J Genet Resour2021;7(1): 72-86 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2021.20190.1226*

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Quantitative Genes Controlling Chlorophyll Fluorescence Attributes in Barley (*Hordeum vulgare* L.)

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ARTICLE INFO

Article history:
Received 28 August 2020
Accepted 31 October 2020
Available online 15 November 2020

Keywords:
Barley
Chlorophyll fluorescence
Hordeum vulgare L
Molecular markers
QTL

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p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Chlorophyll fluorescence is one of the very useful techniques in plant physiology because of the ease with which the user can gain detailed information on the state of photosystem II (PSII) at a relatively low cost. Detection of quantitative traits loci related to chlorophyll fluorescence have a major role in understanding the genetic mechanisms of photosynthesis. In the present research, to mapping, the genome regions controlling chlorophyll fluorescence traits, barley (*Hordeum vulgare* L) from 106 F_8 recombinant inbred lines caused by crossing two cultivars of Badia × Kavir was used and these lines were cultured in a complementary randomized design with two replications. Traits studied include ABS/CS_o, TR_o/CS_o, DI_o/CS_o, ABS/CSm, DI_o/CSm, psi (E_o), TR_o/RC, RE_o/RC, ABS/RC, DI_o/RC, Area, Fv/Fm, Sm. Linkage maps were prepared using 152 SSR polymorphic markers, 72 ISSR, 7 IRAP, 29 CAAT, 27 Scot, and 15 iPBS alleles. Molecular markers were assigned on 7 chromosomes of barley. The linkage map covered 999.2 cM of the barley genome and the average distance between two flanking markers was 3.387 cM. Three major QTLs were identified for Area, psi (E_o), and Di_o/Rc on Chromosome 6 between ISSR31-1-Bmag0867 in position 62 Centimorgan that explained 17.2%, 31.5%, and 15.9%, respectively. Also, another colocation was detected for ABS/CS_o, TR_o/CS_o, ABS/CSm, and DI_o/CSm QTLs on chromosome 6 in position 72 Centimorgan. The results obtained in the present research provide valuable information on the genetic basis of the Chlorophyll fluorescence parameters that can be used in the barley breeding program, including marker-assisted selection.

Please cite this paper as: Makhtoum S, Sabouri H, Gholizadeh A, Ahangar L, Katouzi M. 2021. Quantitative genes controlling chlorophyll fluorescence attributes in barley (*Hordeum vulgare* L.). *J Genet Resour* 7(1): 72-86. doi: 10.22080/jgr.2021.20190.1226.

Introduction

Barley (*Hordeum vulgare* L.) is considered one of the four most important grains in the world. This cereal has a very long history in most of its producing countries and since ancient times, its seeds, in addition to being used in human nutrition, are used in confectionery and its malt is also used in industry and pharmacy (Khodarahmi *et al*., 2006). Chlorophyll fluorescence is a method to utilize excitation energy in photosynthesis, which is widely used in photosynthesis research. Chlorophyll fluorescence is also used to determine the physiology of the plant and the degree of damage to the photosynthetic apparatus (Hakam *et al*., 2000). Although this index is easy to measure, it requires special care. Also, it does not cause the degradation of plant tissue. Chlorophyll fluorescence is also an indicator of excitation of energy in leaf photosynthetic structures and a non-destructive diagnostic system for determining plant resistance to environmental stresses (Percival and Henderson,

2003). The parameters of chlorophyll fluorescence consist of three parts: the parameters related to light absorption (ABS), excited energy trapping (TR), and excited energy transfer to the electron transfer chain (ET) through the Reaction Center (RC), genetic differences between different plant lines affect the physiological parameters of this photosystem (Strasser *et al*., 2004).

Fo and Fm indices are important parameters that are measured for comparative evaluation of other fluorescence parameters. Although these two parameters form the basis for calculating other fluorescence variables, their value is variable. Increasing Fo and decreasing Fm indicate damage to the transfer of photons absorbed from antennas to reaction centers (Schreiber *et al*., 1998). It has been reported that Fv has a positive correlation with performance in a high temperature, but in controlled conditions, it shows a negative correlation with grain yield (Moffatt *et al*., 1990). The amount of chlorophyll fluorescence indicates the health of the thylakoid membrane and the relative efficiency of electron transfer from photosystem II to photosystem I. When the Quinone molecules of the photosystem II electron receiver are in the fully oxidized state of the photosystem (II) reaction center, the system has the lowest fluorescence (Fo), which as it gradually increases with the revival of this molecule fluorescence increases. This process continues until the complete reduction of its molecules. In such a case, the center of the photosystem is in a state of complete regeneration and has the maximum fluorescence (Fm). On the other hand, with increasing light intensity, the photosynthetic system is induced by a regulatory method to reduce the energy (Induced energy loses excess energy as a non-radiative process by increasing non-photochemical extinction. This regulatory mechanism, while protecting the reaction center, causes the least damage to this center (Behra *et al*., 2002).

In the study of the population of barley cultivars, Strepto and Morx for Fv, Fo, and Fv/Fm were determined as 2, 3, and 2 gene places, respectively which explained 81.50, 29.81, and 63.39% of variations of phenotypes (Aminfar *et al*., 2011). To identify quantitative sites related to chlorophyll fluorescence, 228 recombinant inbred lines of maize were examined (Yin *et al*., 2015) (ABS/CS_o), (ET_o/CS_o) and (RCs) were measured. The three main genomic regions on chromosomes 1, 5, and 9 were related to chlorophyll-dependent parameters. Fakheri and Shahraki (2016) determined the genomics loci that control some physiological traits in double haploid populations caused Steptoe and Morex cross. A total of 13 QTLs were detected for the studied traits. The phenotypic variances explained by these QTLs ranged from 9.06 to 30.28% for minimum fluorescence (qFO1m) and maximum photosystem II efficiency, respectively. The QTL locus of 81.2 cm on chromosome 2 was stable for maximum photosystem II efficiency, and they suggested that if the above results were repeated in more environments.

Guo *et al*., (2008) identified a total of nine and five barley genomic regions, under well-watered and drought stress conditions, respectively, that were significantly associated with the expression of the five target traits at the post-flowering stage. No common QTL was detected except one for chlorophyll content, which was identified in both growth conditions, demonstrating that the genetic control of the expression of the traits related to photosynthesis differed under different water conditions. In this study, a QTL for Fv/Fm, which is related to the drought tolerance of photosynthesis was identified on chromosome 2H at 116 cM in the linkage map under drought stress. This QTL alone explained more than 15% of the phenotypic variance of the maximum quantum yield of PSII.

