

QTL mapping for seedling morphology under drought stress in wheat cross synthetic (W7984)/Opata

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Abstract

Drought stress ‘particularly at seedling stage’ causes morpho-physiological differences in wheat which are crucial for its survival and adaptability. In the present study, 209 recombinant inbred lines (RILs) from synthetic wheat (W7984) × ‘Opata’ (also known as SynOpRIL) population were investigated under well-watered and water-limited conditions to identify quantitative trait loci (QTL) for morphological traits at seedling stage. Analysis of variance revealed significant differences ($P < 0.01$) among RILs, and water treatments for all traits with moderate to high broad sense heritability. Pearson’s coefficient of correlation revealed positive correlation among all traits except dry root weight that showed poor correlation with fresh shoot weight (FSW) under water-limited conditions. A high-density linkage map was constructed with 2639 genotyping-by-sequencing markers and covering 5047 cM with an average marker density of 2 markers/cM. Composite interval mapping identified 16 QTL distributed over nine chromosomes, of which six were identified under well-watered and 10 in water-limited conditions. These QTL explained from 4 to 59% of the phenotypic variance. Six QTL were identified on chromosome 7B; three for shoot length under water-limited conditions (*QSL.nust-7B*) at 64, 104 and 221 cM, two for fresh root weight (*QFRW.nust-7B*) at 124 and 128 cM, and one for root length (*QRL.nust-7B*) at 122 cM positions. *QFSW.nust-7B* appeared to be the most significant QTL explaining 59% of the phenotypic variance and also associated with FSW at well-watered conditions. These QTL could serve as target regions for candidate gene discovery and marker-assisted selection in wheat breeding.

Keywords: analysis of variance (ANOVA), broad sense heritability, drought, International Triticeae Mapping Initiative (ITMI), Pearson’s correlation, QTL mapping, wheat

Introduction

In developing countries, 50% of the area under wheat cultivation is rainfed, which could be the main reason for

severe drought stress (Gupta *et al.*, 2017). Grain yield is a complex trait with strong genotype by environment interactions. Grain yield is significantly correlated with seedling traits such as root length (RL), shoot length (SL) and dry root weight (DRW) (El-Rawy and Hassan, 2014). Therefore, adaptive traits less influenced by environment like physiological and seedling emergence traits are targeted to understand the genetics of drought adaptability

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(Acuna-Galindo *et al.*, 2015). For example, smaller leaf area and low dry weight of flag leaf in soft red winter wheat lead to higher yield (Balota *et al.*, 2017).

Recent advancement in genotyping technologies has facilitated the identification of underlying mechanisms of important agronomic traits and candidate gene discovery in many crops (Michelbart *et al.*, 2015; Rasheed *et al.*, 2017). Acuna-Galindo *et al.* (2015) reported 502 quantitative trait loci (QTL) associated with drought tolerance using meta-analysis approach based on the positions and confidence interval of QTL regions reported in the literature. Similarly, about 800 QTL have been reported in wheat related to drought stress (Gupta *et al.*, 2017). For drought adaptive traits at the seedling stage, thirty QTL were identified for root growth angle in a recombinant inbred lines (RILs) population and association mapping panel, and only six were co-localized to previously reported QTL (Maccaferri *et al.*, 2016). Eighteen QTL were identified in RILs of 168 lines ('Iran #49' × 'Yecora Rojo') for root traits, out of which two QTL were on chromosome 4B for deep root weight, three were for shallow root, total root biomass and deep root weight on 2D and 4A chromosomes (Ehdaie *et al.*, 2016). Eleven QTL have been reported in previous studies for

RL on nine chromosomes (Gupta *et al.*, 2017). Acuna-Galindo *et al.* (2015) reported one meta-QTL for DRW on chromosome 1B, three for RL on chromosome 1AL, 6DL and 7DL, and two QTL for SL on chromosome 2DS and 5BL (Fig. 1).

