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Linkage Map Construction and QTL Identification for Yield Related Traits of Rice (Oryza sativa L.)

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ABSTRACT

The study was undertaken to construct a linkage map and to identify the QTLs for heading date, plant height, and culm strength using a F2 population derived from Ayeyarmin/IR 64, two varieties distinctly different in heading date and plant height. A total of 233 F2 individuals were genotyped using 70 SSR primers, and phenotypic data were collected for heading date, plant height and culm strength traits. Fifty-four out of 70 marker loci were mapped across twelve rice chromosomes spanning a total map length of 1592.30 cM with an average distance of 29.49 cM between adjacent marker loci using MAPMAKER/EXP 3. Composite interval mapping for QTL analysis was conducted with 1000 permutation time at 0.05 probability level using WinQTL Cartographer version 2.5. One major QTL for heading date was mapped between marker RM204 and RM50 on chromosome 6. One major QTL for culm strength was mapped between marker RM1003-RM5931, and two major QTLs for plant height were mapped between RM84-RM1003 and RM1003-RM5931 on chromosome 1. The molecular markers linked to the major QTLs could be potentially used in marker-assisted selection for the rainfed rice improvement breeding program.

1. Introduction

Ayeyarmin variety of rice (*O. sativa* L.) is not only one of the popular varieties but also the third widely grown cultivar in the monsoon season of Myanmar. It has extremely good eating quality and market acceptability. It has long maturity duration, tall plant height, intermediate amylose content and gelatinization temperature, medium gel consistency, and translucent grain. It is usually grown under rainfed and irrigated lowland rice ecosystems. It has excessive plant height that leads to lodging during bad weather conditions such as heavy rain and wind. Similarly, long maturity duration makes it less seasonal adaptable. Therefore, it is necessary to develop the adapted varieties, especially early maturing varieties, to fit in the summer rice growing season of Myanmar for rice improvement program. Early maturing variety that has the ability to escape drought and to increase cropping intensity, plays a major role in plant adaptation to the environment. Lodging is also a common problem in rice and it mainly occur as a result of strong winds, heavy rain, water management, planting density, and an excessive use of nitrogen fertilizer (Anitha Shalini, 2015).

In Myanmar, most of the current rice breeding strategies depend on direct phenotypic selection and multi-generation inbreeding which are time-consuming and labor-intensive. The accuracy and efficiency of conventional breeding will not be effective if the phenotypic selection will be used. To improve rice breeding efficiency, it is required to the apply the modern molecular biological tools and techniques for rice genetic improvement. Molecular plant breeding will allow rice breeders to achieve breeding goals quickly and efficiently. In general, rice crop improvement is carried out with the major objectives such as high yield potential, early maturity, resistance to lodging and shattering, resistance to stress environments, disease and insect resistance, acceptable grain quality, and enhancement of nutritional components using biotechnological techniques (Ansari et al., 2015).

Genetic improvement in crop plants largely relies on the handling of quantitative traits because important agronomic traits, yield and yield contributing traits are quantitatively inherited. Quantitative traits are complex because these are controlled by many genes having small and cumulative effect (Kumar et al., 2017). There are limitations to observe the inheritance of polygenes following using classical Mendelian methods because of the environmental influences. Genetic linkage analysis of quantitative trait loci (QTL) has become a common technique to overcome this limitation. For a successful rice breeding program, it is one of the priorities to identify quantitative trait loci (QTLs) associated with these economically important target traits. QTL analysis offer information to identify genome-specific regions affecting agronomic and yield-related traits. A variety of statistical tools have been developed to detect the inheritance of polygenes. The nature of quantitative traits can be insight with the integration of biometrical and molecular techniques by mapping of QTL (Xu, 2010).

