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Mapping QTLs for grain yield components in bread wheat under well-watered and rain-fed conditions

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Abstract
Wheat production is often decreased by water limitation in world particularly in drought-prone environments. To map quantitative trait loci for grain yield as an important economic traits affecting wheat production, a recombinant inbred lines (RIL) population obtained from a cross between two spring bread wheats (Iran #49 and Yecora Rojo) was evaluated for grain yield and its components under well-watered and rain-fed conditions. Linkage map of the population was constructed using 203 SSR and retrotransposon markers spaning a total of 687.29cm of wheat genome with an average distance of 3.32cm between two adjacent markers. A total of 12 QTLs with LOD score from 2.5 to 4.5 (seven for well-watered and five for rain-fed condition) were mapped on six chromosomes for the studied traits. Out of these QTLs, 3, 2, 1 and 1 QTLs were identified for spike length (SL), gain number per spike (NG), grain yield (GY) and thousand kernel weight (TKW) under well-watered condition. Two QTLs for plant height (PH) and three QTLs for each NG, GY and TKW traits were also mapped under rain-fed condition. Detected QTLs explained from 7.78 to 13.89% of phenotypic variation. QTLs of GY and PH in rain-fed condition co-localized in same position.

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Introduction

Wheat (*Triticum aestivum* L.) is the most important crop which is widely cultivated on more than 215 and 5.7 million hectares annually around the world and Iran, respectively (FAOSTAT, 2016). It is estimated that in 2050, the world population will be about 9.8 billion. To feed this population in 2050, some 900 MMT wheat is required (Alexandratos *et al.*, 2012). In the recent years, due to climate change nutrient, water and energy scarcity threaten crop production overall world as well as Iran. Therefore, production of new wheat genotypes adapted to changing environment is the major challenge in wheat breeding programs which require the dissection of quantitative trait loci controlling the drought tolerance related traits (Gahalaut *et al.*, 2017).

Production of wheat as a major cereal cultivated in dry and semi-dry regions is affected by water deficit mostly at flowering stage and due to this stress yield of wheat reduced up to 50% in comparison suitable rainfall environment (Edae, 2013). Due to the short life-cycle under drought stress, reduction in size and number of tillers, spikes, spikelets and kernel resulted in low grain yield (Lascano *et al.*, 2001). Therefore, the main challenges of wheat breeders in water limited environment is to improve wheat productivity and yield under drought and water deficit conditions (Balouchi, 2010).

Due to low heritability of drought associated traits and high genotype × environment interaction, in the recent years large number of studies have been conducted to map QTLs for these traits (Cuthbert *et al.*, 2008, Bennett *et al.*, 2012, Gao *et al.*, 2015, Li *et al.*, 2015b and Gahlaut *et al.*, 2017). Cuthbert *et al* (2008) using a population of 402 doubled haploid at six environments identified 53 QTLs for grain yield and its components explaining 4.5 to 17% of traits phenotypic variation. Some of the QTLs for yield were constant across the environments and co-localized with QTLs of yield components. Studying 192 DH lines with 392 DArT and SSR markers under irrigated and rain-fed environments, Gahlaut *et al.* (2017) reported 98 QTLs with PVE value from 3.58 to 20.43% for agronomic traits at nine environments. Gao *et al.* (2015) and Li *et al.* (2015b) used SNP markers to QTL mapping in RIL populations and reported 86 and 29 QTLs for yield component traits respectively. The objective of this study was to identify QTLs associated with grain yield related traits under well-watered and rain-fed conditions.

Materials and methods

Plant Materials and Field Trials

The mapping population comprised 149 F8 recombinant inbred lines (RILs) bred by single seed descent from crossing genotype Iran #49 9 Yecora Rojo. Iran #49 (hereafter referred to as #49) is a tall late spring landrace collected at Allary, 30_560, 61_390, alt. 530m, average rainfall = 50mm, in Bluchestan, southeast Iran with a large root system. Yecora Rojo is a spring modern Mexican derived, two gene dwarf cultivar with small root system growing in Southern California for more than 40 years (Ehdaie *et al.*, 2016).

For phenotyping, the RIL population and parental lines were evaluated under well-watered condition at Makoo, Iran during 2013 and rain-fed condition at Maragheh, Iran during 2010 using randomized block design with two replicates. In this study, we measured spike length (SL), grain number per spike (NG), plant height (PH), thousand grain weight (TKW), spike number per m² (SN) and grain yield (GY).