International Triticeae Mapping Initiative (ITMI) mapping population is a useful resource derived from synthetic hexaploid wheat 'W7984' and bread wheat cultivar 'Opata'. This population facilitated the development of the first RFLP (van Deynze *et al.*, 1995) and SSR (Röder *et al.*, 1998) linkage maps in wheat (Rasheed *et al.*, 2018). 'W7984' is a drought tolerant synthetic hexaploid wheat (Altar 84/*Ae. tauschii* 219), whereas 'Opata M85' is a drought susceptible bread wheat developed by International Maize and Wheat Improvement Center (CIMMYT), Mexico. Initially, ITMI population consisted of 150 RILs developed by the single seed descent method, but anomalies have been accumulated due to labelling errors and chromosomal instability. Therefore, reconstruction of the population was followed using same parents and with more precautions. A double haploid population from 250 F₁ hybrids referred as 'SynOpDH' and a RIL population consisted of 2043 F₆ lines referred as 'SynOpRIL' were constructed (Sorrells *et al.*, 2011). The objective of the present study is to identify

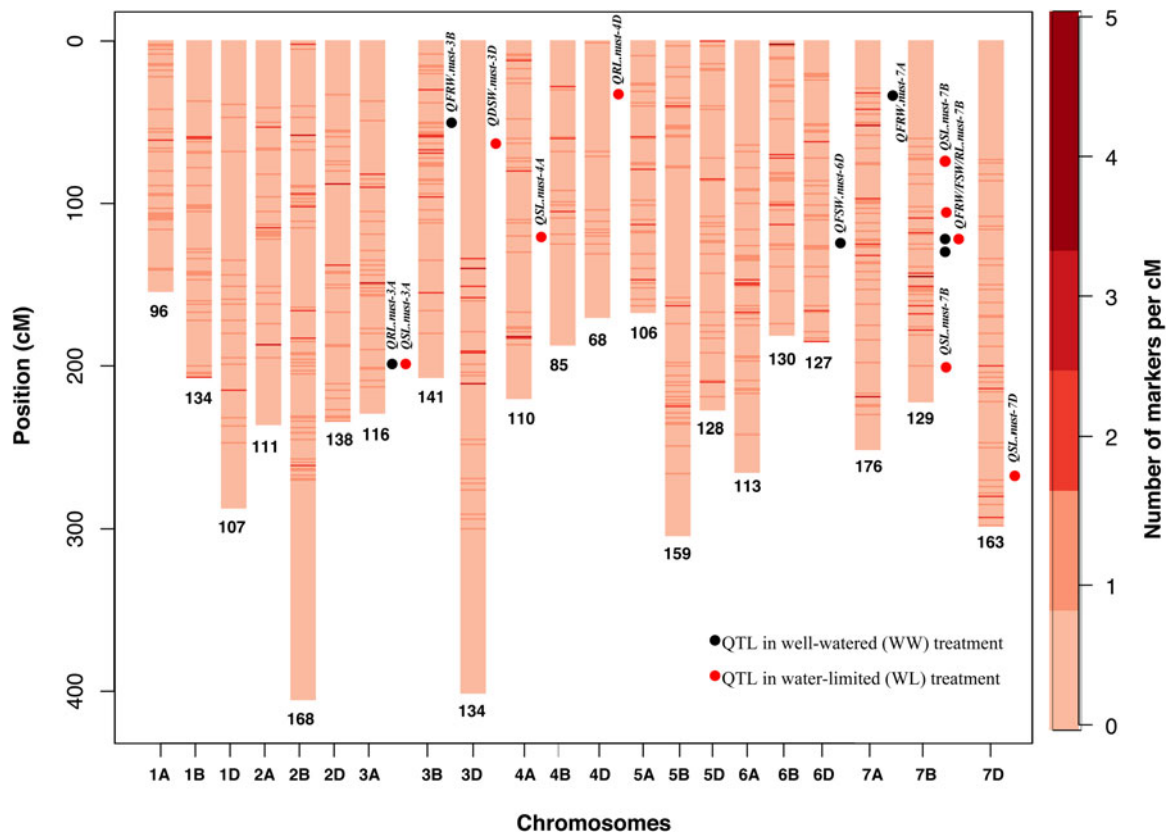


Fig. 1. Linkage map of Synthetic (W7984) × Opata RILs population (SynOpRIL) consisting of 21 chromosomes using 2639 GBS markers. Numbers below each chromosome are the number of markers, Y-axis represents the length of linkage group and scale on the right side represent the density of markers at a given position.

QTL for seedling morphology in SynOpRIL under water-limited and well-watered conditions.