Bertholdsson *et al*., (2015) detected QTLs of chlorophyll fluorescence parameter quantum yield (QY) of electron transport of PSII from leaves of hypoxia-stressed in barley. Five quantitative trait loci (QTL) for QY, with a narrow-sense heritability of 0.87, were identified on chromosomes 4, 6, and 7H. They had additive effects ranging from 0.74 to 1.35 % with LOD scores from 3 to 12 and explained variance from 6 to 29 %. QTL mapping of fluorescence chlorophyll attributes was done in the other research (Czyczyło-Mysza *et al*., 2013 in wheat; Azam *et al*., 2015 in wheat; Bhusal *et al*., 2018 in wheat; Mathur *et al*, 2011 in wheat; Zheng-Bin *et al*, 2010 in wheat; Yin *et al*., 2010 in soybean; Kiani *et al*., 2008 in sunflower;

Christen *et al*., 2007 in grapevine). Detected QTLs dependent on chlorophyll parameters will help elucidate plant responses to environmental changes and help develop plant breeding programs. Since chlorophyll fluorescence represents the capacity of plants to convert light energy into biochemical energy during photosynthesis, as well as non-destructive measurement and measurement speed. With the help of portable equipment, in this study, their controlling QTLs in the barley were detected in the F8 population resulting from the crossing of Badia × Kavir cultivars.

Materials and Methods

To identify QTLs controlling chlorophyll fluorescence in barley, 106 samples of F8 families from crosses of two cultivars Badia × Kavir were used. Outstanding features of Badia parent include higher yield, susceptibility to lodging and parent of Kavir, lower yield, and tolerance to lodging (Kavianicharati *et al*., 2016). The research was conducted at Gonbad Kavous University in 2019. Seeds of 106 lines, as well as parents, were planted in pots. First, the seeds were placed in 5 kg pots in the greenhouse to plant the seeds, then the farm soil was poured in equal amounts in the pots. The physical and chemical properties of soil were measured including, soil texture by hydrometric method (Bouyoucos, 1962), pH and EC in saturated extract (pH, EC in saturated extract) (Haluschak, 2006), percentage of carbon organic carbon based on Walkley and Black Method (Walkley and Black, 1934) equivalent calcium carbonate by hydrochloric acid neutralization (Allison and Moodie,1965). Total nitrogen in the soil by total nitrogen digestion method (Bremner and Mulvaney,1982), phosphorus extractable with 0.5 M sodium bicarbonate by Olsen method (Sparks *et al*., 2020), absorption of potassium was recorded by extraction with normal ammonium acetate were recorded (Table 1).

Table 1. Soil physical and chemical properties of the experiment site (0-30 cm depth).

Sand (%)	Silt (%)	Clay (%)	Potassium (ppm)	Phosphorus (ppm)	N (%)	Organic carbon (%)	Neutral substances (%)	pH	EC (dS/m)
1.19	7.6	9.5	0.90	0.09	11.4	316	29	58	13

And seven seeds were placed in each pot and field operations were applied during plant growth. Chlorophyll fluorescence was measured on a flag leaf using a portable fluorometer (PEA; Hansatech Instrument, King's Lynn, UK). Fluorescence activating light was provided by an array of three light-emitting diodes focused on the leaf surface to produce homogeneous lighting. All measurements were performed on the upper surface of the flag leaves using leaf clips. All samples were adapted to darkness for 10 minutes before measurements. Traits related to chlorophyll fluorescence were recorded as follows: minimum fluorescence yield of PS II (Fo), variable chlorophyll fluorescence yield (Fv), maximum fluorescence yield of PS II (Fm), maximum quantum yield of primary photosystem II (Fv/Fm), lateral reactivity of PSII (Fv/Fo), total complementary area between fluorescence induction (OJIP) curve and the line F = FM (Area), normalized total complementary area above the OJIP transient or total electron carriers per RC (Sm), phenomenological electron transport flux per excited CSo (ET_o/CS_o), absorbed energy flux per cross-section (ABS/CS_o), Phenomenological trapping flux per excited CSo (TR_o/CS_o), Phenomenological dissipated energy flux per excited CSo (DI_o/CS_o), phenomenological absorption flux per excited cross-section CSm (ABS/CSm), penomenological dissipated energy flux per excited CSm (DI_o/CSm), trapped energy flux per excited cross section (TR_o/CSm), electron transport flux percs (ET_o/CSm), the flux of electrons from QA- to final PSI acceptors per cross section of PSII at maximum time (RE_o/CSm), trapping flux per RC (TR_o/RC), electron flux reducing end electron acceptors at the photosystem I acceptor side per RC (RE_o/RC), absorption flux of antenna chlorophylls per RC (ABS/RC), electron transport flux per RC (ET_o/RC), dissipation energy flux per active reaction center at t = 0 (DI_o/RC), quantum yield consequent of the reduction in end electron acceptors of photosystem I (psi(E_o)), primary photochemical quantum performance (phi(P_o)), relative variable fluorescence at phase J of the fluorescence

induction curving in 2 ms (vj), relative variable fluorescence at phase I of the fluorescence induction curve in 30 ms (vi), initial slope of relative variable fluorescence (Dv/dt_o), expresses the excitation (DVG/DT_o) (Strasser and Tsimilli-Michael, 1998; Lazár and Pospíšil, 1999; Strasser *et al*., 2000).