The development of genetic linkage map for many plant species provides the foundation and tools for QTL mapping, marker-assisted breeding and map-based cloning of gene. Several molecular markers, such as simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and amplified fragment length polymorphism (AFLP), have been used in constructing linkage maps to identify QTLs associated with economically important traits in rice. The SSR markers are distributed throughout the genome uniformly and detects a high level of allelic diversity in cultivars and related species (Singh & Singh, 2015). The SSR markers are one of the most useful markers to construct the linkage map for rice. Owing to the genetic linkage map, DNA markers can detect the allelic variation of the genes underlying these traits and it offers great scope for improving selection efficiency. A linkage map for Ayeyarmin/IR64 population is the is the first one in Myanmar rice cultivars. The aim of the study was to identify QTLs linked to heading date, plant height and culm strength and to construct the linkage map using F_2 population derived from Ayeyarmin/IR64.

2. Materials and methods

2.1 Plant materials and F_2 population development

Myanmar's popular variety, Ayeyarmin which is late flowering and tall was crossed with IR64, an early maturing and semi-dwarf variety developed by International Rice Research Institute,

Philippines. Ayeyarmin and IR64 were crossed to develop F_1 generation and subsequently F_2 population by self-pollination during the post-monsoon season in 2018.

Ayeyarmin F_1 IR64

Figure 1. Plant morphology of *O. sativa* cv. Ayeyarmin, IR64 and their F_1

2.2 Phenotypic evaluation

Field experiment was conducted at the field of Yezin Agricultural University situated at 19˚10'N latitude, 96˚07'E longitude with an elevation of 102 meters above sea level during the summer season, 2019. Five tallest tillers were evaluated for the yield-related traits and measured as follows; heading date (number of days from seeding to 50 % flowering), plant height: length from the soil surface to the tip of the panicles excluding awn at physiological maturity (cm), culm strength; reading value of prostrate tester in which plants at the field for ten days after harvest, were cut off at 40 cm height and tied at 20cm. The prostrate tester (DIK-7400, Daiki Rika Kogyo Co. Ltd., Tokyo, Japan) was set perpendicularly and the tied tillers were pushed to an angle of 45° at the lower part of the cut plant (20 cm). Pushing resistance was measured as tester reading (g).

2.3 Molecular marker analysis

Genomic DNA of 233F_2 lines and two parents was extracted with a little modification using the CTAB method following Murray & Thompson (1980). The 250 SSR primers were used in the parental polymorphism survey. The genomic DNA of 233F_2 lines and two parents was amplified using the parental polymorphic primers in 10 µl of mixture containing 10X buffer with $MgCl_2$, 10 mM dNTP mix, 10 µM of each of primer, and 5U/µl of Taq DNA polymerase (MP Biomedicals India Private Limited, India). A thermal profile was as follows, 1 cycle at 95˚C for 5 minutes (initial denaturation), followed by 35 cycles at 94˚C for 45 seconds (denaturation), at 48-62˚C for 45 seconds (annealing), at 72˚C for 1 minute (extension), 1 cycle for final extension at 72˚C for 10 minutes and at 10˚C for 15 minutes (store). PCR products were separated by electrophoresis in 3-4% ultra-agarose. Omega Lum G imaging system was utilized to photograph the gel for genotypic data scoring. The genotypic data was achieved by scoring on the banding pattern of each F_2 plant as "A" for the allele size similar to the female parent, Ayeyarmin, "B" for the alleles similar to the male parent, IR64, and "H" for the heterozygotes i.e. showing alleles for both the parents as per Collard et al. (2005).

2.4 Parental polymorphism survey

A total of 250 SSRmarkers were used to identify the markers that have the ability distinguishing the morphism of two parents. The level of polymorphism (%) was calculated as the ratio of number of polymorphic markers and total markers used in the parental polymorphism survey.

2.5 Segregation analysis

Marker segregation on F_2 population was analyzed for the goodness of fit with the Mendelian segregation ratio (1:2:1) on genotypic data of 233 F_2 plants by chi square test.

