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in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 355-361

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QTL mapping of morphological traits associated with drought adaptation in a Iranian mapping population of durum wheat

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Abstract. Drought stress represents one of the most important abiotic constraints to durum wheat production. The identification of genotypes carrying drought-related resistance traits and the mapping of the corresponding genomic regions are fundamental requirements to sustain the selection of new cultivars more adapted to semiarid conditions. Toward these aims a new genetic linkage map was constructed based on a cross between the local durum wheat local variety Zardak and the landrace 249 collected in the semiarid Kermanshah region (Iran). One hundred thirty single-seed descent derived F_6 recombinant inbred lines (RILs) were evaluated in a drought stress (rainfed) and supplemental irrigation conditions to investigate QTLs of drought tolerance and its associated traits. A primary skeletal molecular map constructed using 71 SSR markers has been developed and it is currently under implementation with AFLP markers. The linkage analysis defined 15 linkage groups. Several QTLs were found for morphological (peduncle length, awn length and flag leaf length) and physiological traits (relative water loss and exited leaf water retention) under rainfed and supplemental irrigation conditions.

Keywords. Quantitative trait loci (QTLs) – Durum wheat – Simple sequence repeat (SSR) markers – Drought stress.

Cartographie de QTL des caractères morphologiques liés à l'adaptation à la sécheresse dans une population de cartographie iranienne de blé dur

Résumé. *La sécheresse représente l'une des plus importantes contraintes abiotiques pour la production de blé dur. L'identification des génotypes portant des caractères de résistance à la sécheresse et la cartographie des régions génomiques correspondantes sont des exigences fondamentales pour soutenir la sélection de nouveaux cultivars plus adaptés aux conditions semi-arides. Face à ces objectifs, une nouvelle carte de liaison génétique a été élaborée sur la base d'un croisement entre la variété locale de blé dur Zardak et la variété primitive 249, collectées dans la région semi-aride de Kermanshah (Iran). On a évalué cent trente lignées pures recombinantes (RIL) F6, en filiation monograine, sous stress hydrique (régime pluvial) et on a exploré les conditions d'irrigation d'appoint pour identifier des QTL de tolérance à la sécheresse et ses caractères associés. Une carte moléculaire squelettique préliminaire a été élaborée en utilisant 71 marqueurs SSR est actuellement, on est en passe de la compléter par des marqueurs AFLP. L'analyse de liaison a défini 15 groupes de liaison. Plusieurs QTL ont été trouvés pour des caractères morphologiques (longueur du pédoncule, longueur des arêtes et longueur de la feuille étendard) et physiologiques (perte d'eau relative et rétention de l'eau provenant de la feuille) sous régime pluvial et d'irrigation d'appoint.*

Mots-clés. *Loci des caractères quantitatifs (QTL) – Blé dur – Marqueurs de répétition de séquence simple (SSR) – Sécheresse.*

I – Introduction

Durum wheat (*Triticum turgidum* L.), possessing the A- and B genomes, is an important cereal crop used mainly for different food products such as pasta, couscous, and burghul (Kubaláková *et al.,* 2005). Durum wheat is traditionally grown in semiarid environments where drought stress is the main constraint. Iran, with about 220 mm of average annual rainfall is a generally dry country with the exception of some northern provinces located in the vicinity of the Caspian Sea (Nouri-Ganbalani *et al.,* 2009). Landraces from Iran should therefore carry a number of adaptive traits allow them to growth under limited water availability, the identification of the genetic bases of such adaptation mechanisms might contribute to improve drought tolerance in modern durum wheat cultivars. Drought tolerance is a complex trait controlled by many genetic factors characterized by strong interactions with the environment (Reynolds *et al.,* 2006).