Statistical analysis

The data obtained in each environment were subjected to analysis of variance (ANOVA) using the SAS 9.2 (SAS Institute Inc., 2006). Correlation analysis was used to quantify the relationship between traits.

DNA Extraction

Leaf tissue was harvested from 2- to 3-week-old seedlings and genomic DNA was isolated from leaf tissues following the protocol described by Saghai-Maroof *et al.* (1984). DNA quality was examined using agarose-gel electrophoresis 0.8%, and the concentration was assessed using a spectrophotometer. Finally, DNA samples were diluted to a concentration of 25ng/μL, used TE (Tris EDTA) and stored at -20ºC.

PCR amplification and electrophoresis running

The population was genotyped using 160 SSRs and 43 retrotransposon markers. PCR was carried out in a 10μL volume containing 2μL DNA (25ng), 0.5μL of each primer, 0.3μL MgCl2, 0.2μL dNTPs, 1μL reaction buffer, 0.1μL Taq DNA polymerase and 5.4μL dH2O. 1 cycle was performed at 94ºC for 3 min, 35 cycles of 45 s at 94ºC, annealing for 45 s at 58-62ºC (depending on various primers), extension for 45 s 72ºC, were followed by a final extension step of 5 min at 72ºC.

The amplified products were separated on 4% non-denature polyacrylamide and running with gel-scanning 3000 electrophoresis system (Corbett Robotics Australia company). The time of electrophoresis varied between 20-45min.

Linkage map construction and QTL identification

The linkage map was constructed using Map Disto 1.7 (Lorieux, 2012) software based on the Kosambi mapping function (Kosambi 1944) and LOD score ≥3.0. The Chi-square test and p-value <0.05 was used to examine the deviations of segregation ratios from the Mendelian ratio.

Markers that presented segregation distortion from (1:1) ratio were excluded from the linkage analysis. QTL mapping was performed with Windows QTL Cartographer V2.5 (Wang *et al.*, 2012) using composite interval mapping (CIM).

Significant LOD thresholds were set by 1000 times of permutations along with model 6 and forward and backward regression to select cofactors. We used MapChart v2.1 (Voorrips 2002) to generate graphical linkage maps and QTLs positions.

Results

Phenotypic analysis

Frequency distributions of the studied traits under well-watered and rain-fed conditions displayed continuous and normal distribution for all traits (Fig. 1). Transgressive segregation was observed for all traits. In the rain-fed condition, maternal parent (#49) consistently showed higher values for all of traits compared with Yecora Rojo.

Under rain-fed condition, Yecora Rojo had higher mean values for GY, NG and TKW, whereas #49 was taller with long spike length compared with Yecoro Rojo. The mean and range values of traits for population and parental line are shown in table 1.

The phenotypic correlations between the traits are presented in Table 2. Significant correlations were estimated between GY and PH, SL, NG and TKW under both conditions.

The correlation between GY and NG was stronger compared with the others. The correlations between PH and other traits were positive and significant except NG under well-watered condition, however the values were not high.

In total, correlation between traits were stronger under rain-fed condition compared with well-watered condition.

Table 1. Means and range of plant height (PH), spike length (SL), grain yield(GY), number of grain per spike (NG) and 1000 grain weight (TGW) for Iran #49 x Yecora Rojo (YR) recombinant inbred lines (RILs) population and parental lines under well-watered and rain-fed conditions.