Genotype evaluations

Four hundred-microliter of extraction buffer was added to ground leaf samples (50 mM - HCl, pH 0.8, 2.5 mM EDTA, 300 mM NaCl and 1% SDS) for extraction. The contents were centrifuged for 12 min at 12,000 g/g per µl, and approximately 400 µl of the resulting lysate was formed with 400 µl of chloroform. The supernatant above the water was transferred to another 1.5 ml tube and the DNA was precipitated with ethanol. The contents were then centrifuged at full speed for 3 min and the supernatants were discarded. The DNA strand was washed with 70% ethanol and then exposed to air and dried in 50 µl of TE buffer (10 mMTris-HCl, pH 0.8, 1 mM EDTA, pH 0.8) and was suspended. The 365 SSR markers that were appropriately distributed on 7 chromosomes in the barley, according to (Li *et al*., 2003, Ramsay *et al*., 2000; Struss and Plieske, 1998; Varshney *et al*., 2007; Marcel *et al*., 2007 and Thiel *et al*., 2003) were elected. These SSR primer pairs were examined for polymorphism between two parents, and polymorphic primers were used to amplify the DNA of each plant from the RIL population. 152 polymorphic SSR markers were used to prepare the primary map. To amplify the DNA, 50 ng DNA, 0.67 M primers, 10 M reaction buffer, 2.5 M $MgCl_2$, 0.2 M dNTP, 0.5 units of Taq DNA polymerase, with water deionized twice (deionized) to a volume of 15 µl for SSR markers were used based on 30 cycles as follow: denaturation at 94 °C for 1 min, binding at 58 °C for 1 min, elongation at 72 °C for 1.5 min, final extension at 72 °C for 5 min. The polymerase chain reaction was performed by a thermocycler (iCyclerBIORAD, USA). The amplified product was isolated on polyacrylamide gel electrophoresis 6 GE (PAGE) and made visible by silver staining (Xu *et al*., 2002). For saturation of primary maps of markers, iPBS (Kalendar *et al*., 2010), IRAP (Kalendar *et al*., 1999; Boronnikovaa and Kalendar, 2010), ISSR (University of British Columbia - UBC), ScoT (Collard *et al*., 2009), CAAT (Singh *et al*., 2014), were used. To investigate the polymorphism of the above markers in parents, 21 IPBS markers, 8 IRAP markers, 15 SCoT markers, 15 CAAT markers, and 1 combination of ISSRiPBS markers were used, and finally 7 IRAP markers, 29 markers. CAAT, 27 SCoT markers, 72 ISSR markers, 15 IPBS markers, and 5 combinations of ISSRiPBS polymorphic alleles were used to establish genetic linkage. For genetic mapping, a score of 1 (for male parent band) and 2 (for female parent band) in SSR markers were used. For ISSR, iPBS, IRAP, SCoT, and CAAT markers, scores of 1 (for the presence of the band) and 3 (for the absence of the band) (for the absence of the band) for when the band was observed in the paternal parent and also for the score (score 2) (for the presence of the band) and 4 (for the absence of the band) were also used when the band was present in the parent. It should be noted that the connection of randomized markers to microsatellite markers for chromosomes was done separately. Polymorphic bands for each of the primers iPBS, IRAP, ISSR, SCoT, CAAT are numbered in descending order of molecular weight (from top to bottom of the gel) (from top to bottom of the gel).

Linkage map construction and QTL analysis

The IPBS, IRAP, ISSR, SCoT, CAAT, and polymorphic SSR markers were evaluated using the QGene program individually with χ^2 test for 1: 1 segregation ratio at the probability level of 0.01. (Nelson, 1997). Map preparation was done with software map manager QTX17 (Manly and Olson, 1999). Assignment of linkage groups to relevant chromosomes was performed based on Li *et al*., 2003, Ramsay *et al*., 2000; Struss and Plieske 1998; Varshney *et al*., 2007; Marcel *et al*., 2007 and Thiel *et al*., 2003. Map distances (SantiMorgan) were carried out based on Kosambi, 1994 function, and critical LOD threshold 3 was used to determine linkage groups. QGENE 4.0 (Nelson, 1997) was used to find QTLs. CIM method was used to determine QTLs and estimate their effects (Estimation of effects) and the point with the highest LOD was identified as the region with the highest probability of QTL.

Results

Phenotypic evaluation

Phenotypic evaluation of the studied traits indicated the existence of continuous quantitative variation (Fig. 1). For all of these traits, values higher than the parent with the maximum value of the trait and less than the parent with the minimum trait were observed.