Genetic linkage maps are powerful tools for many studies, such as gene tagging, genome characterization, QTL analysis and evolutionary studies (Chu *et al.,* 2010). Construction of a genetic map plays a vital role in linkage analysis of agronomic traits and can be used to detect QTL for both abiotic and biotic stresses and therefore facilitate marker-assisted selection (MAS) (Peleg *et al.,* 2008). Identification of QTLs influencing grain yield and related traits in dry environments is needed to expand our knowledge on the adaptation of durum wheat under limited conditions and support the selection of more stress-tolerant cultivars, thus improving yield and yield stability in marginal regions. The application of molecular markers, such as amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR) and random amplified polymorphism DNA (RAPD), has provided effective approaches to dissect complicated quantitative traits into component loci to study their relative effects on a specific trait (Langridge *et al.,* 2001; Doerge, 2002.

The aims of this study were 1) to construct a genetic linkage map of Zardak × 249 (local variety and landrace from Kermanshah province, Iran), 2) to evaluate the performance of the RILs population under rain-fed and irrigated conditions and 3) to determine the chromosomal locations of the QTLs controlling yield and yield-related traits under drought conditions.

II – Material and methods

1. Materials and methods

One hundred-thirty F_6 recombinant inbred lines (RILs) deriving from the cross between the durum wheat genotypes ‘Zardak’ and ‘249’ (local variety and landrace from Kermanshah province, Iran, respectively) were used in this study. Field experiments were carried out at the Research Station of Faculty of Agricultural, Razi University, Kermanshah, Iran (latitude 34˚ 21′, longitude 47˚ 9′, altitude 1319 m) in 2009-2010 cropping season. Young leaves from F_6 seedling were cut as tissue samples for DNA extraction. Total genomic DNA was isolated according to the protocol described by Murray and Thompson (1980).

2. Phenotyping

The experiment was laid out in one-replicate within RCBD augmented design. To study the environmental effects on the expression of the traits, the RILs were sown at two locations namely, rain-fed and irrigated (supplemental irrigation) conditions. Fourteen days after anthesis, a supplemental irrigation was applied to one of the two field trials, whilst other field was carried out under rain-fed conditions. Morphological characters, including peduncle length (PED), awn length (AL) and flag leaf length (FL) were measured in rain-fed and irrigated conditions. Physiological traits including RWL (relative water loss) and ELWR (excised leaf water retention) were evaluated under both conditions. For RWL (%)) five young fully expanded flag leaves were sampled from each plot at anthesis stage. The leaf samples were weighed (FW), wilted for 4 h at 35°C, reweighed (W4h), and oven-dried for 24 h at 72°C to obtain dry weight (DW). RWL % = [(FW - W4h)/(FW - DW)] × 100 and ELWR % = [1- ((FW- W4h)/FW)] × 100, according to method of Farshadfar *et al. (*2002).

3. Molecular characterization

A total of 382 molecular markers, in particular 360 simple sequence repeats (SSRs) and 22 RAPDs were tested to detect polymorphism between parents. The forward primer of each pair of SSR markers was synthesized with the universal M13 tail at the 5′ end. The M13 tail was labeled either with carboxyfluorescein (FAM) or hexachlorofluorescein (HEX) fluorescent tags. Multiplexes of SSR fragments, different for color and size, were separated using an ABI 3130xl Genetic Analyzer sequencer (Applied Biosystems) and the GeneScan ROX 500 was used as size standard. Visualizations and sizing of the SSR fragments were performed using the GeneMapper software version 4.0 (Applied Biosystems). The RAPDs were analyzed on 1.2% agarose gel and stained with ethidium bromide.

4. Linkage analysis and map construction and QTL analysis

The genetic linkage map was constructed with JoinMap v. 4 (J.W. van Ooijen, 2006) and the Kosambi mapping function was used to calculate map distance (Kosambi, 1944). Markers were placed with a LOD threshold of 3.0 and a maximum REC frequency= 0.40. The association between phenotype and marker genotype was investigated using different mapping procedures, Simple Interval Mapping (SIM, Lander and Botstein, 1989) and Multiple QTL Model (MQM, Jansen and Stam, 1994) implemented in the MapQTL 6.0 software (Van Ooijen, 2009). Simple interval mapping (SIM) was used to identify the markers most significantly associated with variation. To enhance the power of QTL detection, the analyses were repeated using these markers identified by SIM as co-factors in a multiple QTL model (MQM).