Trait	Well-watered				Rain-fed			
	Yecora Rojo	#49	RILs		Yecora Rojo	#49	RILs	
			Mean	range			Mean	Range
PH	52.40	88.24	80.51	49.95 - 111.50	32.95	34.50	43.14	27.1 - 66.7
SL	9.75	10.15	9.38	7.28 - 12.03	7.30	7.60	6.21	3.55 - 8.80
GY	5.15	3.83	4.63	1.90 - 7.07	6.52	7.82	5.59	1.93 - 13.46
NG	5.38	4.88	5.26	3.61 - 6.49	22.80	25.80	18.10	8.10 – 30.00
TKW	40.00	36.38	39.22	27.75-47.63	26.87	27.56	29.51	14.62-58.78
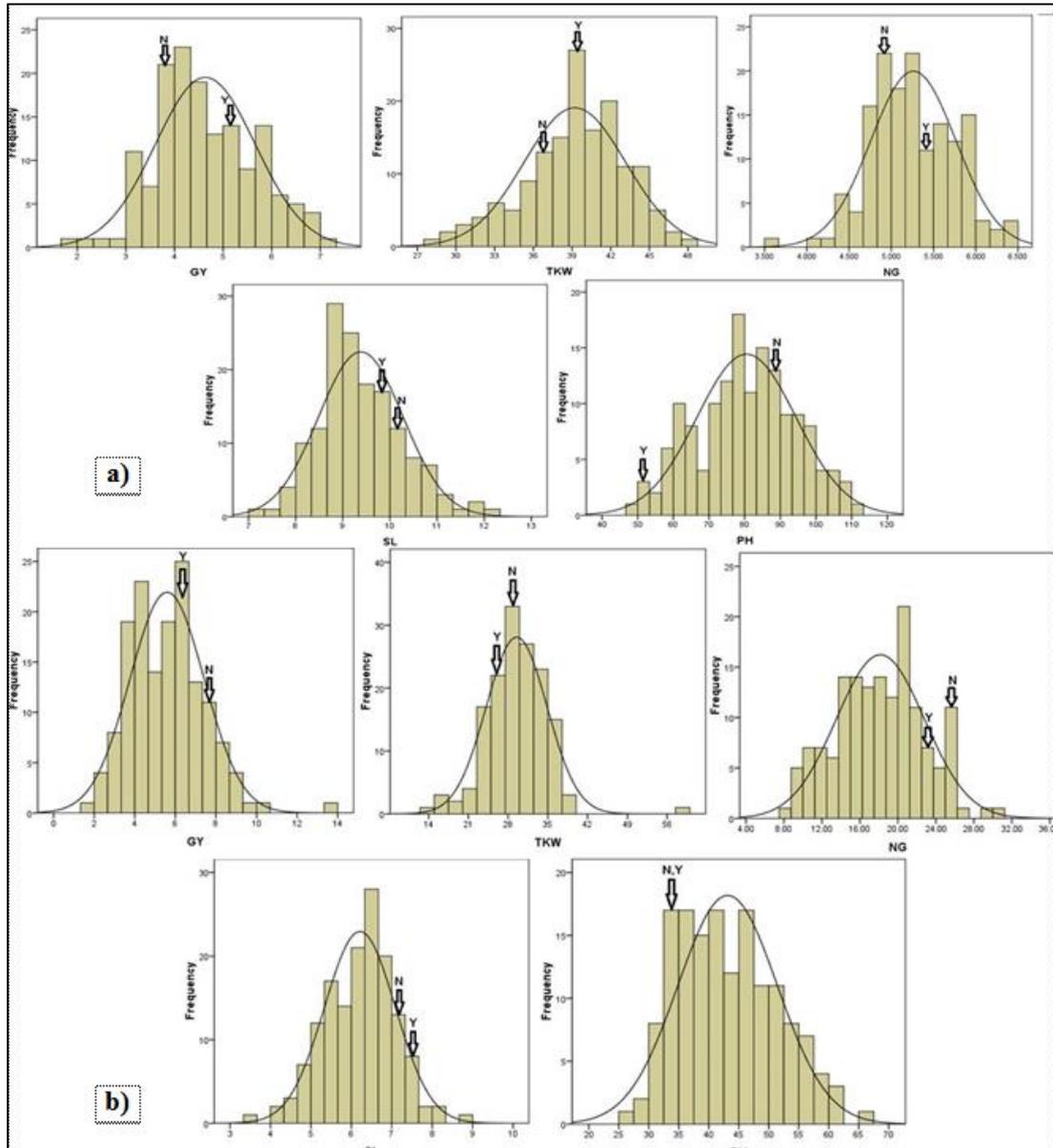
Fig 1. Frequency distribution of plant height (PH), spike length (SL), grain yield (GY), number of grain per spike (NG) and 1000 grain weight (TGW) in Iran #49 (N) x Yecora Rojo (YR) recombinant inbred lines (RILs) population under a) well-watered and b) rain-fed conditions.

Table 2. Phenotypic correlation between plant height (PH), spike length (SL), grain yield(GY), number of grain per spike (NG) and 1000 grain weight (TGW) in Iran #49 x Yecora Rojo (YR) recombinant inbred lines (RILs) population under well-watered and rain-fed conditions.