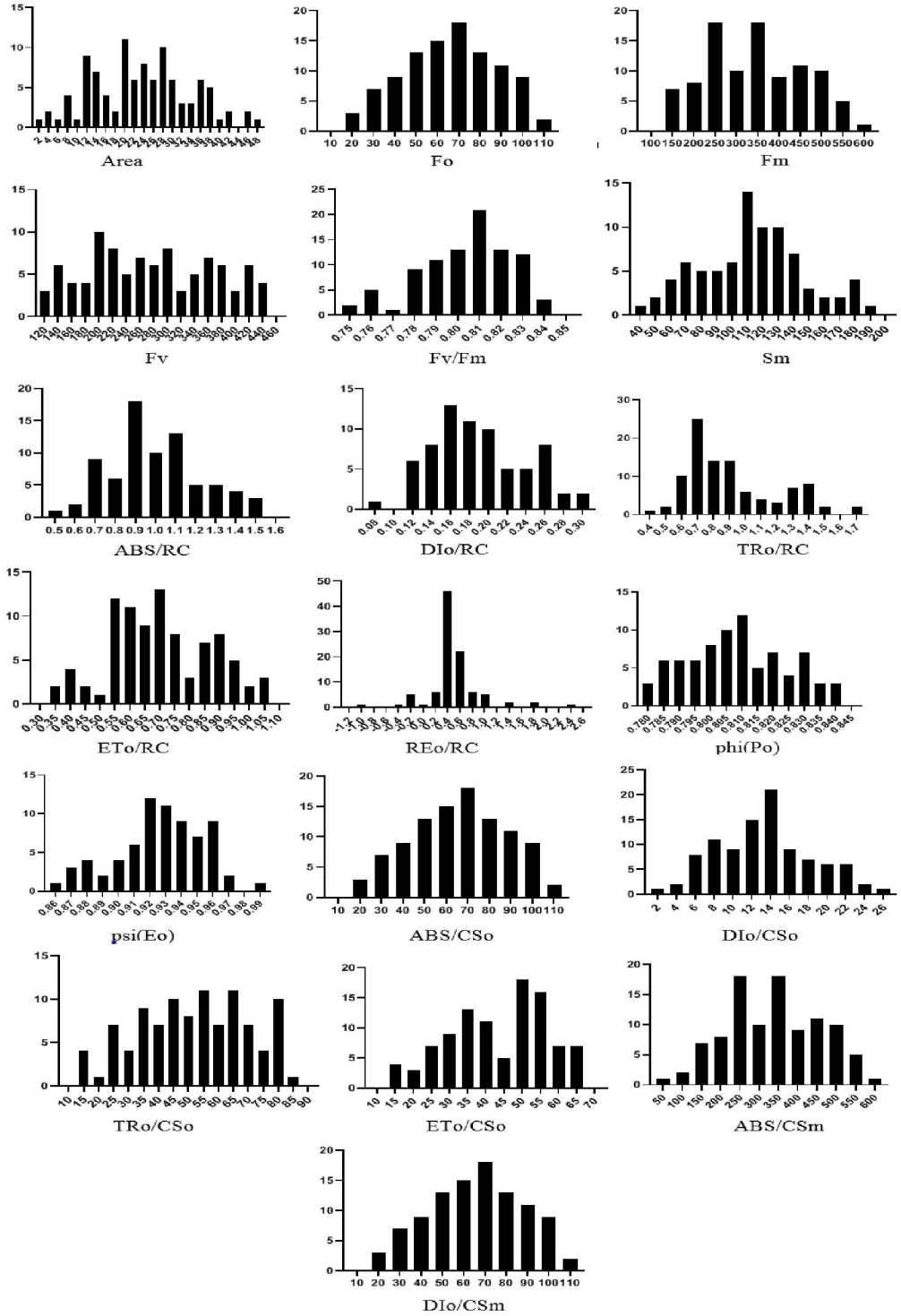

Fig. 1. Histogram for chlorophyll fluorescence parameters in RIL Population-based on Kavir and Badia Cross.

The correlation results between the parameters in Table 2 showed that the maximum fluorescence (Fm) with the parameters Fv, ABS/CS_o, DI_o/CS_o, TR_o/CS_o, ET_o/CS_o, ABS/CSm, and DI_o/CSm has a positive correlation but has a negative and significant correlation with Sm and PSI (E_0) parameters. Minimum fluorescence (Fo) was also positively and significantly correlated with Fm, Fv, ABS/CS_0, DI_0/CS_0, TR_o/CS_o, ET_o/CS_o, ABS/CSm, and DI_o/CSm parameters, but with Sm parameters. RE_o/RC and PSI (E_o) had a significant negative correlation. Variable fluorescence (Fv) had a positive and significant correlation with ABS/CS_o, DI_o/CS_o, TR_o/CS_o, ET_o/CS_o, ABS/CSm, and DI_o/CSm parameters, but a negative correlation with Sm and PSI (E_o) parameters was found. Maximum photochemical productivity (Fv/Fm) had a strong and significant correlation with the phi (P_o) parameter (Fig. 2).

Cluster analysis divided the studied lines into three groups based on all parameters. In the first group, genotypes 45, 87, 80, 81, 94, 77, 88, 62, 90, 21, 56, 35, 64, 36, 93, 91, 26, 102, 32 and 63 were in the second group of genotypes 27, 59, 85, 34, 58, 92, 68, 76 and 75 and finally the third group of genotypes 30, 57, 95, 78, 33, 71, 67, 79, 98, 69, 103, 84, 31, 89, 99, 96 and 97 were recorded (Fig. 3). The difference between the groups created in cluster analysis for chlorophyll fluorescence traits was significant so that Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root indices to 1.435, 040 0.03, 12.038, and 10.961 were significant at the 1% probability level (Table 2).

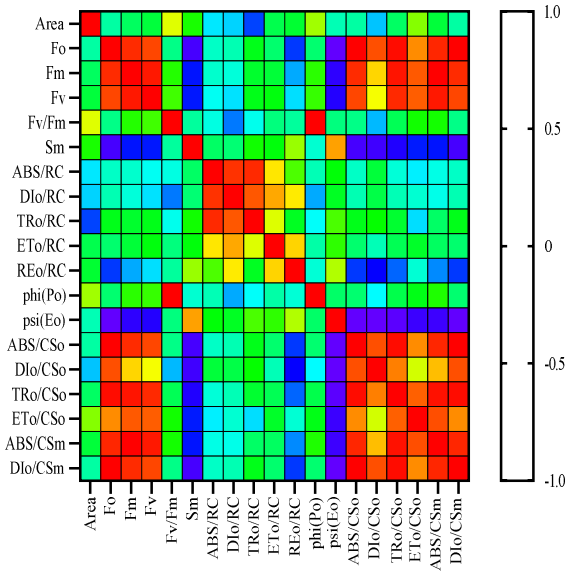

Fig. 2. Correlation diagram for chlorophyll fluorescence parameters in RIL Population caused Kavir and Badia Cross. The red to yellow color spectrum indicates a correlation between 1 and 0.5. Next, the green color spectrum indicates a correlation between 0.5 and 0. Also, the light blue spectrum indicates a correlation between 0 and -0.5. Finally, the dark blue to navy blue color spectrum indicates a correlation between -0.5 and -1.

There was a significant difference at the level of 0.01 between groups created in terms of parameters Area, Fo, Fm, Fv, Sm, ABS/RC, DI_o/RC, ABS/CS_o, DI_o/CS_o, TR_o/CS_o, ET_o/CS_o, ABS/CSm, DI_o/CSm, But the difference for Fv/Fm, TR_0/RC, ET_0/RC, RE_0/RC, PHI (P_0), PHI (E_0) parameters was significant at the level of 5% (Table 2).

Table 2. Comparison mean of three groups for chlorophyll fluorescence parameters caused cluster analysis

Cluster	Area	Fo	Fm	Fv	Fv/Fn	Sm	ABS/RC	DI_o/RC	TR_o/RC
Cluster 1	28.471^{b}	65.712^{b}	337.476^{c}	271.763^{c}	0.805^{b}	105.175^{b}	0.986^{a}	0.192^{a}	0.749^{ab}
Cluster 2	27.854^{b}	49.454^{c}	252.790^{b}	203.335^{b}	0.803^{b}	140.130^{a}	1.049^{a}	0.207^{a}	0.842^{a}
Cluster 3	35.102^{a}	79.058^{a}	441.823^{a}	362.764^{a}	0.819^{a}	99.421^{b}	0.859^{b}	0.154^{b}	0.704^{b}
Cluster 1	0.727^{ab}	0.497^{b}	0.805b	0.913^{b}	65.712^{c}	12.864^{a}	52.847^{b}	48.191^{b}	337.47b	65.712^{b}
Cluster 2	0.788^{a}	0.587^{a}	0.803b	0.935^{a}	49.454^{b}	9.741^{b}	39.713^{c}	37.048^{c}	252.79c	49.454^{c}
Cluster 3	0.637^{b}	0.420^{a}	0.819a	0.903^{b}	79.058^{a}	14.233^{a}	64.824^{a}	58.370^{a}	441.82a	79.058^{a}

