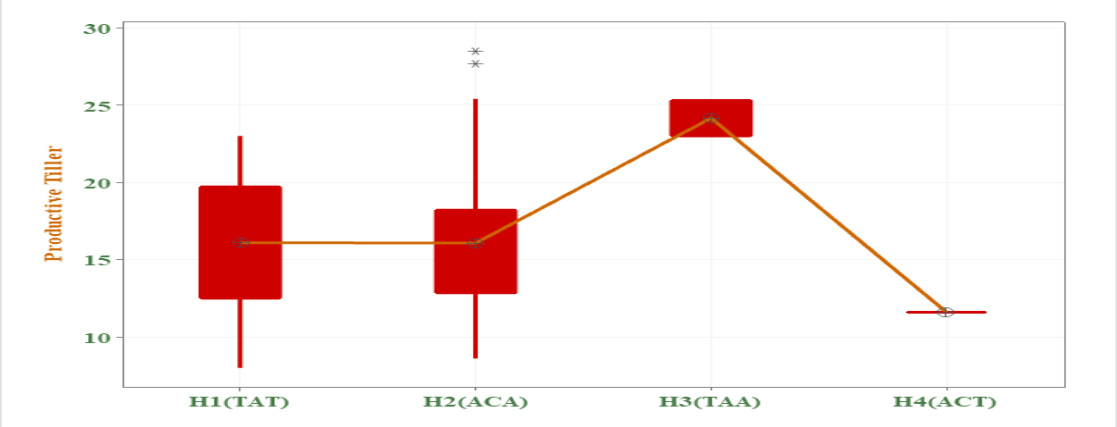

Fig5. Box plot curve for different haplotypesgroups for total productive tillers.

The above results from the haplo-pheno analysis revealed that haplotype group H3 is the superior haplotype group based on its statistical significance over the other haplotype groups. The genotype CN 44-40-7 has the highest number of total tillers as well as productive tillers with 27 and 25 tillers respectively. This data supports that the genotype CN 44-40-7 can be a potential donor in the crop improvement programmes for increasing both tiller number and productive tiller.

CONCLUSION

A growing world population necessitates increasing the production of rice grains. A plant's total number of tillers is very important because it directly affects grain yield, resource use efficiency, and climate tolerance. One of the most important approaches to meet the difficulties of the food demand is to improve rice varieties for increased tillers. So, it is essential to find superior genotypes with more tillers since these can make excellent candidates for breeding initiatives that would lead to crop improvement.Based on this analysis, haplotype group with the allelic combination of H3 (TAA) shows the maximum mean value for both total tillers and productive tillers thereby leading to the identification of a novel donor that can be used in the future crop breeding programmes.

FUTURE SCOPE

This research may facilitatethe identification of alleles leading to higher yield in rice.

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REFERENCES

1. Allen TT, Allen TT. Software overview and methods review: Minitab. Introduction to Engineering Statistics and Lean Six Sigma: Statistical Quality Control and Design of Experiments and Systems. 2019:575-600.
2. Al-Babili S, Bouwmeester HJ. Strigolactones, a novel carotenoid-derived plant hormone. Annual review of plant biology. 2015 Apr 29;66:161-86.
3. Barrett JC, Fry B, Maller JD, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005 Jan 15;21(2):263-5.
4. Bashir K, Ishimaru Y, Nishizawa NK. Identification and characterization of the major mitochondrial Fe transporter in rice. Plant signaling & behavior. 2011 Oct 1;6(10):1591-3.
5. Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids: handbook. Springer Science & Business Media; 2004.
6. Dhande VN, Williams M, Ranjani RV, Sakthi AR, Sudha M, Raveendran M. Identifcation of superior haplotypes for CCD8 regulating tiller number and grain yield in rice. Electronic Journal of Plant Breeding. 2023;14(1):148-53.
7. Gross BL, Zhao Z. Archaeological and genetic insights into the origins of domesticated rice. Proceedings of the National Academy of Sciences. 2014 Apr 29;111(17):6190-7.
8. Li Y, Xiao J, Chen L, Huang X, Cheng Z, Han B, Zhang Q, Wu C. Rice functional genomics research: past decade and future. Molecular plant. 2018 Mar 5;11(3):359-80.
9. Liu L, Xie T, Peng P, Qiu H, Zhao J, Fang J, Patil SB, Wang Y, Fang S, Chu J, Yuan S. Mutations in the MIT3 gene encoding a caroteniod isomerase lead to increased tiller number in rice. Plant Science. 2018 Feb 1;267:1-0.
10. López-Ráez JA, Matusova R, Cardoso C, Jamil M, Charnikhova T, Kohlen W, Ruyter-Spira C, Verstappen F, Bouwmeester H. Strigolactones: ecological significance and use as a target for parasitic plant control. Pest Management Science: Formerly Pesticide Science. 2009 May;65(5):471-7.
11. Lopez-Obando M, Ligerot Y, Bonhomme S, Boyer FD, Rameau C. Strigolactone biosynthesis and signaling in plant development. Development. 2015 Nov 1;142(21):3615-9.
12. Liao Z, Yu H, Duan J, Yuan K, Yu C, Meng X, Kou L, Chen M, Jing Y, Liu G, Smith SM. SLR1 inhibits MOC1 degradation to coordinate tiller number and plant height in rice. Nature communications. 2019 Jun 21;10(1):2738.
13. Małas K, Kabała K. Identification and Functional Analysis of Two Mitoferrins, CsMIT1 and CsMIT2, Participating in Iron Homeostasis in Cucumber. International Journal of Molecular Sciences. 2023 Mar 6;24(5):5050.
14. Mishra R, Joshi RK, Zhao K. Genome editing in rice: recent advances, challenges, and future implications. Frontiers in Plant Science. 2018 Sep 19;9:1361.
15. Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Frontiers in plant science. 2017 Apr 18;8:537.
16. Ruyter-Spira C, Al-Babili S, Van Der Krol S, Bouwmeester H. The biology of strigolactones. Trends in plant science. 2013 Feb 1;18(2):72-83.3.
17. Sundaramoorthy M, Rajagopalan VR, Ayyenar B, Kambale R, Muthurajan R. Surveying allelic diversity of Os DEP1 regulating panicle architecture in rice.
18. Varshney RK, Singh VK, Kumar A, Powell W, Sorrells ME. Can genomics deliver climate-change ready crops?. Current opinion in plant biology. 2018 Oct 1;45:205-11.
19. Wang Y, Lu J, Ren T, Hussain S, Guo C, Wang S, Cong R, Li X. Effects of nitrogen and tiller type on grain yield and physiological responses in rice. AoB Plants. 2017 Mar;9(2):plx012.

20. Xu CF, Lewis K, Cantone KL, Khan P, Donnelly C, White N, Crocker N, Boyd PR, Zaykin DV, Purvis IJ. Effectiveness of computational methods in haplotype prediction. Human genetics. 2002 Feb;110:148-56..
21. Yan Y, Ding C, Zhang G, Hu J, Zhu L, Zeng D, Qian Q, Ren D. Genetic and environmental control of rice tillering. The Crop Journal. 2023 Jul 5.