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Linkage Mapping and Identification of QTLS Responsible for Earliness in Bread Wheat (*Triticum aestivum* L.) in $F_{2:3}$ Mapping Population

N. A. Delvadiya[1]*, D. R. Mehta[2], A. K. Nandha[1] and R. R. Rathod[1]

[1]Department of Biotechnology, [2]Department of Genetics and Plant Breeding, Junagadh Agriculture University, Junagadh-362001, India

**Corresponding author*

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A B S T R A C T

Earliness, an adaptive trait and factor of variation for agronomic characters, is a major trait in plant breeding. In present investigation, the experimental material comprised of P_1, P_2, F_1, F_2 and $F_{2:3}$ generations of wheat crossDL-788-2 X GW-322 for earliness related traits with objective of linkage and QTL mapping in bread wheat. Out of 200 SSR markers screened for parental polymorphism for earliness related traits, only 11% of SSR markers showed good polymorphism between two parental lines. Out of 22 tests, all the test markers showed non-significant chi-square which revealed that observed data were agreement with expected ratio of 1:2:1 segregation ratio. The linkage map was constructed using software Ici Mapping v.4.1 and recombination frequencies were converted into map distance using Kosambi's mapping function. The markers were grouped with minimum logarithm of the odds (LOD) of 3.0 with walking speed was set at 1.0 cM. Four linkage groups with a total map length of 267.12 cM were constructed using data from 22 marker loci for 74 F_2 plants that ranged from minimum of 8.62 cM (LG4) to maximum of 126.56 cM (LG1).Genotypic data of F_2 and phenotypic data of on 74 $F_{2:3}$ lines were analyzed for identification of the main effect QTLs using the software ICIM-ADD mapping in QTL IciMappingV4.1. A linkage map of earliness related traits output data file was used for the construction of QTL mapping. One QTL was identified for days to 50% flowering (LG1 at 58.0 cM, LOD 3.06, 18 PVE %) and two QTLs for days to maturity (LG1 at 21 cM, LOD 8.89, 31.51 PVE% and LG3 at 38 cM, LOD 12.83, 45.16 PVE%).with use of molecular marker and QTL mapping complex from of earliness traits and their underlying genes are now far more accessible which can be routinely used by breeders in marker assisted selection in wheat breeding programs.

Introduction

The wheat belongs to the genus *Triticum* of the family Poaceae and its origin is believed to be Middle East Region of Asia (Lupton, 1987). Three species of wheat viz., *Triticum aestivum L*. (bread wheat), *Triticum durum* Desf. (macaroni wheat) and *Triticum dicoccum* Schulb. (emmer wheat) are presently grown as commercial crop in India, covering 86, 12, and 2% of the total area, respectively(Anonymous, 2013).The bread wheat (hexaploid with chromosome number 2n=6x=42) is cultivated in all the wheat

growing areas of the country, the macaroni or durum wheat is mostly grown in the Northern (Punjab) and Southern states, while the emmer wheat (tetraploid, 2n=4x=28) (Feldman *et al.,* 1995; Kihara, 1944; McFadden and Sears, 1946) is confined to the Southern states (mainly Karnataka) and some parts of Gujarat.

Heading time of wheat is a complex character comprised of three genetic factors: vernalization requirement, photoperiodic response, and earliness *per se*. Earliness *per se*, different from the other two, is independent of environmental factors and is recognized as the earliness by nature which is specific to varieties. This character is controlled by several minor genes (Kato and Sawada, 2000) and they were assigned to different chromosomes. Miura and Worland (1994) reported a gene on chromosome 3A and Hoogendoorn (1985) reported genes on chromosomes 3A, 4A, 4D, 6B, and 7D. On the contrary, vernalization requirement and photoperiodic response depend on environmental factors and they ensure safer heading (reproduction) by delaying heading time until environmental condition becomes favorable. A very good understanding of, and ability to manipulate oligogenic and polygenic traits is offered to the plant breeders by recent advances in genetic marker technology (Young, 1999). A major advantage of using molecular markers for the introgression of resistance genes into cultivars is a gain in time (Tanksley *et al.,* 1989; Melchinger, 1990) by guiding and expediting conventional plant breeding programme by reducing number of breeding cycles. The second major advantage is that it facilitates effective selection even when phenotypic selection is likely to be ineffective. The development and availability of abundant, naturally occurring, molecular markers (RFLP, RAPD, ISSR, SSRs, Isozymes, etc.) (Kochert, 1994) during the last two decades has generated renewed interest in counting, locating and measuring the effects of genes (polygenes or QTLs) controlling quantitative traits(Wu and Tanksley, 1993; Morgante and Olivieri, 1993). When there is a marker map and a segregating population for a character of interest, it is often possible to obtain information about the number, effects and positions of the QTLs affecting the trait (Paterson *et al.,* 1988). Marker assisted selection could be more efficient than purely phenotypic selection in quite large populations and for traits showing relatively low heritabilities (Moreau *et al.,* 1998).