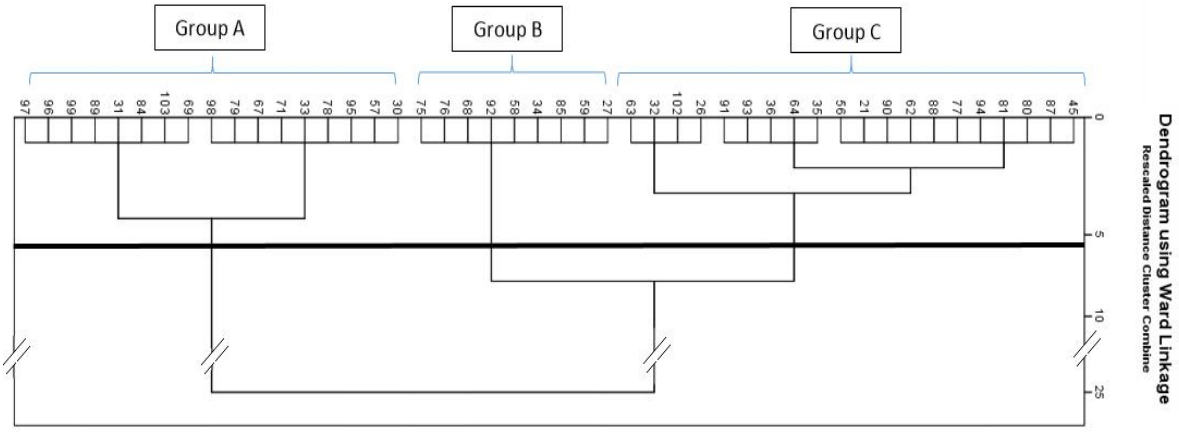

Fig. 3. Dendrogram of clustering barley lines based on chlorophyll fluorescence parameters in RIL population caused Kavir and Badia Cross.

The third group in terms of parameters Area, Fo, Fm, Fv, Fv/Fm, PHI (P_0), ABS/CS_o, DI_o/CS_o, TR_o/CS_o, ET_o/CS_o, ABS/CSm, and DI_o/CSm more value than group one. But the second group of Sm, ABS/RC, DI_o/RC, TR_o/RC, ET_o/RC, RE_o/RC, and PHI (E_o) parameters had more value than the first and third groups (Table 2).

Genotypic evaluation

Linkage mapping was done using 152 SSR markers, 72 ISSR alleles, 7 IRAP alleles, 29 CAAT alleles, 27 Scot alleles, and 15 iPBS alleles. Mendelian ratio 1: 1 for all amplified alleles was examined using the Chi-square test and non- Mendelian transgressive segregation markers were not used for mapping. Molecular markers used were assigned to 7 linkage groups according to the 7 chromosomes of the barley. Molecular markers on 7 chromosomes of barley were assigned. The resulting map covered 999.2 cM of the barley genome and the average distance between two flanking markers was 3.387 cM (Fig. 4). For Area, five QTLs were detected on Chromosomes 4 (QTL2), 6 (QTL2), and 7. The QTLs detected on chromosome 4 were in positions 58 and 140 cM with LODs of 3.818 and 3.342, respectively, and were located between markers EBmac0635-scssr14079 and MGB84-Bmac0144. qAREA-4a and qAREA-4b reduced the Area value by -4696.396 and -4837.759 and were transferred from Kavir parent to progenies. The mentioned QTLs were 1.6% and 14.1% of phenotypic variation. QTLs were detected on chromosome 6 at positions 62 and 74 cM with LODs of 4.141 and 4.31 and were located between ISSR31-1-Bmag0867 and MGB84-Bmac0144 markers. qAREA-6a and qAREA-6b reduced the Area value by -7313.787 and -4837/546, respectively, and were transferred from Kavir parent to the progenies. These QTLs explained 17.2% and 17.8% of the phenotypic changes of this parameter and had the role of large QTLs. The QTL detected on chromosome 7 was in position 98 with LOD = 3.346 and between the markers, Bmag0135-scssr07970 and qAREA-7 reduced the area value by -5030.259 and moved from Kavir's parent were transferred to progenies, this QTL explained 14.1% of the phenotypic variation of the Area (Table 3). For ABS/CS_o, a QTL on chromosome 6 at positions of 72 cM with LOD = 3.151 was detected between Bmag0867-HVM65 markers. $QABSCS_0$-6 had an additive effect and from parent Badia was transferred to progenies and while increasing the value of ABS/CS_o 9/063, it explained 13.5% of ABS/CS_o phenotypic variation. For DI_o/CS_o a QTL was detected on chromosome 6 at position 70 with LOD = 3.962 and placed between Bmag0867-HVM65 markers. This QTL explained 1.7% of the DI_o/CS_o phenotypic variations and increased the DI_o/CS_o value by 2.739. For TR_o/CS_o, a QTL with LOD = 7.48 on chromosome 6 was detected in the position of 72 cM and was located between the Bmag0867-HVM65 marker and explained 13.9% of the TR_o/CS_o phenotypic variations. The expression of the qTR_oCS_o-6 increasing allele was transferred from parent kavir to progenies and increased TR_o/CS_o by 3.287. A QTL for ABS/CSm was located on chromosome 6 with LOD = 3.253 at position 72 cM between Bmag0867-HVM65 marker and explained 13.9% ABS/CSm phenotypic variation.

Table 3. QTLs Detected for chlorophyll fluorescence parameters in a RIL Population Obtained from Kavir and Badia Cross.