2.6 Linkage map construction and QTLs identification

MAPMAKER/EXP 3 developed by Lander et al., (1987), was used for the linkage mapping. Composite interval mapping for QTL analysis was conducted with 1000 permutation time at 0.05 probability level using WinQTL Cartographer version 2.5 (Wang, 2007). The putative QTLs were nomenclature using the method on rice (*Oryza sativa* L.) developed by McCouch (1997).

3. Results and discussion

3.1 Phenotypic performance

The mean performance of parents, F_1 and F_2 populations for three traits: heading date, plant height, and culm strength was shown in Table 1. The heading date of the female parent, Ayeyarmin, was recorded as 158.67 ± 2.08 days, while the male parent, IR64, had a heading date of 83.67 ± 1.16 days. The mean values of heading date in the F_1 and F_2 progenies closely resembled the early parent, IR64, with values of 81.00 and 88.57 ± 8.08 days, respectively. The heading dates observed in the F_2 population ranged from 72 to 135 days.

The plant height of the female parent, male parent, and F_1 plant were 145.39 cm, 86.00 cm, and 117.67 cm, respectively. Among the F_2 plants, there was a range of height from 73.70 cm to 150.30 cm, with a mean value of 115.46 cm ± 18.10 cm.

The culm strength values for the female parent, male parent, and F_1 plant were 12.50 g, 13.00 g, and 12.33 g, respectively. The culm strength varied from 1.50 g to 33.00 g, with a mean value of 11.29 g ± 6.04 g in the F_2 population.

The frequency distribution of heading date, plant height, and culm strength in the F_2 population was described in Figure 1. Some F_2 progenies were heading dates earlier than the early parent (IR64), indicating transgressive segregation for this trait. Similarly, transgressive segregants were observed for plant height among the F_2 plants, with some F_2 plants being shorter or taller than the early parent (IR64). The study population also showed transgressive segregants with higher or lower culm strength values than the parents; Ayeyarmin and IR64. Therefore, this F_2 segregating population was potential to further investigate and improve in subsequent generations, for the development of a variety with desirable plant height and growth duration.

Table 1. Phenotypic performance of parents, F_1 and F_2 population for plant height, heading date and culm strength

Trait	Ayeyarmin	IR64	F_1	F_2			
	Mean			Mean	Minimum	Maximum	Standard deviation
Heading date (day)	158.67	83.67	81.00	88.57	72.00	135.00	8.08
Plant height (cm)	145.39	86.00	117.67	115.46	73.70	150.30	18.10
Culm strength (g)	12.50	13.00	12.33	11.29	1.50	33.00	6.04