The building up of a saturated linkage map using molecular markers like microsatellites (SSR) makes it possible to dissect Mendelian factors underlying a complex trait such as earliness and consequently enhance the effectiveness and accelerate the rate of breeding programmes to improve pure line varieties of self-pollinated crops and parental lines of hybrid in cross-pollinated crops. Linkage drag and confounding effects of environmental variation associated with conventional plant breeding can also be reduced. With QTL mapping, the role of specific loci can be described and interactions between genes, plant development, and environment can be analyzed. As the molecular-marker-based genetic linkage map for wheat has been constructed (William *et al.,* 1997) and extended (Nelson *et al.,* 2006; Ramya *et al.,* 2010), QTL analysis is now possible utilized in molecular breeding. Earliness is an important trait in plant breeding. Its constituent traits such as flowering time and days to heading are largely controlled by vernalization genes (Vrn), photoperiod response genes (Ppd) and developmental rate genes (‘earliness per se’, Eps). Mapping of major genes controlling quantitative traits, flowering time (FT) and days to heading (DTH) was carried out in an intervari *et al.,* wheat cross by Nalini *et al.,* (2006).

Materials and Methods

The complete set of experiment was carried out a tthe Biotechnology Laboratory of the Department of Genetics and Plant Breeding as well as Wheat Research Station, J.A.U., Junagadh during the year 2014 to 2017.

Mapping population and phenotyping

The experimental materials comprised two diverse parents viz., DL 788-2, and GW-322 collected from Wheat Research Station, Junagadh Agricultural University, Junagadh. The parental lineDL-788-2 has character of early maturity and parental line GW-322 has character of late maturity. The seeds of pure lines DL 788-2 and GW-322 for earliness and related traits were used as parents and sown at Wheat Research Station JAU, Junagadh during winter 2013-14. The parental lines and F_1 hybrids seeds were sown during winter 2014-15 to obtain selfed seeds of F_2. Whole spikelet of F_1 plant was covered with white parchment paper bags to prevent any unwanted cross pollination. Along with parental lines and saved F_1, selfed seeds of F_2 were sown during winter 2015-16. All the necessary observations were recorded in parental lines, F_{1S}, F_{2S}. Plant leaf samples were also collected from every single plant for DNA extraction 20 days after sowing and genotyping was done. To obtain selfed seeds of F_3, whole spikelet of selected F_2 plants were covered with white parchment paper bags to prevent any unwanted cross-pollination. Along with parental lines, selfed seeds of F_3 were sown in two replications at Wheat Research Station, JAU, Junagadh during winter 2016-17 for $F_{2:3}$ phenotyping.

DNA isolation, polymorphism and genotyping

Total genomic DNA extraction was carried out by CTAB method as described by Stein *et al.*(2001) with minor modifications. To identify SSR primer pairs detecting polymorphism between parents, initial screening of parental lines was conducted before actual genotyping of individuals in segregation F_2 mapping population. For this, DNA from DL 788-2 (taken as first parent i.e. P_1) and GW-322 (taken as second parent i.e. P_2) and their corresponding F_1 hybrids were subjected to PCR amplification with each of the available SSR primer pairs. A total of 200 SSR primers pairs were used to screen the parental polymorphism of the population. Simple Sequence Repeat (SSR) which showed good scorable polymorphic pattern in parental lines was used for characterization of F_2 population. Primers required for SSR were synthesized from Merck Bioscience, Bangalore. The amplified products of SSR were analyzed on 3 % agarose gel.

Construction of Linkage Map

QTL IciMapping v4.0 (Meng *et al.*, 2015) was used for linkage group construction using all the polymorphic markers. Three general steps were involved in linkage map construction: Grouping, Ordering and Rippling. First of all, markers were grouped based on a Likelihood of odd ratio (LOD) of 3.0, recombination frequency of 0.3 and Window size 5cM. To include additional markers on the map, Try and move to commands were used. Finally, linkage map based on SSR marker was constructed.