III – Results

1. Linkage analysis

The preliminary genetic linkage map consists of 71 loci on 15 linkage groups including 62 SSR loci, 7 EST-SSR markers and one RAPD locus (Figure 1). These loci were mapped on chromosomes 1A, 1B, 2A, 2B, 3A, 4B, 5B, 6B, 7A and 7B. Total map coverage is 913.5 cM, excluding chromosome 3B, 4A, 5A and 6A which contained unlinked markers. This map provides an average distance of 12.86 cM between loci.

2. QTL analysis with simple interval mapping

Peduncle length (PED). Six QTLs were detected for PED under rain-fed conditions located on chromosomes 1B, 3A, 5B and 7A (Table 1). Under irrigated conditions, two QTLs were identified on chromosomes 5B and 6B (Table 1). One overlapping peak for PED under rain-fed and irrigated conditions was detected on chromosome 5B.

Table 1. Genetic characterization of QTL linked to PED under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	1B	Xgwm18	2.52	8.5	Zardak	1.04
	1B	Xbarc181	2.27	7.7	Zardak	1.11
	3A	XDuPw227	2.29	7.8	Zardak	1.11
	3A	Xwmc264	2.16	7.4	249	-1.05
	5B	Xgwm234	4.66	9.80	Zardak	1.41
	7A	XDuPw254	2.08	7.1	249	-0.92
Irrigated	5B	Xgmw234	2.00	6.8	Zardak	0.85
	6B	BF483631	3.26	10.9	249	-1.03

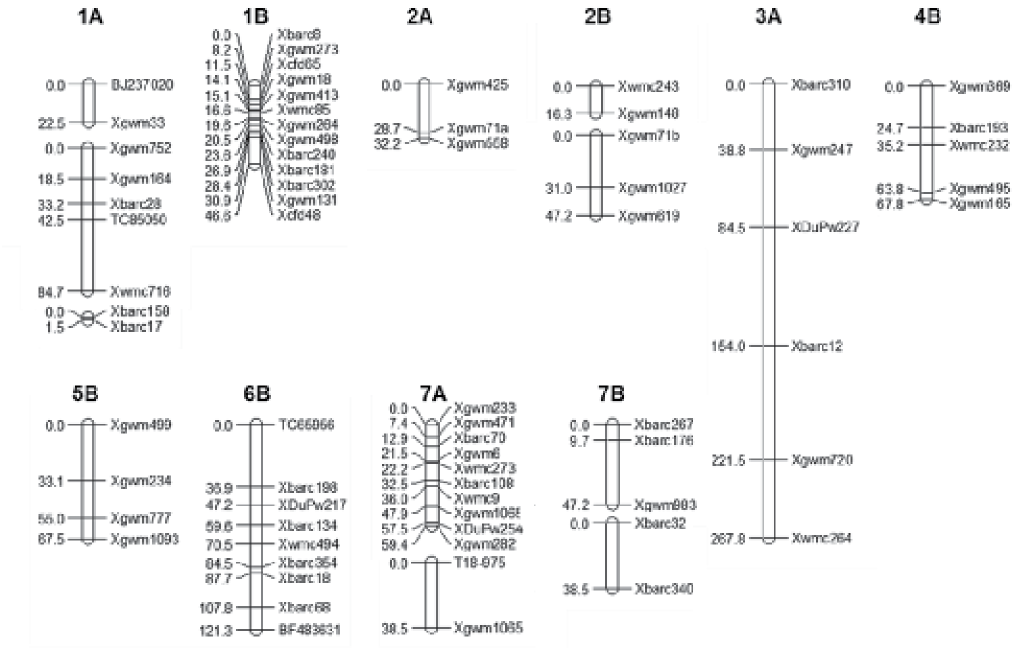

Figure 1. Linkage groups for the Zardak/249 F6 population.