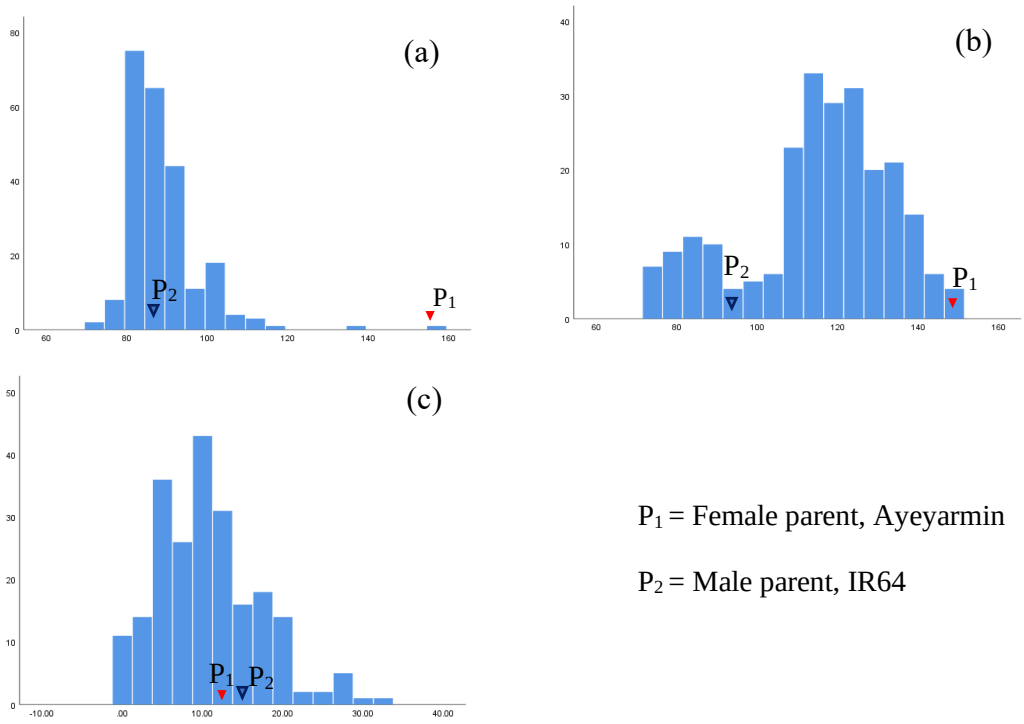


Figure 1. Frequency distribution of F_2 population derived from Ayeyarmin/IR64 (a) heading date (day), (b) plant height (cm), (c) culm strength (g)

3.2 Information on parental polymorphism survey

In the present study, seventy markers out of two hundred and fifty SSR markers were polymorphic between the parents; Ayeyarmin and IR64 indicating the level of polymorphism between the parental genotypes be 28 % (Table 2). Parental polymorphism percent was low because both parents were of indica type only. Naturally, the levels of DNA sequence variation are generally lower in the self-pollinated crop than that of the cross-pollinated crop. Low parental polymorphism event was found by Tripathi et al. (2018).

Table 2. Polymorphism percent on 250 tested markers

Chromosome	No. of SSR marker used	No. of polymorphic markers	Polymorphism (%)
1	23	9	39.13
2	23	7	30.43
3	23	8	34.78
4	19	6	31.58
5	22	6	27.27
6	14	7	50.00
7	23	5	21.74
8	15	5	33.33
9	24	6	25.00
10	21	4	19.05
11	18	4	22.22
12	25	3	12.00
Total	250	70	28.00

3.3 Marker segregation

Segregation analysis helps to identify markers that express Mendelian inheritance, indicating their suitability in the linkage map construction. Marker segregation for seventy SSR markers was investigated using chi square test (Table 3). As the tested population was F_2 population, the expected genotypic ratio would be 1:2:1 for homozygous Ayeyarmin: heterozygous Ayeyarmin/IR64, homozygous IR64. A total of twenty-four out of seventy SSR markers were skewed towards Ayeyarmin or IR64. The skewed markers found to distribute across all the chromosomes. Allelic frequency in an F_2 population without selection would be 25% Ayeyarmin, 50% Ayeyarmin/IR64, and 25%IR64. The twenty-four markers were skewed toward either Ayeyarmin or IR64. Seventeen markers were skewed to IR64 and seven to Ayeyarmin. Xiong et al., (1999) also documented skewness of markers towards one or other parents. Although F_2 seeds were randomly selected without biasness, segregation distortion in the present study population had occurred. Segregation distortions may result in reduction in recombination.

Table 3. Chi square values ($\chi 2$) for identification of marker distorsion

No.	Marker	Chromosome	$\chi 2$	Skewness
1	RM5536	1	32.43**	Ayeyarmin
2	RM6	2	8.98*	Ayeyarmin
3	RM211	2	7.3*	IR64
4	RM231	3	21.83**	IR64
5	RM232	3	61.24**	IR64
6	RM3525	3	6.11*	IR64
7	RM251	3	6.66*	IR64
8	RM261	4	7.09*	IR64
9	RM5506	4	8.89*	IR 64
10	RM18452	5	15.21**	Ayeyarmin
11	RM50	6	54.61**	IR64
12	RM6403	7	6.09*	IR64
13	RM51	7	122.09**	IR64
14	RM210	8	73.49**	Ayeyarmin
15	RM506	8	37.96**	IR64
16	RM3819	8	7.84*	IR64
17	RM23654	9	59.53**	Ayeyarmin
18	RM5526	9	66.48**	IR64
19	RM107	9	10.10*	IR64
20	RM7492	10	6.63*	Ayeyarmin
21	RM222	10	9.69*	IR64
22	RM209	11	7.51*	IR64
23	RM27326	11	157.63**	Ayeyarmin
24	RM1227	12	30.21**	IR64