QTL Mapping

Trait data from $F_{2:3}$ was averaged for each entry and sorted to correspond with the progeny order of the genotypes (marker data). The total number of progeny individuals from the cross with trait and genotype information was 74. QTL mapping was performed using the Inclusive Composite Interval Mapping Additive (ICIM-ADD) method of QTL

IciMapping v4.0. A threshold LOD score 3.0 was used to confirm significant QTL. Other parameters settings for ICIM were the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables with threshold of 0.001 for removing variables and 1cM walking speed along chromosome. QTL was considered to have a significant effect when LOD statistics exceeded a threshold of 3.0(Meng *et al.*, 2015).

Results and Discussion

Parental polymorphism for earliness

The parental lines P_1 (DL-788-2, early maturity) and P_2 (GW-322, late maturity) were screened against 200 SSR (microsatellite) markers to identify parental polymorphic combinations. A total of 22 polymorphic SSR markers between two parental lines were used to screen the mapping population of F_2 developed for earliness. Out of 200 markers screened, only 11% of SSR marker showed good polymorphism between two parental lines for traits related to earliness. All the 200 SSR makers used in the present study were previously reported and available in the public domain.

The markers consisted primary of barc (Song *et al.,* 2005), cfd (Guyomarc'h *et al.,* 2002), gwm (Röder *et al.,* 1995, 1998), wmc (Gupta *et al.,* 2002; Somers *et al.,* 2004) markers. A total of 22 very clear and scorable polymorphic SSR markers between two parental lines (Fig. 1) were used to screen the mapping population of F_2 developed for earliness.

The low level of polymorphism obtained from SSR markers in the present was akin to the results reported in rice and wheat (Chao *et al.*, 1989; Devos *et al.,* 1992).

Segregation of markers and their distortion

The segregation pattern of marker loci (SSR) for the mapping population of 74 F_2 plants was compared with the expected ratio of 1:2:1 [1 homozygote (A) from P_1: 2 heterozygote (H): 1 homozygote (B) from P_2]. The calculated chi-square values using observed frequency of A: H: B and its expected frequency for each and every individual marker locus is presented in Table 1.

The calculated chi-square values were compared with tabulated values for 5% and 1% probability levels at two degrees of freedom. Out of 22 tests for 22 SSR, all the test markers showed non-significant chi-square as expected ratios at both probability levels. This revealed that observed data were agreement with expected ones, indicating fulfillment of 1:2:1 segregation ratio.

Distorted segregation of molecular marker loci appears to be a common phenomenon in crop species (Cloutier *et al.*, 1991; Yarnagishi *et al.*, 1996).

Construction of genetic linkage map for earliness and related traits

The main objective of the present experiment is to develop a new intra-specific genetic linkage map DL-788-2 (early maturity) X GW-322 (late maturity) for cultivated bread wheat. The linkage map was constructed using software IciMapping v.4.1 (Meng *et al.*, 2015).A total of 22 polymorphic markers were integrated into four linkage groups (LGs) with a total map length of 267.12 cM which was constructed using data from 22 marker loci for 74 F_2 progenies. The map lengths of individual linkage groups ranged from a minimum of 8.62 cM (LG4) to maximum of 126.56 cM (LG1), as shown in Fig. 2.

A linkage map of 267.12 cM (Kosambi) was constructed using 22 SSR markers loci spread on four linkage groups in the present study. Gorji *et al.*, (2014) constructed a linkage map of 224 cM from 22 well-distributed SSR markers in wheat. Wu Hong *et al.*, (2015) constructed high-density genetic linkage map in the wheat population (Yanda 1817 × Beinong) and reported genetic coverage of each chromosome which varied from 19.1 cM to 292.9 cM with 150 polymorphic markers in 269 F_8 to F_{12} recombinant inbred lines (RILs) derived fromYanda1817x Beinong by single seed descent procedure.

The complete linkage map consisted of total 22 molecular markers in present investigation distributed on four linkage group with a total length of map accounted 267.12 cM. The total marker number was highest in linkage group 1 (10 loci) with total map length of this linkage group was 126.56 cM. Linkage group 4 has the lowest number of markers (2 loci) and lowest map length (8.62 cM) in the present study. None of the polymorphic markers remained unlinked, shorter map distance was observed in present study might be due touse of only single molecular markers (SSR markers).