Materials and methods

Germplasm

Two hundred and nine RILs from ITMI (SynOpRIL) mapping population including both parents were used for this study. These lines constitute a subset of new ITMI population consisting of 2043 F6 RILs (Sorrells *et al.*, 2011).

Phenotyping

All lines were phenotyped at seedling stage under control and drought stress conditions using the cigar roll method (Zhu *et al.*, 2006; Placido *et al.*, 2013). Seeds were first surface sterilized with 10% bleach followed by 70% ethanol and then rinsed three to five times with distilled water. Sterilized seeds were placed in Petri plates containing filter paper and germinated in dark. After 5 d, five uniformly germinated seeds were placed 1 cm below the top centre of germination paper which was then rolled to a final dimension of 2 cm in diameter and 35–38 cm in height supported vertically by a metal mesh with 2 × 2 cm² holes. The rolled papers were placed in 1 litre beakers so that each beaker have a seedling from six accessions to ensure 30 seedlings in each beaker. For well-watered conditions, beakers were filled with 100 ml tap water. For water-limited conditions, 50 ml of tap water was supplied. Water levels were maintained until taking phenotype. Seedlings were grown in a controlled environment with temperature 25/27°C and 11.5/12.5 h day/night. After 7–10 d, RL, SL, fresh root weight (FRW), fresh shoot weight (FSW), DRW and dry shoot weight (DSW) were measured both in well-watered (WW) and water-limited (WL) conditions. All data for each trait in all replications and both treatments were collected in the same day. All trait abbreviations were suffixed by condition names, e.g. RL in WW and WL were expressed as RL_{WW} and RL_{WL}, respectively. For RL, roots were separated from the root–shoot junction without any breakage in root system and then laid down on flat surface and stretched to measure its length and weight using a sophisticated weighing balance. It was then dried in an incubator at 60°C for 24 h to measure DRW. The same procedure was done for shoots of all lines. Three technical replicates were performed. All data were collected and mean values were noted.

Genotyping

DNA extraction was done by the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). A

genotyping-by-sequencing (GBS) library was constructed according to protocol proposed by Poland *et al.* (2012) with two restriction enzymes *Pst*I (CTGCAG) and *Msp*I (CCGG), and barcoded adapters were ligated to each sample. It was then pooled by plate into libraries and amplified by polymerase chain reaction. The library was constructed in 95-plex using P38-4A adapter set and was sequenced to 100 bp on a single lane of Illumina HiSeq 2000 at Kansas State University, KA, USA.

Statistical analysis

Genotypic and phenotypic variances were calculated by following formulas:

$$\text{Phenotypic variance}(\sigma^2 p) : \sigma^2 g + \sigma^2 \text{err}$$

$$\text{Genetic variance}(\sigma^2 g) : (MS_f - MS_{ft}) \times (rt)$$

$$\text{Genetic} \times \text{treatment interaction variance}(\sigma^2 gt) :$$

$$(MS_{ft} - MS_e) \times (r)$$

where r is the number of replications; t , the number of treatments; MS_f , genotype mean square; MS_{ft} , genotype × treatment interaction mean square; $\sigma^2 \text{error} = MS_e$, Error mean square. All phenotypic data were analysed by SAS 9.2 software. Following are the formulas. Broad-sense heritability (H) were estimated by:

$$\text{Broad sense heritability}(H) : \sigma^2 g$$

$$[(\sigma^2 g + \sigma^2 gt + \sigma_e^2) \times (r)] \times (rt)$$

where $\sigma^2 g$ is the genetic variance; $\sigma^2 gt$, genotype × treatment interaction; r , replication; t , treatments and $\sigma^2 p$, phenotypic variance. Statistical differences were estimated by threshold probability of $P \leq 0.05$.