	Water Condition	PH	SL	GY	NG	TKW
PH	Rain-fed	1				
	Well-watered	1				
SL	Rain-fed	0.46**	1			
	Well-watered	0.26**	1			
GY	Rain-fed	0.50**	0.72**	1		
	Well-watered	0.30**	0.21*	1		
NG	Rain-fed	0.41**	0.75**	0.82**	1	
	Well-watered	0.13	0.22**	0.78**	1	
TKW	Rain-fed	0.28**	0.16*	0.57**	0.03	1
	Well-watered	0.36**	0.09	0.42**	-0.01	1

QTL mapping

The genetic map containing 160 SSRs and 43 retrotransposon markers was used for detecting QTLs underlying traits of interest. The 201 markers were mapped in 34 linkage groups corresponding to the 21 hexaploid wheat chromosomes spanning 687.29cm of wheat genome. The length of linkage groups ranged from 4.88cm (5D) to 124.57cm (3A) (Table 3). The A, B and D genomes consisted of 95 markers (47%) and length of 347.26cm, 82 markers (41%) and length of 252.38cm and 24 markers (12%) and length of 87.64cm, respectively. Distance between two adjacent markers varied from 1.76 to 4.94 with an average of 3.22cm.

Table 3. The detail of linkage map of SSR and retrotransposon markers in Iran #49 x Yecora Rojo (YR) recombinant inbred lines (RILs) population.

Genome	Chromosome	No. of linkage group	No. of marker	CM	Distance between adjacent markers
A	1A	1	3	9.36	3.12
	2A	3	13	30.23	2.33
	3A	3	30	124.57	4.15
	4A	2	10	31.71	3.17
	5A	3	22	71.28	3.24
	6A	2	11	50.43	4.58
	7A	1	6	29.68	4.95
			95	347.26	
B	1B	2	14	32.39	2.31
	2B	1	9	15.90	1.77
	3B	1	3	9.23	3.08
	4B	2	16	74.24	4.64
	5B	2	17	38.77	2.28
	6B	2	14	53.49	3.82
	7B	2	9	28.36	3.15
			82	252.38	
D	1D	1	6	22.67	3.78
	2D	1	6	29.31	4.88
	5D	1	2	4.88	2.44
	6D	1	3	5.45	1.82
	7D	3	7	25.34	3.62
			24	87.65	
Total		34	201	687.29	3.32

A total of 12 putative QTLs were identified with a single QTL explaining 7.7 to 13.9%. of phenotypic variation observed for traits. These QTLs were distributed on six chromosomes, 1B, 2D, 3A, 4A, 4B and 6B. The LOD values ranged from 2.60 to 4.50 and the additive effect values ranged from 0.15 to 30.2. The additive effects for five QTLs were positive with Yecora Rojo allele increasing the effects of QTLs, whereas the other seven QTLs were negative with #49 allele increasing the effects (Table 4). Among the studied traits, no QTL was mapped for number of spike per m² under both conditions.

Table 4. QTLs for grain yield (GY), number of grain per spike (NG), 1000 grain weight (TGW), plant height (PH) and spike length (SL) in the Iran #49 X Yecora Rojo RIL population under well-watered and rain-fed conditions.

Trait	Environment	Marker interval	Chromosome	Position CM	LOD	Add effect	PVE%	Favorable parents
GY	Well-watered	LTR6149/ISSR2.260-Psp2999	4B	5.01	3	0.32	8.77	#49
	Rain-fed	LTR6149/Sukkula.600-Sukkula/ISSR7.610	3A	66.71	3.8	1.28	13.89	Yecora Rojo
NG	Well-watered	5LTR.2/Nikita.770-3.LTR.180	6B	41.51	3.3	0.18	12.05	Yecora Rojo
		Sukkula/ISSR7.230-LTR6149/ISSR2.180	1B	2.81	3	0.15	8.90	#49
	Rain-fed	Sukkula/ISSR4.590-LTR6149/Sukkula.600	3A	63.41	2.6	30.20	8.31	Yecora Rojo
TKW	Well-watered	Psp2999-Wmc336	4B	7.31	3.3	1.12	7.91	#49
	Rain-fed	LTR6150/ISSR10.260-	3A	84.21	3.1	1.62	7.78	Yecora Rojo