3.4 Construction of linkage map

A total of seventy marker loci were used to construct a linkage map employing the 233 individuals of F_2 population derived from Ayeyarmin/IR64. Sixteen markers showed as an unlinked group. Fifty-four marker loci excluding sixteen unlinked markers were mapped on 12 rice chromosomes spanning a total map length of 1592.30 cM with an average distance of 29.49 cM between adjacent marker loci and the minimum distance of 5.5 cM. Whereas, there was the gaps between linkage groups on chromosome 1, 2, 3, 4,5 and 9 (Figure 2). The polymorphic markers were not evenly distributed across the genome indicating poor linkage map quality. To construct a good linkage map, it should use not only phenotypically and genotypically diverse parents for target traits but also the large

number of molecular markers which are evenly distributed across the whole genome of rice. Additional markers should be mapped in the gap chromosomal region in the future.

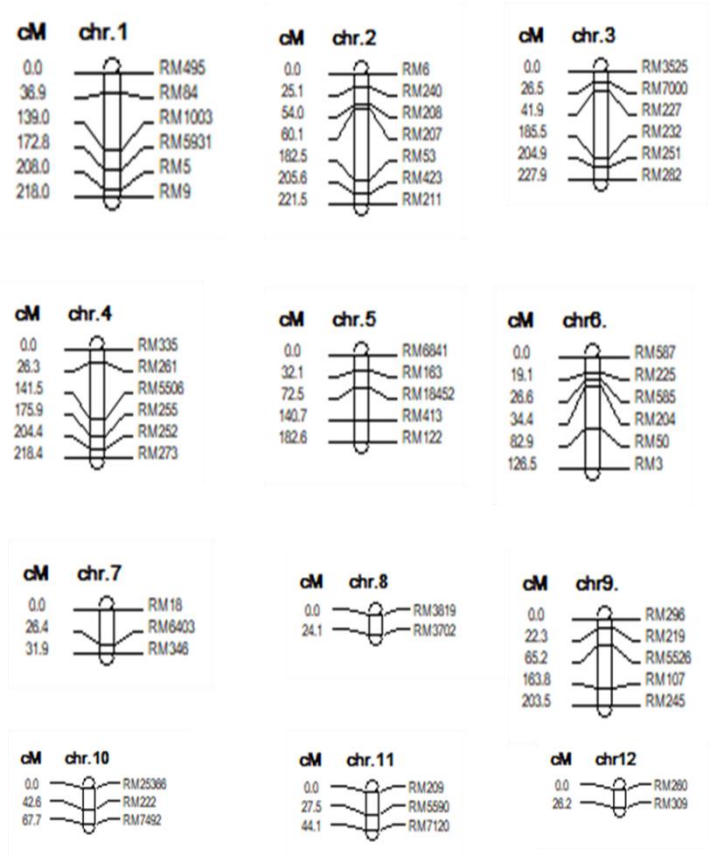


Figure 2. Linkage map constructing using 233 F_2 individuals of fifty-four SSR markers

3.5 Identification of QTLs for yield related traits

A total of four QTLs for yield related traits viz., heading date, plant height and culm strength were detected on chromosome 6 and 1. The additive and dominance effect, estimated LOD and regression coefficient values of these QTLs were shown in Table 4 & Figure 3 a, b,c.