The raw GBS data were filtered to remove markers with distorted segregation (departure from the expected 1:1 segregation ratio). Such individuals were flagged as DISTORTED in the raw data file and, in general, conformed to markers with MAF < 0.4. GBS markers were grouped using IciMapping 4.0 software (<http://www.isbreeding.net>). Linkage analysis was performed using JoinMap 4.0 (Stam, 1993). The linkage map was then constructed using R package 'synbreed' (Wimmer *et al.*, 2012). Map distances between markers were calculated with Kosambi mapping function. QTL analysis was performed using inclusive composite interval mapping (ICIM) with IciMapping 4.0 software (Li *et al.*, 2007). Phenotypic values of all RILs in both WW and WL treatments were used for QTL detection. Missing phenotypic data was deleted using 'Deletion' command. The walking speed chosen for all QTL was 1.0 cM, with $P = 0.001$ in step-wise regression. Based on 2000 permutations at the probability level of 0.01, the LOD scores declare significant QTL for all traits ranged from 2.0 to 2.5. Thus, LOD threshold of 2.5 was chosen for a declaration of putative QTL. The phenotypic variance

explained (PVE) was estimated using step-wise regression (Li et al., 2007). QTL were named according to the rules of International Rules of Genetic Nomenclature (<http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>).

Results

Phenotypic variation and coefficient of correlation

The average, maximum and minimum values of each trait varied among different lines in both treatments (Table 1). The bar plots showing variation in population for each trait are shown as online Supplementary Fig. S1. Estimate of variance components showed significant differences ($P < 0.05$) among genotypes, between treatments and genotype by treatment interaction (Table 2). Both RL and SL were least affected and were reduced by 18% in WL condition, followed by DSW (19.9%). DRW was most affected and reduced by 91% followed by FRW (37.7%) and FSW (23%) in WL treatment. Broad-sense heritability ranged from 0.51 (DSW) to 0.96 (FRW).

All seedling traits showed moderate to strong positive correlation with each other in both treatments (Table 3). The highest positive correlation was observed between SL and FSW ($r = 0.74$) in WL treatment. In WW treatment, RL showed highly positive correlation with SL ($r = 0.68$). Similarly, FRW showed significant correlation with RL ($r = 0.70$). DRW was not strongly correlated with any of

the traits. On the other hand, FSW showed high correlation with SL ($r = 0.72$).

Linkage mapping and QTL analysis

In the present study, 2639 SNPs from GBS platform were used for QTL study, which facilitated the development of a high-density map (online Supplementary Table S1). In total, 16 QTL for seedling morphology were identified explaining 4.7% (*QSL.nust-7B*) to 59% (*QFSW.nust-7B*) of phenotypic variation (Table 4). Ten QTL were identified in WL for RL, SL, DSW and six in WW treatment for FRW and FSW. No QTL was identified for DRW in both conditions. The LOD values ranged from 2.51 (*QFSW.nust-6D*) to 14.27 (*QFSW.nust-7B*) (Table 4). QTL were distributed on nine chromosomes, of which seven QTL were mapped to chromosome 7B, two were mapped on 3A and one on each of chromosomes 3B, 3D, 4A, 4D, 6D, 7A and 7D. The additive effects of 12 QTL were from synthetic allele and remaining 4 QTL were from 'Opata' allele.

Three QTL for RL_{WL} were identified on chromosome 3A (209 cM), 4D (37 cM) and 7B (122 cM) (Table 4). The phenotypic variation ranged from 6.98% (*QRL.nust-7B*) to 12.57% (*QRL.nust-3A*) (Table 4). The positive additive effect for *QRL.nust-3A* and *QRL.nust-7B* were from synthetic allele and negative additive effect of *QRL.nust-4D* was from 'Opata' allele.

Six QTL for SL_{WL} were detected on 3A, 4A, 7B (3) and 7D chromosomes. The phenotypic variation ranged from 4.7 (*QSL.nust-7B*) to 14.6% (*QSL.nust-7B*). Three QTL for

Table 1. Maximum, minimum and average values of agronomic traits at early seedling stage of RILs and their parents in well water and water-limited conditions

Treatment	Traits	Parents		RILs		
		Synthetic (W7984)	Opata	Min	Max	Average
Well-watered	RL (cm)	10.10	20.20	0.40	21.50	12.11
	SL (cm)	12.97	14.30	2.60	17.33	10.68
	FRW (g)	0.040	0.099	0.002	0.099	0.043
	DRW (g)	0.008	0.006	0.001	0.048	0.057
	FSW (g)	0.089	0.135	0.009	0.150	0.074
	DSW (g)	0.011	0.009	0.001	0.063	0.009
Water-limited	RL (cm)	10.30	6.50	1.70	19.25	9.91
	SL (cm)	10.25	8.30	1.20	21	8.71
	FRW (g)	0.039	0.028	0.004	0.089	0.027
	DRW (g)	0.007	0.006	0.001	0.585	0.004
	FSW (g)	0.099	0.055	0.005	0.164	0.057
	DSW (g)	0.012	0.006	0.001	0.082	0.007

RL, root length; SL, shoot length; FRW, fresh root weight; DRW, dry root weight; FSW, fresh shoot weight; DSW, dry shoot weight.