Trait	Environment	Marker interval	Chromosome	Position CM	LOD	Add effect	PVE%	Favorable parents
PH	Rain-fed	5LTR.2/ISSR5.530 LTR6149/Sukkula.600-Sukkula/ISSR7.610	3A	69.71	2.7	2.82	9.91	Yecora Rojo
		Gwm160-Barc78	4A	1.01	3.3	2.50	8.36	#49
SL	Well-watered	Sukkula/ISSR7.920-Sukkula/ISSR4.100	1B	16.51	2.6	0.28	8.92	#49
		Wmc216.2-Wms131	1B	24.81	4.5	0.31	11.34	#49
		Wms102-Wmc18	2D	6.01	3.2	0.28	9.25	#49

At well-watered condition, three QTLs were detected for SL on chromosome 1B and 2D that, respectively explained 8.92, 11.34 and 9.25% of the trait variation and #49 parent contributed increasing alleles for these QTLS. For NG, two QTLs were mapped on chromosomes 1B and 6B that, respectively explaining 8.90 and 12.05% of total trait variation. In addition, one QTL was detected for each GY and TGW on chromosome 4B explaining, respectively 8.7 and 7.91% of the variation. The QTLs of GY and TGW were co-localized at 5.01 and 7.31cm apart microsatellite Xwmc. For these QTLs, the icreasing alleles were contributed from #49 parental line. For PH, no QTL were identified

under well-watered condition.A total of five QTLs were mapped at rain-fed condition for PH, GY, NG and TKW and distributed on chromosome 3A and 4A. For PH, two QTLs on chromosomes 3A and 4A explained, respectively 9.90 and 8.40% of the trait variation. One QTL was detected for each of NG, GY and TGW on chromosome 3A that, respectively explaining 8.31, 13.89 and 7.78% of the variation. The QTLs for PH and GY were co-localized on chromosome 3A between markers LTR6149/ Sukkula. 600 and Sukkula /ISSR7. 610. For all the detected QTLs, except of QTL for PH on chromosome 4A, the increasing alleles were inherited from Yecora Rojo parent.
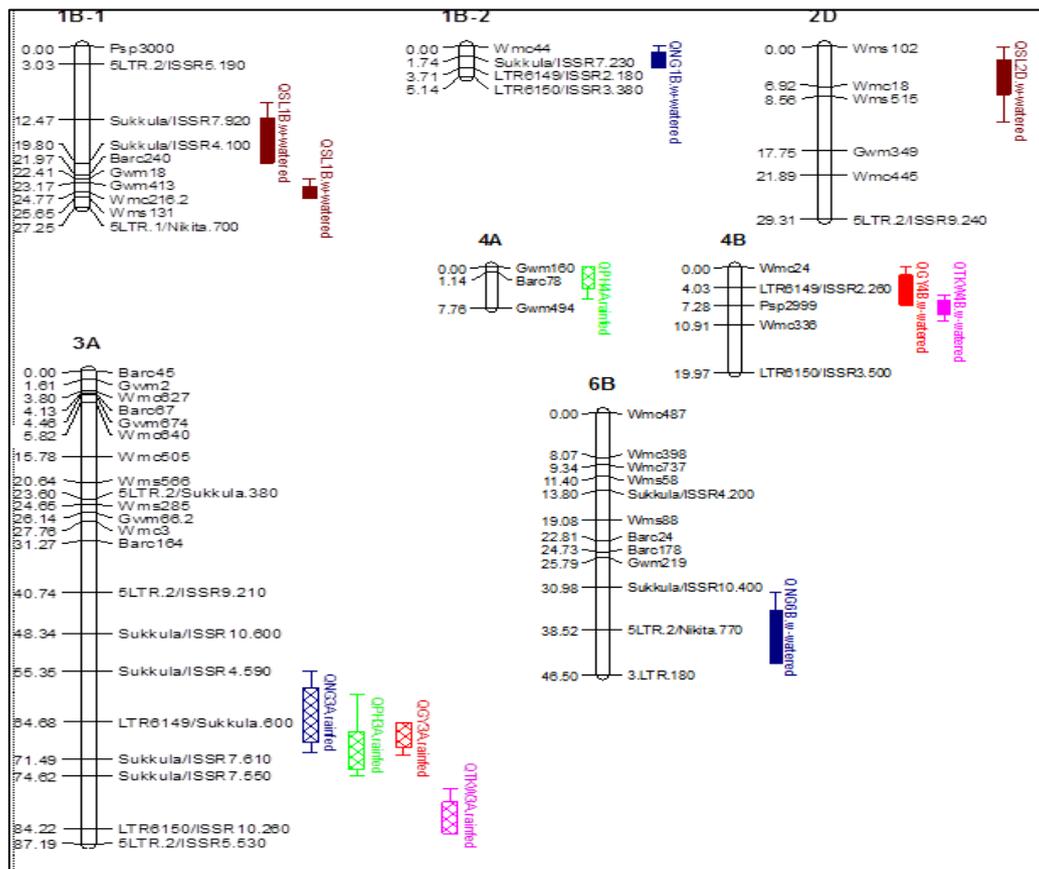
Fig 2. Locations of QTLs for the traits evaluated under well-watered and rain-fed conditions in the population of 149 RILs derived from the cross Iran #49 X Yecora Rojo. QTLs bars and markers are indicated on the right side of each chromosome and vertical lines on bars show confidence intervals of QTL position in cM. Detected QTLs under well-watered and rain-fed conditions are shown by solid filled and stripes filled, respectively.