Table.1 Chi-square tests for 22 SSR markers used to discriminate 74 F_2 equivalents to P_1, P_2, and F_1

Sr. No	Marker Name	Position	hmzA	htz	HmzB	Missing Marker	Chi-Square	Pr>ChiSq	Degree of Dominance
1	Xgwm337	0.00	19	40	15	0	0.9189	0.6316	Codominant
2	Xgwm106	5.69	22	37	14	1	1.7671	0.4133	Codominant
3	Xgwm136	18.52	23	37	14	0	2.1892	0.3347	Codominant
4	Xgwm33	28.62	19	37	18	0	0.0270	0.9866	Codominant
5	Xgwm642	42.90	22	40	12	0	3.1892	0.2030	Codominant
6	GPW4431	60.94	19	37	18	0	0.0270	0.9866	Codominant
7	Xbarc240	79.63	22	35	17	0	0.8919	0.6402	Codominant
8	Xgwm99	92.11	21	40	13	0	2.2162	0.3302	Codominant
9	Xgwm259	96.27	19	40	15	0	0.9189	0.6316	Codominant
10	Xgwm18	126.56	26	32	16	0	4.0541	0.1317	Codominant
11	Xgwm55.2	0.00	23	36	15	0	1.7838	0.4099	Codominant
12	Xgwm484	4.87	20	39	15	0	0.8919	0.6402	Codominant
13	Xgwm148	12.71	22	40	12	0	3.1892	0.2030	Codominant
14	Xgwm566	0.00	20	38	16	0	0.4865	0.7841	Codominant
15	Xgwm389	8.61	21	38	15	0	1.0270	0.5984	Codominant
16	GPW4225	20.41	21	36	17	0	0.4865	0.7841	Codominant
17	Xgwm162	37.12	20	38	16	0	0.4865	0.7841	Codominant
18	Xgwm533.1	59.75	23	36	15	0	1.7838	0.4099	Codominant
19	Xwmc513	92.33	25	32	17	0	3.0811	0.2143	Codominant
20	Xgwm583	119.23	20	37	17	0	0.2432	0.8855	Codominant
21	Xgwm194	0.00	18	42	14	0	1.7838	0.4099	Codominant
22	Xgwm608	8.62	20	40	12	0	3.1892	0.2030	Codominant

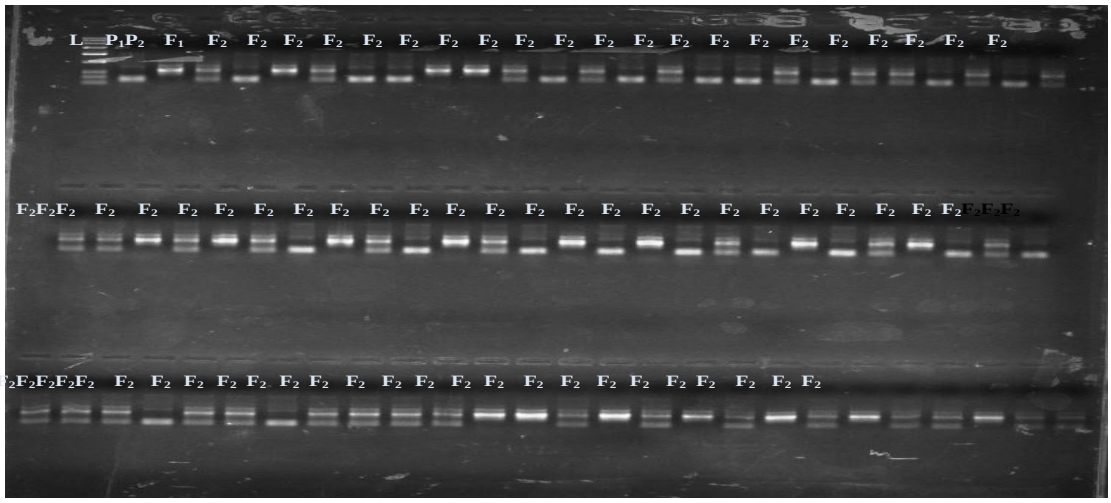
hmzA= Homozygous for P_1,hmzB= Homozygous for P_2, htz=Heterozygous F_1 .
*,** Significant at 5% an 1% levels respectively

Table.2 QTL identification for earliness and related traits with LOD score, PVE (%), additive and dominance effect

Sr No	Trait Name	LG	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
1	Days 50%flowering (DF)	1	58.00	Xgwm642	GPW4431	3.06	18.7	-2.72	3.48
2	Days to maturity(DTM)	1	21.00	Xgwm136	Xgwm33	8.89	31.5	2.39	0.59
		3	38.00	Xgwm162	Xgwm533	12.83	45.1	2.77	1.24