Table 2. Mean square from combine analysis of variance (ANOVA) for agronomic traits at early seedling stage evaluated in glass house for well-watered and water-limited conditions

Source	df	RL	SL	FRW	DRW	FSW	DSW
Genotype (G)	201	25.2	11.2	4.50E-04	2.85E-05	6.66E-04	3.93E-05
Treatment (T)	1	490	395	2.68E-02	1.17E-04	2.98E-02	3.37E-04
G × T	202	7.5	3.1	1.63E-04	2.35E-05	2.23E-04	3.81E-05
Error	407	7.6	3.1	1.61E-04	2.33E-05	2.24E-04	3.78E-05
Heritability		0.75	0.79	0.96	0.54	0.71	0.51

All mean square values are significant at $P < 0.05$; RL, root length; SL, shoot length; FRW, fresh root weight; DRW, dry root weight; FSW, fresh shoot weight; DSW, dry shoot weight.

SL_{WL} viz. *QSL.nust-7B*, *QSL.nust-7B* and *QSL.nust-7B* were identified at 64, 104 and 221 cM, respectively. These are likely to be different QTL on the same chromosome due to the distance between them. The additive effect of four QTL, *QSL.nust-3A*, *QSL.nust-7B*, *QSL.nust-7B* and *QSL.nust-7D* were contributed by the synthetic allele.

Four QTL for FRW_{WW} were mapped on chromosome 3B (at 54 cM), 7A (at 38 cM) and two QTL on 7B (at 124 and 128 cM). These QTL explained 7.92% (*QFRW.nust-3B*) to 10.06% (*QFRW.nust-7B*) of the phenotypic variation and the additive effect was contributed by synthetic allele. Two QTL for FSW_{WW} were detected on chromosomes 6D and 7B. *QFSW.nust-7B*, is a major QTL and explained 59% of the phenotypic variance, while of *QFSW.nust-6D* explained 9.56% of the phenotypic variance. Both were contributed by a synthetic parent. A major QTL for DSW_{WL} was also identified on chromosome 3D at 63 cM, explaining 14.1% of the phenotypic variance with additive effect from synthetic allele. QTL were not detected for RL_{WW}, SL_{WW}, FSW_{WL}, FRW_{WL}, DSW_{WW}, DRW_{WW} and DRW_{WL}.

Table 3. Pearson's coefficient of correlation for six seedling traits

	RL	SL	FRW	DRW	FSW	DSW
RL		0.68**	0.66**	0.17*	0.59**	0.37**
SL	0.71**		0.54**	0.18*	0.74**	0.43**
FRW	0.70**	0.64**		0.31*	0.67**	0.35**
DRW	0.24*	0.28*	0.29*		0.08 ^{ns}	0.08 ^{ns}
FSW	0.56**	0.72**	0.69**	0.26*		0.49**
DSW	0.17*	0.21*	0.16*	0.06 ^{ns}	0.09 ^{ns}	

Upper diagonal represent values in water-limited treatment, and lower diagonal represent correlation in well-watered treatment.

*Significant at $P < 0.05$; **significant at $P < 0.01$; ns, non-significant; RL, root length; SL, shoot length; FRW, fresh root weight; DRW, dry root weight; FSW, fresh shoot weight; DSW, dry shoot weight.

Two QTL were identified for RL_{WL} and SL_{WL} on chromosome 3A. These QTL were identified by the same markers at 209 cM (*QRL.nust-3A*, *QSL.nust-3A*). Seven QTL were identified on chromosome 7B; of which one was for RL_{WL} (*QRL.nust-7B*), three for SL_{WL} (*QSL.nust-7B*), two for FRW_{WW} (*QFRW.nust-7B*) and one for FSW_{WW} (*QFSW.nust-7B*) (Table 4).