Parameters	QTL	Chr	Position	Flanking markers	Distance to closer marker	LOD	Add effect	R^2	Allele Direction
Area	qAREA-4a	4	58	EBmac0635-scssr14079	0.88(EBmac0635)	3.818	-4696.396	1.6	KAVIR
	qAREA-4b	4	140	MGB84-Bmac0144	0.72(MGB84)	3.342	-4837.759	14.1	KAVIR
	qAREA-6a	6	62	ISSR31-1-Bmag0867	1.77 (ISSR31-1)	4.141	-7313.787	17.2	KAVIR
	qAREA-6b	6	74	HVM65-EBmac0874	1.26(EBmac0874)	4.31	-5693.546	17.8	KAVIR
	qAREA-7	7	98	Bmag0135- scssr07970	2.15(Bmag0135)	3.346	-5030.259	14.1	KAVIR
ABS/CS_o	$qABSCS_o$-6	6	72	Bmag0867-HVM65	0.33(HVM65)	3.151	9.064	13.5	BADIA
DI_o/CS_o	q DI_o CS_o-6	6	70	Bmag0867-HVM65	2.33(HVM65)	3.962	2.739	1.7	BADIA
TR_o/CS_o	q TR_o CS_o-6	6	72	Bmag0867-HVM65	0.33(HVM65)	7.48	3.287	13.9	KAVIR
ABS/CSm	qABSCSm-6	6	72	Bmag0867-HVM65	0.33(HVM65)	3.253	49.673	13.9	KAVIR
DI_o/CSm	q DI_o CSm-6	6	72	Bmag0867-HVM65	0.33(HVM65)	3.151	9.064	13.5	BADIA
TR_o/RC	qTR_oRC-6	6	74	HVM65-EBmac0874	1.26(EBmac0874)	3.64	0.157	14.7	KAVIR
RE_o/RC	qREoRC-1	1	22	CAAT2-B-Scot5-C	2.36(Scot5-C)	3.044	-456.693	12.8	KAVIR
	qRE_oRC-6	6	64	ISSR31-1-Bmag0867	3.77 (ISSR31-1)	3.127	-0.336	13.2	KAVIR
psi(E_o)	q psi E_o-1	1	24	CAAT2-B-Scot5-C	0.36(Scot5-C)	3.239	-604.77	13.5	BADIA
	q psi E_o-3	3	46	HVM33-HVM44	0.98(HVM44)	4.273	-0.095	17.4	BADIA
	q psi E_o-6	6	62	ISSR31-1-Bmag0867	1.77 (ISSR31-1)	8.478	-0.171	31.5	BADIA
	q psi E_o-7	7	98	Bmag0135- scssr07970	2.15(Bmag0135)	3.629	-0.088	1.5	BADIA
Fv/Fm	qFvFm-1	1	22	CAAT2-B-Scot5-C	2.36(Scot5-C)	3.107	-47.466	1.3	BADIA
	qFvFm-6	6	62	ISSR31-1-Bmag0867	1.77 (ISSR31-1)	3.007	-0.038	12.6	BADIA
Sm	qSm-6	6	62	ISSR31-1-Bmag0867	1.77 (ISSR31-1)	3.3	-33.027	13.7	BADIA
ABS/RC	q ABSRC-7	7	100	scssr07970-HVCMA	1.23(scssr07970)	3.512	0.285	14.7	BADIA
DI_o/RC	q DI_oRC -6	6	62	ISSR31-1-Bmag0867	1.77 (ISSR31-1)	3.845	0.203	15.9	BADIA

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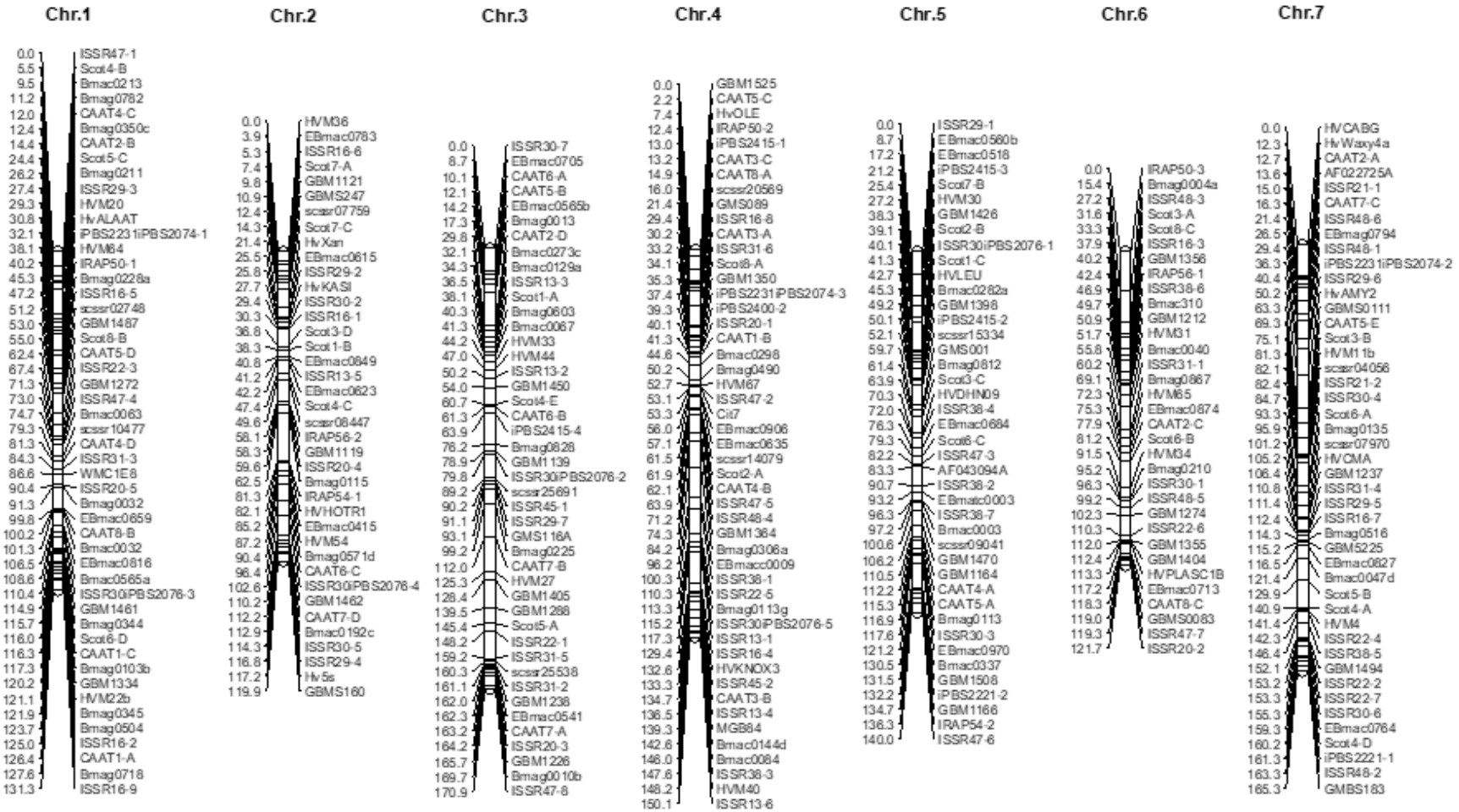

Fig. 4. Linkage maps of barley chromosomes based on SSR, ISSR, IRAP, iPBS, CAAT, and Scott markers in RIL population-based on Kavir and Badia Cross.