Fig.1 Agarose gel from genotyping of the SSR loci (A) Xgwm 106, (B) Xgwm 259 markers differing in size of PCR-amplified DNA in individual F_2plants

(A)



(B)

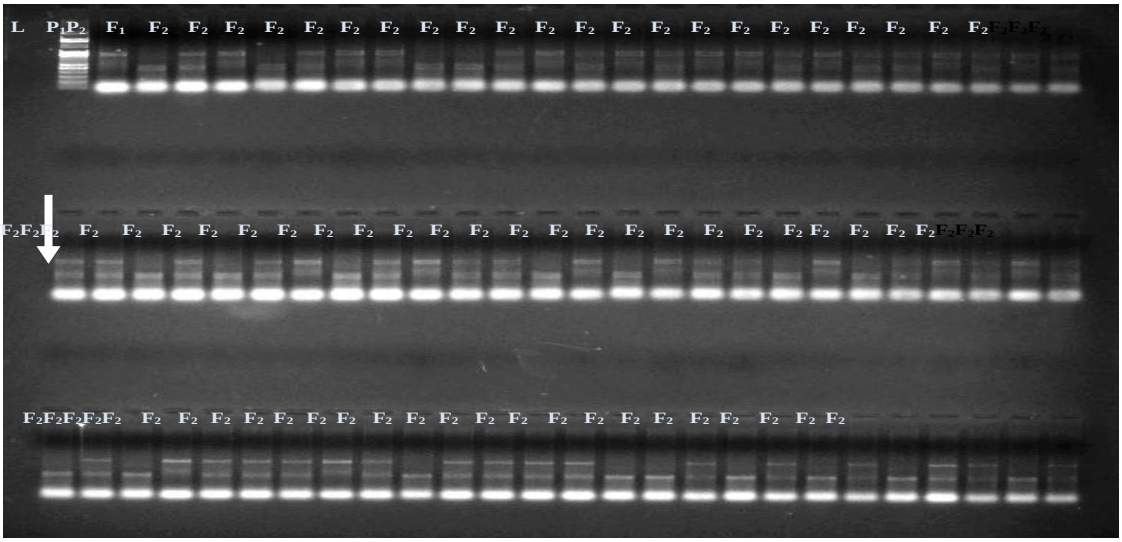


Fig.2 Genetic linkage group of bread wheat (LG-1) to (LG-4) indicates marker position on chromosome NO.1 to 4, respectively

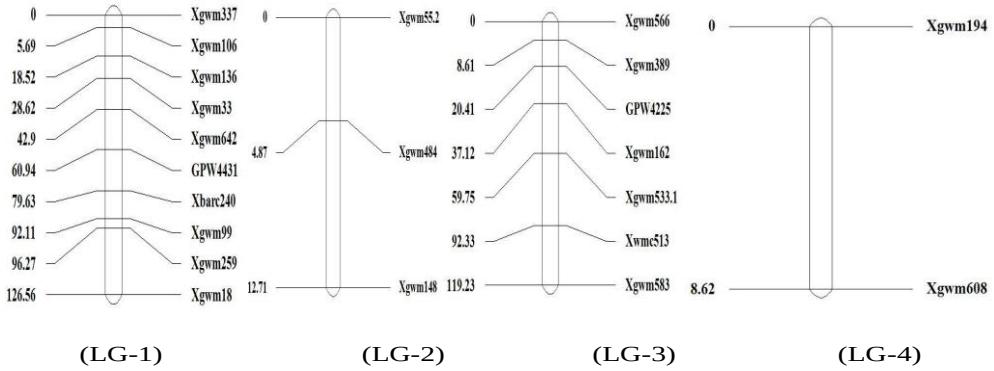


Fig.3 Position of earliness and related traits in the whole genome with LOD score