Heading date

The QTL designed *qHD6* was mapped between the markers RM204 and RM50 at map position 40.41 cM on chromosome 6. It had a LOD score value of 5.49 and explained 25 % phenotypic variation with the positive additive effect of 3.72 indicating the delayed heading about 4 days at this locus was from Ayeyarmin. Allele of qHD*6* showed complete dominance effect on early flowering.

Plant height

Two QTLs for plant height were mapped between the markers RM84-RM1003 at 118.91cM and RM1003-RM5931 at 152.01 cM on chromosome 1. These QTLs had the positive additive effect and large phenotypic variation effect. Positive allele of the resultant QTLs came from Ayeyarmin increased plant height by 23-27 cm. They had positive partial dominance effect.

Culm strength

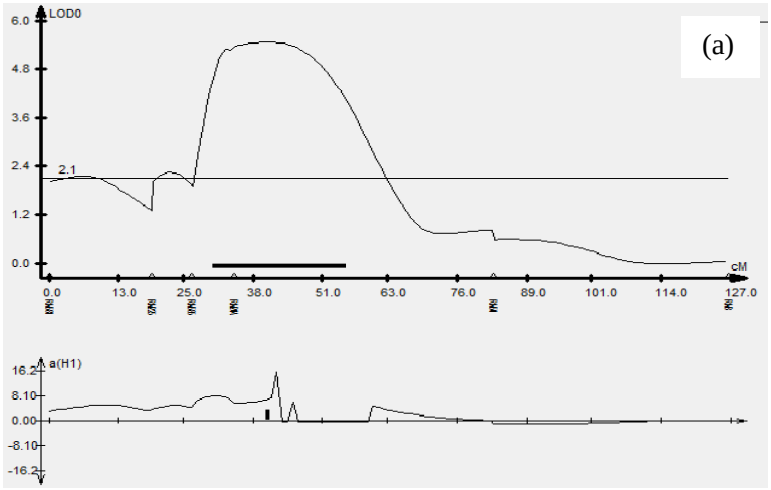
A QTL associated with culm strength expressing a large phenotypic effect was mapped between RM1003 and RM5931 at 159.01 cM on chromosome 1. It showed a positive additive effect indicating that the allele came from Ayeyarmin. It had negative incomplete dominance effect.

In the study, only a few QTLs were detected for heading date, plant height, and culm strength although the parents distinctly differed for these traits, and transgressive segregants were also observed in the in the F_2 population. This might be because of the low density linkage map of the F_2 population which happened due to less polymorphic markers. Further, the markers did not distributed evenly across the genome. However, all the mapped QTLs in the present study were the major QTLs. QTLs for heading date contributing the major effect was mapped between RM240 and RM50 on chromosome 6 in the present study. Suji et al. (2012) also identified QTLs for heading date near RM 204 on chromosome 6 using 232 recombinant inbred lines derived from IR62266 and Norungan. Heading date QTLs were mapped between PSM677 and RM204 on chromosome 6 using two secondary segregation populations in rice by Hua et al. (2018). In the present study, the QTLs associated with plant height and culm strength were mapped between RM1003 and RM5931 on chromosome 1. Sahu et al. (2017) also identified the QTL for plant height near RM1003 on chromosome 1 in a F_5 population derived from Swarna (a high-yielding semi-dwarf widely adapted indica variety) with IR86931B-6 (semi-tall, interspecific line derived from Nagina22). Khahani et al. (2021) also identified the 24 QTLs on chromosome 1 using different populations and molecular markers. Yadav et al. (2017), and Kashiwagi (2014) discovered culm strength QTLs on chromosome 1 by analyzing backcross population and chromosomal segment substitution lines in rice. Therefore, the resultant QTLs in the present study are reliable for the consistency of the results on the repeatability in various studies with different genetic backgrounds or different segregating populations.