Increasing allele's qABSCSm-6 was transferred from parent progenies and increased the value of this parameter by 49.673. Only QTL detected for DI_o/CSm was detected on chromosome 6 with LOD = 3.151 at 72 cM Positions. This QTL was located between the Bmag0867-HVM65 marker and its Increasing alleles were transferred from parent to progenies and increased DI_o/CSm value by 9.064. This QTL explained 13.5% of the DI_o/CSm phenotypic variation.

For TR_o/RC, a QTL was detected on chromosome 6 at 74 cM between HVM65-EBmac0874 markers. This QTL explained 14.7% of the TR_o/RC phenotypic variation and increased the value of this parameter by 0.157. Two QTLs for RE_o/RC with LODs of 3.044 and 3.127 on chromosomes 1 and 6 at positions 22 and 64 cM and mapped between CAAT2-B-Scot5-C and ISSR31-1-Bmag0867 markers: qRE_oRC-1 and qRE_oRC-6 reduced the RE_o/RC value by -456.693 and -0.336 and were transferred from parent Kavir to the progenies. The mentioned QTLs explained 12.8 and 13.2% phenotypic variations of RE_o/RC.

Four QTLs (q psi E_o-1, q psi E_o-3, q psi E_o-6, and q psi E_o-7) for psi (E_o) were identified on chromosomes 3, 1, 6, and 7 with LODs of 3.239, 4.273, 8.478, and 3.629 in positions 24, 46, 62 and 98 cM. The QTLs were located between CAAT2-B-Scot5-C, HVM33-HVM44, ISSR31-1-Bmag0867, and Bmag0135-scssr07970 markers, which together explained 63.9% of the psi phenotypic variation (E_o). q psiE_o-3 and q psiE_0-6 were accounted for 17.4% and 31.5%, respectively, among the major effect QTLs in this parameter. All detected QTLs had a reducing role and reduced the value of psi (E_o) by -604.77, -0.095, -0.171, and -0.088, and were transferred from Badia parent to the progenies.

Two QTLs with LODs of 3.107 and 3.007 were detected on chromosomes 1 and 6 at Positions 22 and 62 cM for Fv/Fm between CAAT2-B-Scot5-C and ISSR31-1 Bmag0867 markers: and explained 1.3% and 12.6% of Fv/Fm phenotypic variations. These QTLs qFvFm-1 and qFvFm-6 from each parent of Badia were transferred to the progenies and reduced the amount of Fv/Fm by -47.466 and -0.038. For Sm, a QTL with LOD= 3.3 at position 62 cM was located on chromosome 6 between ISSR31-1-Bmag0867 marker and explained 13.7% of phenotypic variation of Sm, reducing allele The qSm-6 donor was transferred from parent Badia to the progenies and reduced the Sm value by -33.027. For ABS/RC a QTL with LOD= 3.512 on chromosome 7 detected in the 100 cm variation position which was located between scssr07970-HVCMA marker and explained 14.7% of ABS/RC phenotypic variation. Increasing allele's q ABSRC-7 increased the ABS/RC value by 0.285 and was transferred from parent Badia to progenies. DI_0/RC was detected with a QTL with LOD= 3.845 on chromosome 6 at 62 cM and between ISSR31-1 Bmag0867 and 15.9% of the phenotypic variation could be one of the major effects of QTLs in this trait. The qDI_oRC-6 increasing allele was transferred from parent Badia to progenies, increasing the value of this parameter by 0.203.

Discussion

Transgressive segregation was observed for all traits in this research and other QTL studies (Tian *et al*., 2005) (Zeng, 1994) Tian *et al*. (2005). This phenomenon can be due to recombinant small effect QTLs, epistasis, genotype interaction with the environment, and mutations in the production of recombinant inbred lines populations.

Positive and negative correlations were obtained in this study. These relationships can be used to achieve lines with higher photosynthetic ability. Keshavarznia *et al*., (2017) found that the Fv has a very high positive and significant correlation with the Fm index and a high and significant negative correlation with the Fo index. This study also showed a very high and significant positive correlation between Fv/Fm index and Fm and Fv indices. Zamanian *et al*., (2013) showed in their research that Fv has the highest correlation with the Fm parameter, and also Fv/Fm has a significant correlation with Fm and Fv parameters.

The 50, 39, 44, 49, 42, 33, and 45 markers were assigned to chromosomes 1, 2, 3, 4, 5, 6, and 7 (Fig. 2). Arrangement of SSR markers with (Li *et al*., 2003, Ramsay *et al*., 2000, Struss and Plieske, 1998 and Thiel *et al*., 2003) was different, but their chromosome location matched the maps and their difference can be attributed to the difference in parents.

The first linkage maps of the barley were prepared by Graner *et al*. (1991) using 71 lines of the double haploid population from Igri and Franka cross and RFLP markers. Kleinhofs *et al*. (1993) prepared linkage maps using a double haploid population from crossing Steptoe and Morex and RFLP markers. Wenzl *et al*. (2006) provided a linkage map using SSR, RFLP, STS, and DArT markers. SSR, SST, and EST combined with AFLP and used for preparing of genetic linkage map by Qi *et al*. (1998), Jafary *et al*. (2006), and Thiele *et al*. (2003), respectively. Sato (2004) used SNP markers, Varshney *et al*. (2004) used RFLP, SNP, SSR and Rostoks *et al*. (2005) applied markers SNP and SSR markers for QTL mapping of barley population.

Relatively little is known of the genetic control of chlorophyll fluorescence (CF) in determining the efficiency of photosynthesis in wheat and its association with biomass productivity.