Discussion

Phenotypic variation for seedling morphology

Numerous methods have been used for phenotypic evaluation of morphological traits under drought stress which is subsequently required for genetic analysis (Iannucci *et al.*, 2017; Afzal *et al.*, 2017). In the present study, RIL population showed transgressive segregation for DRW and RL as compared to other traits under both treatments. It is likely that transgressive segregation in root traits might be a consequence of small leaf size, loss of turgidity and minimized transpiration (Foito *et al.*, 2009). Our phenotypic results were in accordance with Ayalew *et al.* (2017) for DRW and RL. Comparatively lower heritability of all traits under WL might be due to the fact that the genetics of drought adaptability is complex as compared to optimal environment. It has been reported that water stress at seedling stage leads to higher DRW, which can be utilized as selection criteria for drought tolerance at seedling stage (Takele, 2000). In addition, variation within RILs population indicated the presence of valuable alleles for these traits, which can be exploited in gene manipulation for drought adaptability.

QTL identified for seedling traits in ITMI mapping populations

Several association mapping and linkage mapping studies have been conducted for drought tolerance in bread wheat (Zhu *et al.*, 2005; Liu *et al.*, 2013; Ain *et al.*, 2015). These

Table 4. Summary of QTL detected in SynOpRIL ITMI population using GBS markers

Trait	QTL	Position (cM) ^a	Left marker	Right marker	LOD ^b	PVE (%) ^c	Add ^d	References
RL _{WL}	<i>QRL.nust-3A</i>	209	chr3A_21408488	chr3A_20841712	4.39	12.57	1.40	Ayalew et al., 2017
	<i>QRL.nust-4D</i>	37	chr4D_11509632	chr4D_16607007	3.39	9.67	-1.23	Czyczyło-Mysza et al., 2014
	<i>QRL.nust-7B</i>	122	chr7B_611967289	chr7B_610782563	2.54	6.99	1.07	Zhang et al., 2013
SL _{WL}	<i>QSL.nust-3A</i>	209	chr3A_21408488	chr3A_20841712	4.64	9.19	0.79	
	<i>QSL.nust-4A</i>	130	chr4A_3383160	chr4A_3351311	4.69	8.93	-0.78	Zhang et al., 2013
	<i>QSL.nust-7B</i>	64	chr7B_739128842	chr7B_732570779	2.61	4.79	-0.57	
	<i>QSL.nust-7B</i>	104	chr7B_659612030	chr7B_657034962	7.32	14.60	1.00	
	<i>QSL.nust-7B</i>	221	chr7B_5156956	chr7B_1384484	3.03	5.96	0.63	
	<i>QSL.nust-7D</i>	295	chr7D_5365880	chr7D_4175447	4.38	8.36	0.75	
	<i>QSL.nust-7D</i>	295	chr7D_5365880	chr7D_4175447	4.38	8.36	0.75	
FRW _{WW}	<i>QFRW.nust-3B</i>	54	chr3B_69622431	chr3B_64066119	2.58	7.92	0.01	
	<i>QFRW.nust-7A</i>	38	chr7A_723652273	chr7A_721520274	3.52	10.77	-0.01	
	<i>QFRW.nust-7B</i>	128	chr7B_597213328	chr7B_594383475	4.04	12.42	0.01	
	<i>QFRW.nust-7B</i>	124	chr7B_609103054	chr7B_606740729	2.73	10.06	0.01	
FSW _{WW}	<i>QFSW.nust-6D</i>	153	chr6D_27859416	chr6D_27356480	2.51	9.56	0.01	Liu et al., 2017
	<i>QFSW.nust-7B</i>	129	chr7B_587492916	chr7B_580857474	14.27	59.02	0.01	Zhang et al., 2013
DSW _{WL}	<i>QDSW.nust-3D</i>	63	chr3D_363865612	chr3D_554158392	2.73	14.13	0.002	

^aPosition of QTL located on chromosome: as cM distance from the top of each map.

^bA LOD threshold of 2.5 was used for declaration of QTL, based on 2000 permutations at a significance level of 0.01.

^cPhenotypic variance explained by QTL.