Table 4. QTLs associated with yield related traits detected in the F_2 population derived from yeyarmin/IR64

Trait	QTL	Chr.	Marker interval	Position (cM)	LOD	Additive (a)	Dominance (d)	d/a	R^2 (%)
Heading date	*qHD6*	6	RM204-RM50	40.41	5.49	3.72	-3.76	-1.01	25
Plant height	*qPH1.1*	1	RM84-RM1003	118.91	22.98	23.14	7.31	0.32	17
Plant height	*qPH1.2*	1	RM1003-RM5931	152.01	26.79	23.45	8.30	0.35	36
Culm strength	*qCS1*	1	RM1003-RM5931	159.01	5.37	3.62	-2.53	-0.69	23

Chr. = chromosome; LOD = logarithm of odd; d/a = degree of dominance; R^2 = total phenotypic variance explained by the QTL



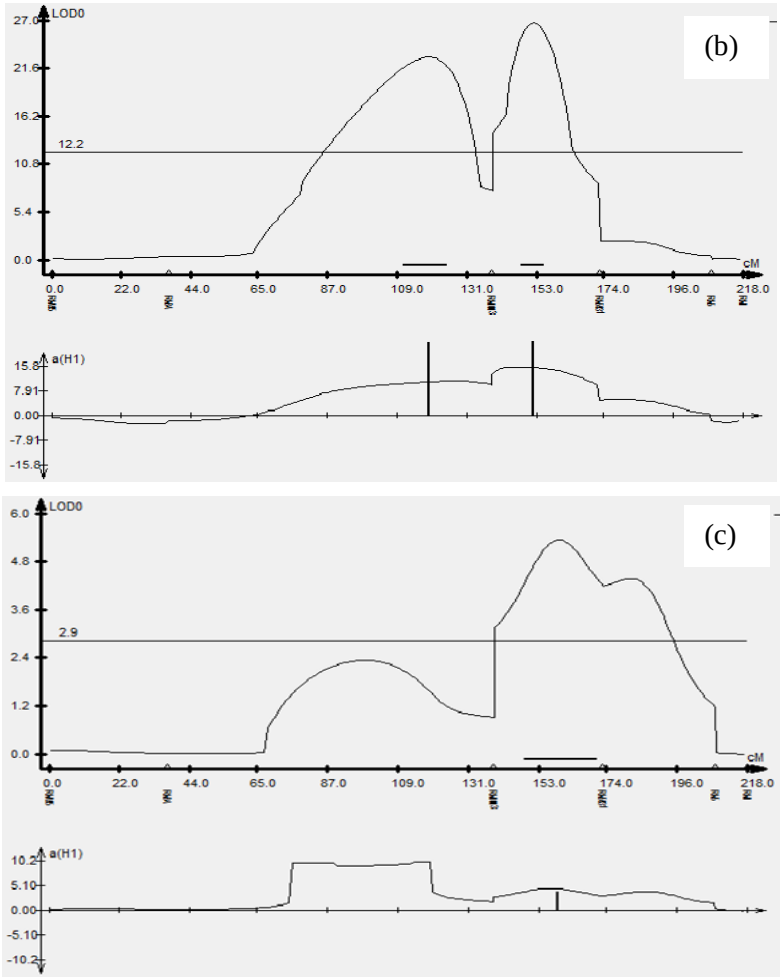


Figure 3. Chromosomal region of (a) heading date QTL on chromosome 6, (b) plant height QTLs on chromosome 1, and (c) culm strength QTL on chromosome 1 of rice genome

4. Conclusion

The study was undertaken to construct a linkage map using 233 F_2 individuals derived from the cross Ayeyarmin/IR64 and to identify the QTLs for yield related traits; heading date, plant height and culm strength. These yield related traits belong to quantitative nature indicating selection based on phenotype alone is not enough. The linkage map provides a valuable genetic framework for understanding the genomic organization and inheritance. Therefore, a linkage map was constructed using fifty-four SSR molecular markers and the 233 F_2 segregating lines derived from Ayeyarmin/IR64. One each major QTL for heading date on chromosome 6, culm strength on chromosome 1, and two QTLs for plant height on chromosome 1 were mapped in the F_2 population of Ayeyarmin/IR64. The resultant QTLs should be validated using different populations testing in multi-environment trials. Molecular markers linked the major QTLs can be of potential value in the application of marker assisted selection of the corresponding traits for rainfed rice improvement breeding program.