Discussion

Improving of wheat production in drought-prone environment such as Iran required the identification of genomic regions related to traits such as grain yield and yield-components under various water conditions. QTL analysis has been widely utilized to characterize the associated QTL related to drought tolerance. To map QTLs for grain yield and its components, a set of 149 RILs from #49 x Yecora Rojo cross along with the parents were evaluated under well-watered and rain-fed conditions. Transgressive segregation was observed for all the studied traits at both conditions indicating the allelic complementation in the RILs (Poehlman and Sleper, 1995). Significant correlations between grain yield and some of its components except NG could be due genetic linkage of the genes controlling the traits or their pleiotropic effects. The significant correlations between grain yield and its components were also reported in previous studies (Kuchel *et al.*, 2006, McIntyre *et al.*, 2010 and Wu *et al.*, 2012). The correlations between traits were stronger under rain-fed condition compared with well-watered condition.

Grain yield and its associated traits are complex quantitative traits controlled by multiple genes and highly affected by environments, therefore genetic dissection is necessary for their improvement. We identified two QTLs for grain yield under well-watered and rain-fed conditions on 4B and 3A, respectively explaining 8.77 and 13.89% of trait variation. In previous studies QTLs for grain yield were reported on all wheat chromosomes (McIntyre *et al.*, 2010, Li *et al.*, 2015a, Li *et al.*, 2015b and Bhusal *et al.*, 2017). Identification of grain yield associated QTLs on different chromosomes in different studies could be due differential expression of QTLs at various genetic backgrounds and QTL × QTL and QTL × environment interactions (Barton and Keightley, 2002). Breeding high yielding wheat cultivars by direct selection for yield has generally been slow, due to complex genetic nature and low heritability of grain yield. The coincidence of grain yield QTL with that of at least one yield component with high heritability offers a means for selecting for grain yield by efficient selection for one component (Wu *et al.*, 2012). In the present study, two QTLs 6B and 1B for NG and a single QTL on 4B for TKW were detected under well-watered condition. Under rain-fed condition, a single QTL for each of NG and TKW were mapped on chromosome 3A, respectively explaining 8.3 and 7.78% of trait variation. In the both loci, the favorable alleles were contributed by Yecora Rojo. Two QTLs for PH on chromosome 3A and 4A under rain-fed condition and three QTLs for SL on chromosome 1B and 2D under well-watered condition were detected. For PH, both parents contributed increasing alleles, but at all three QTLs for SL, the favor alleles were inherited from #49 as tall parent.

The reduced height genes (*Rht*) have been utilized in wheat yield breeding for many years as a means to reduce the height of the crop (Wu *et al.*, 2012). Yecora Rojo is a dwarf variety with two dwarfing genes (*Rht-B1b* and *Rht-D1B*). Therefore, yield-related QTL on 4B may be associated with the green revolution gene *Rht1* gene on 4B. The common chromosomal location QTLs for PH and GY under rain-fed condition could offer a means for selecting for grain yield by efficient selection for one component. Co-localized QTLs for yield related traits were also reported by Li *et al.* (2007), Wang *et al.* (2009), McIntyre *et al.* (2010) Li *et al.* (2015b).

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