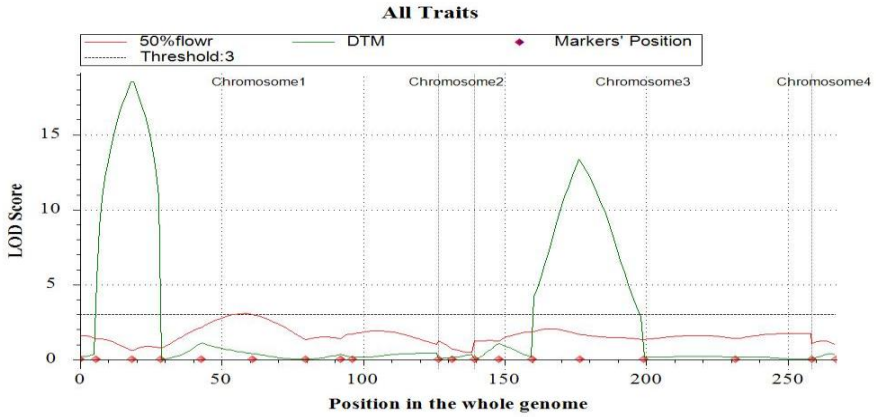
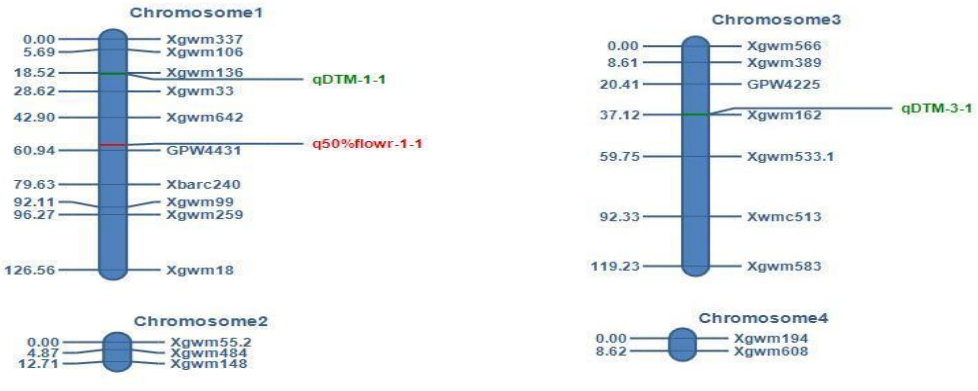


Fig.4 Position of earliness and related QTL in whole genome



Other alternative reasons could be the sizes of the mapping populations, genetic constitution of parental lines, and number and polymorphism of marker loci obtained for both parental lines.

QTL mapping for earlines and related traits

Genotypic data of 74 F_2 and phenotypic data obtained on 74 $F_{2:3}$ lines of the mapping population were analyzed for identification of the main effect QTLs using the software ICIM-ADD mapping in QTL IciMappingV4.1 (Meng *et al.,* 2015). The 267.12 cM linkage map constructed using Kosambi mapping function for 74 F_2 progenies from the cross DL-788-2 (early maturity) x GW-322 (late maturity).QTL analysis was done for phenotypic data using day to 50% flowering and days to maturity collected from Wheat Research Station, Junagadh Agricultural University, Junagadh. QTL Ici Mapping was used for constructing linkage map was also used for QTL mapping. A linkage map output data file was used for the construction of QTL mapping. Overall, one QTL was identified (Table 2) for day to 50% flowering on chromosome 1 and two QTL for day to maturity on chromosome 1 and 3 (Fig. 3 and 4). Many previous studies were done on QTL mapping for day to 50% flowering traits which supported similar results of the present study. *viz.,* Zou *et al.,* (2017) identified QTL position for days to 50% flowering on chromosome 4 named as QFlt. dms-4B, QFlt. dms-4B, QFlt. dms-4B with LOD score 3.0, 2.5 and 2.5, respectively with an additive effect of -0.6, 0.9, 0.9. Another study done by Nguyen *et al.,* (2015) identified QTL for days to 50% flowering on chromosome 4 with LOD score of 3.6 and the additive effect of -7.18.QTL mapping for days to maturity in the present study were supported by the findings. Fatima *et al.,* (2014) they identified two QTL named as QDPM.S.IM.wwc-2D.1 on chromosome 2 with LOD scores 8.68, the additive effect of 4.20 as well as another QTL named as QDPM.C.IM.wwc-6A.7 on chromosome 6 with LOD 4.45 score, the additive effect of 5.94.

In conclusion the most agricultural traits of economic interest are polygenic and quantitative in nature and are controlled by many genes on the same/different chromosome. In wheat earliness is agronomically important trait. Earliness and related character is controlled by several minor genes and they were assigned to different chromosomes.QTL mapping is used to detect the genes which control the trait of interest. It is very useful for the genome-wide scan for QTLs detection in plants. Identification of marker which gives clear polymorphism, development of linkage map and detection of new QTLs associated with earliness should be useful for wheat improvement in the future, especially as these QTLs appear to have relatively large effects. Ideally QTL associated with earliness found at chromosome number 1,3 and the markers attached to the QTL after validation have the potential to be used for marker assisted selection in wheat breeding programs.

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