^dPositive 'additive effect' indicates an increasing effect from Synthetic (W7984); negative 'additive effect' indicates an increasing effect from 'Opata'.

studies have been valuable in identifying chromosomal regions related to drought and have also led to notable phenotypic variability. Furthermore, these studies identified novel haplotypes, which can help in deploying genes for tolerance against drought stress (Iannucci et al., 2017).

High marker density is crucial for gene mapping, and several GBS and SNP arrays have been introduced for gene discovery (Rasheed et al., 2017). Thus, 'SynOpRIL' population represents a valuable resource to identify novel genes for drought-related traits against drought. In the present study, 2639 SNPs from GBS platform were used for QTL study, which facilitated the development of a high-density map. A total of 16 QTL on nine different chromosomes were distributed for all traits except DRW. These QTL explained phenotypic variation ranging from 4.7 to 59%, out of which five QTL were considered as major QTL due to explaining more than 10% phenotypic variation. Among those, *QFSW.nust-7B* explained the highest phenotypic variation (59%) that may harbour an important gene.

Numerous QTL have been identified by utilizing various populations. Some new QTL are identified in the current study. Seven QTL from the present study were already reported in previous studies (Zhang et al., 2013;

Czyczyło-Mysza et al., 2014; Kabir et al., 2015; Luo et al., 2016; Ayalew et al., 2017; Ma et al., 2017) but the markers used for QTL mapping were different. The present study and Ayalew et al. (2017) identified QTL for RL on 3A under stress condition. In addition, QTL identified on chromosome 4D and 7B were previously reported as *QRLlengthPC.csd-4D*, *QRL.WL.7B.1* and *QRL.WL.7B.2* (Zhang et al., 2013; Czyczyło-Mysza et al., 2014). However, *QRLlengthPC.csd-4D* and *QRL.WL.7B.2* were identified under controlled conditions whereas *QRL.WL.7B.1* was found under both conditions. QTL on chromosome 4D was found within the confidence interval of Green-revolution gene (*Rht-D1*), but both were homozygous for *Rht-D1* and contain wild-type *Rht-D1a* allele. Therefore, it is likely that some other genes within the vicinity of *Rht-D1* are responsible for RL increase in the W7984 parent.

For SL_{WL}, *QSL.nust-4A* identified in this study was previously reported as *QSH.WY.4A2* under water stress condition on chromosome 4A (Zhang et al., 2013). To the best of our knowledge, the remaining QTL for SL_{WL} detected in the current study have not been reported in wheat earlier. Similarly, QTL for DSW_{WL} has also not been reported in wheat. Under control conditions, we identified four QTL on 3B, 7A and 7B (2) chromosomes for FRW. QTL on 3B may

agree with *QRFW.WL.3B* reported by Zhang *et al.* (2013). QTL for FSW were identified on chromosomes 6D and 7B. Liu *et al.* (2017) reported similar QTL as *QPFWR-WJ-6D* on chromosome 6D and Zhang *et al.* (2013) reported *QFSW.WY.7B* on chromosome 7B, which are likely to be the same QTL. On the other hand, QTL were not detected for some of the traits such as RL_{WW} , SL_{WW} , FSW_{WL} , FRW_{WL} , DSW_{WW} , DRW_{WW} probably due to the low abundance of markers results in a large gap in linkage map.

Many QTL clusters have been reported in previous studies (Kabir *et al.*, 2015; Luo *et al.*, 2016; Ma *et al.*, 2017). This study also identified one co-located QTL cluster on chromosome 7B. These QTL were detected for SL (3), FRW (2), RL and FSW. Based on a very high phenotypic variance, *QFSW.nust-7B* is likely to harbour a major gene for shoot biomass (Zhang *et al.*, 2013). Co-localization also indicated a closer association of highly correlated traits. These traits are most likely controlled by the same gene. The positive alleles of these traits are from W7984 parent and which could be traced back to its durum parent, i.e. 'Altar 84'.

Conclusion

Conclusively, QTL mapping using GBS markers identified 16 QTL for seedling morphology. Seven QTL detected on chromosome 7B positioned from 64 to 221 cM, could harbour important genes for drought adaptability. These QTL might serve for fine mapping, gene discovery and MAS in wheat breeding.

Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1017/S1479262118000023>.

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