5. Acknowledgement

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6. References

Anitha Shalini, S. (2015). Identification of molecular markers associated with culm strength in rice (*Oryza sativa* l.) using selective line genotyping (Doctoral dissertation). Telangana State Agricultural University, Rajendranagar, Hyderabad.

Ansari, M. U. R., Shaheen, T., Bukhari, S., & Husnain, T. (2015). Genetic improvement of rice for biotic and abiotic stress tolerance. Turkish Journal of Botany, 39(6), 911-919.

Collard, B. C., Jahufer, M., Brouwer, J., & Pang, E. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica, 142(1), 169-196.

Hua, Z., Xu, L., Yongyi, Y., Ning, X., & Fangyin, Y. (2018). Mapping of Hd-6-2 for Heading Date Using Two Secondary Segregation Populations in Rice. Rice Science, 25(3), 161-168.

Kashiwagi, T. J. E. (2014). Identification of quantitative trait loci for resistance to bending-type lodging in rice (*Oryza sativa* L.). Euphytica, 198(3), 353-367.

Khahani, B., Tavakol, E., Shariati, V., & Rossini, L. (2021). Meta-QTL and ortho-MQTL analyses identified genomic regions controlling rice yield, yield-related traits and root architecture under water deficit conditions. Scientific reports, 11(1), 6942.

Kumar, J., Gupta, D. S., Gupta, S., Dubey, S., Gupta, P., & Kumar, S. (2017). Quantitative trait loci from identification to exploitation for crop improvement. Plant Cell Reports, 36(8), 1187-1213.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., & Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, 1(2), 174-181.

McCouch, S. J. R. G. N. (1997). Suggestions for QTL nomenclature for rice. Rice Genetic Newsletter, 14, 11-13.

Murray, M., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic acids research, 8(19), 4321-4326.

Sahu, V. K., Sunil, K. N., Vishwakarma, A. K., Verulkar, S., & Chandel, G. (2017). QTL hotspots detected for yield contributing traits in rice (*Oryza Sativa* L.) using composite interval mapping analysis. Biosciences Biotechnology Research Asia, 14(1), 329-341.

Singh, B., & Singh, A. K. (2015). Marker-assisted plant breeding: principles and practices: Springer.

Suji, K. K., Biji, K. R., Poornima, R., Prince, K. S. J., Amudha, K., Kavitha, S., . . . Babu, R. C. (2012). Mapping QTLs for Plant Phenology and Production Traits Using Indica Rice (*Oryza sativa* L.) Lines Adapted to Rainfed Environment. Molecular biotechnology, 52(2), 151-160. doi:10.1007/s12033-011-9482-7

Tripathi, J., Thagele, S. N., Sharma, A. K., Tripathi, N., Tripathi, P., & Singh, A. K. (2018). Parental polymorphism survey for drought tolerance in rice by using SSR marker. Journal of Biotechnology and Biochemistry, 4(5), 82-86.

Wang, S. (2007). Windows QTL cartographer 2.5. http://statgen ncsu. edu/qtlcart/WQTLCart. Html.

Xiong, L., Liu, K., Dai, X., Xu, C., Zhang, Q. J. T., & Genetics, A. (1999). Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *O. sativa* and *O. rufipogon*. Genetics, 98(2), 243-251.

Xu, Y. (2010). Molecular Plant Breeding, Wallingford. *UK: CAB International*.

Yadav, S., Singh, U. M., Naik, S. M., Venkateshwarlu, C., Ramayya, P. J., Raman, K. A., & Kumar, A. (2017). Molecular mapping of QTLs associated with lodging resistance in dry direct-seeded rice (*Oryza sativa* L.). Frontiers in Plant Science, 8, 1431.