In this study, we analyzed the quantitative trait loci (QTL) controlling chlorophyll content and chlorophyll fluorescence in 103 recombinant inbred lines (RILs) developed from the cross between the cultivar Badia and Kavir. Fo, Fv, Fm, Fv/Fm, Fv/Fo, Area, $SmET_o/CS_o$, ABS/CS_o, TR_o/CS_o, DI_o/CS_o, ABS/CSm, DI_o/CSm, TR_o/CSm, ET_o/CSm, RE_o/CSm, TR_o/RC, RE_o/RC, ABS/RC, ET_o/RC, DI_o/RC, psi(E_o), phi(P_o), vj, vi, Dv/dt_o, DVG/DT_o, were measured.

For Area, psi (E_o), Fv/Fm, Sm, and DI_o/RC in the 62 cM position, chromosome 6 between ISSR31-1-Bmag0867 markers, qTEA qAREA-6a, q psi E_o-6, qFvFm- 6, qSm-6, and qDI_oRC-6 were detected. The distance of the mentioned QTLs to the flanking marker was 1.77 (ISSR31-1) cM. Also, on chromosome 6 for the parameters ABS/CS_o, TR_o/CS_o, ABS/CSm, and DI_o/CSm in the 72 cM region between the markers Bmag0867-HVM65, qTSCs $qABSCS_o$-6, q TR_oCS_o-6, qABSCSm-6, and q DI_oCSm were located. The distance of detected QTLs to the nearest 0.33 (HVM65) marker was 0.33 CM. In the study of Guo *et al.,* (2008) a major QTL was detected, for Fv/Fm, which is related to the drought tolerance of photosynthesis on chromosome 2H at 116 cM. This QTL alone explained

more than 15% of the phenotypic variance of maximum quantum yield of PSII. Also, another QTL for Fv/Fm was also located on the same chromosome (2H) but at 135.7 cM explaining around 9% of the phenotypic variance. The result presented here suggests that two major loci, located on chromosome 2H, are involved in the development of functional chloroplast at the post-flowering stage for drought tolerance of photosynthesis in barley. They also found three QTLs for Fo on chromosomes 1, 2, and 7, which were not detected in the study. This result may be due to the type of population and the different types of markers they have used. Similar results were seen for Fm and Fv.

For Area and TR_0/RC in chromosome 6 on 74 CM, two QTLs (qAREA-6b) and (qTR_oRC-6) were detected between HVM65-EBmac0874 markers and the distance of the detected QTLs was closer to 1.26 CM (EBmac0874). Mousavi *et al*. (2016) in their study located a QTL QFo6H.n on chromosome 6 at position 61.74 cM near the ABC170E marker. Chromosome 6 in position 62 in Area, psi (E_0) and Di_0/Rc parameters could be 17.2%, 31.5%, and 15.9%, among major QTLs in these parameters respectively.

Two QTLs (qAREA-7) and (q psi E_o-7) were detected in the 98 cM region between Bmag0135-scssr07970 markers on chromosome 7 for Area and psi (E_o). Two QTLs (qRE_oRC-1) and (qFvFm-1) was detected on chromosome 1 for RE_o/RC and Fv/Fm in the 22 cM region between CAAT2-B-Scot5-C markers. The distance of detected QTLs closer to the marker (Scot5-C) was 2.36 cM. In stress-free conditions for maximum photosystem II efficiency, three QTL-containing sites (qFvFm2n, qFvFm5, and qFvFm6n) on chromosomes H2, H5, and H6 at the positions of 81.2, 71.8, and 68. 0 cM Markers ABG014, ABC314, and ABG388 were detected (Fakheri and Shahraki, 2016).

In several cases, one QTL was found to be associated with more than one trait. The position of 62 cM on chromosome 6 (ISSR31-1-Bmag0867) controlled Are, psi(E_o), Fv/Fm, sm, and DI_o/Rc. Also, The position of 72 cM on chromosome 6 (Bmag0867-HVM65) controlled ABS/Csm, TR_o/CS_o, ABS/CS_o, and DI_o/CSm. The results showed also overlapping QTLs for some of the chlorophyll fluorescence parameters identified. It appears that markers linked to these traits might be useful for marker-assisted

selection in barley, after being validated in another genetic background.

The identified QTL markers for chlorophyll fluorescence traits will be useful for understanding the genetics background and marker-assisted selection in wheat photosynthetic traits improving.

Chlorophyll fluorescence parameters can provide qualitative and quantitative information about photosynthetic processes in chloroplasts. JIP-test and modulated fluorescence (MF) parameters are commonly used chlorophyll a fluorescence parameters. This study was conducted to identify quantitative trait loci (QTLs) associated with JIP-test parameters, MF parameters, and photosynthetic rate (PN), and to examine the relationships among them in barley.

Three major QTLs were identified for the area, psi (E_0) and Di_0/Rc on chromosome 6 between ISSR31-1-Bmag0867 in position 62 cM that explained 17.2%, 31.5%, and 15.9%, respectively. Also, another colocation was detected for ABS/CS_o, TR_o/CS_o, ABS/CSm, and DI_o/CSm QTLs on chromosome 6 in position 72. The existence of significant positive and negative correlations between traits can be attributed to the pleiotropic effect or strong association between the genes controlling them. Correlated traits are often controlled by quantitative gene loci located in similar regions on chromosomes. Similar results were observed in this study between ISSR31-1-Bmag0867 markers for the area, psi (E_o), Fv/Fm, Sm, and DI_o/RC parameters, which were located on chromosome 6 at positions 62 and also for the parameters ABS/CS_o, TR_o/CS_o, ABS/CSm, and DI_o/CSm were located on chromosome 6 in position 72.

The result presented here suggest that eight major loci, located on chromosome 6 (qAREA-6a, qAREA-6b, qDIoRc-6) and 3 (qpsiEo-3, qpsiEo-3) are involved in the development of functional for photosynthesis in barley. If validated in other populations, chlorophyll fluorescence parameters could be used as selection criteria. These identified genomic regions can be used in marker-assisted breeding after validation.

These QTL hot spot regions along with stable QTLs should be targeted for better understanding the genetic basis of chlorophyll fluorescence kinetics parameters in future mapping studies.

Acknowledgments

We would like to acknowledge the help of Dr. Hossein Ali Fallahi, Crop and Horticultural Science Research Department, Mazandaran Agricultural and Natural Resources and Education Center, AREEO, Sari, Iran. We are also grateful to the Gonbad Kavous University for their financial support.

Conflicts of interest

The authors declare that they have not conflict of interest.

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