Awn length (AL). In total, 6 QTLs were mapped for AL under rain-fed conditions, while eight QTLs were identified for the same trait under irrigated conditions (Table 2). Five QTLs located on chromosomes 6B and 7A were common under both conditions.

Flag leaf length (FL). Nine QTLs were detected on chromosomes1A, 4B, 6B, and 7A for flag leaf length under rained conditions (Table 3). Under irrigated conditions, eight QTLs significantly associated with FL were identified on chromosomes 6B and 7A (Table 3), all of them, except Xbarc108 located on 7A, were coincident with the loci detected in the rain-fed field trial. In the study of Dodig *et al* (2012) the variation in flag leaf width was found to be related with major yield QTL on 7A chromosome, expressed mainly under stressed conditions.

Relative water loss (RWL). Only two QTLs for RWL under rainfed conditions were found on chromosome 4B and 7B (Table 4). These QTLs were found to be environment responsive, most influential or exclusively detected under the dry treatment. No other QTLs were detected under irrigated conditions.

Excised leaf water retention (ELWR). A single QTL for ELWR was located on chromosome 7B under rain-fed conditions (Table 5). This QTL was found to be environment responsive, most influential or exclusively detected under the dry treatment. No other QTLs were detected under irrigated conditions.

Table 2. Genetic characterization of QTL linked to AL under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	5B	Xgwm234	2.34	8.0	Zardak	0.47
	6B	Xbarc68	5.34	17.2	249	-0.65
	6B	BF483631	2.66	9.0	249	-0.47
	7A	Xgwm471	2.74	9.3	249	-0.49
	7A	Xgwm6	3.29	11	249	-0.52
	7A	Xbarc108	2.51	8.5	249	-0.49
Irrigated	4B	Xgwm165	2.28	7.8	249	-0.53
	6B	Xbarc68	2.41	8.2	249	-0.42
	6B	BF483631	4.0	13.2	249	-0.62
	7A	Xgwm471	2.04	7.0	249	-0.40
	7A	Xbarc70	2.41	8.2	249	-0.43
	7A	Xgwm6	3.41	11.4	249	-0.58
	7A	Xbarc108	3.24	10.8	249	-0.59
	7A	Xwmc9	2.68	9.1	249	-0.46

Table 3. Genetic characterization of QTL linked to FL under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Expl.: Var %	Donor	Additive
Rainfed	1A	BJ237020	3.92	13.0	249	-0.022
	4B	Xgwm369	2.92	9.8	249	-0.020
	6B	Xbarc354	3.32	11.1	249	-0.026
	6B	Xbarc68	6.45	20.4	249	-0.028
	6B	BF483631	3.17	10.6	249	-0.019
	7A	Xgwm233	3.53	10.6	249	-0.019
	7A	Xgwm471	4.28	14.1	249	-0.024
	7A	Xbarc70	3.38	11.3	249	-0.022
	7A	Xgwm6	3.52	11.7	249	-0.022
Irrigated	6B	Xbarc354	2.24	7.6	249	-0.020
	6B	Xbarc68	2.44	8.3	249	-0.0177
	6B	BF483631	2.47	8.4	249	-0.0177
	7A	Xgwm233	2.43	8.2	249	-0.0179
	7A	Xgwm471	3.26	10.9	249	-0.0216
	7A	Xbarc70	3.76	12.5	249	-0.0222
	7A	Xgwm6	4.56	14.9	249	-0.0250
	7A	Xbarc108	2.29	7.8	249	-0.0167

Table 4. Genetic characterization of QTL linked to RWL under rainfed conditions.

Environment	Chromosome	Locus	LOD	Expl. var. (%)	Donor	Additive
Rainfed	4B	Xwmc232	2.18	7.4	Zardak	0.027
	7B	Xgwm983	2.21	7.5	Zardak	0.024

Table 5. Genetic characterization of QTL linked to ELWR under rainfed conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	7B	Xgwm983	2.18	7.4	249	-1.54

IV – Discussion

Most periods of drought stress encountered in the major grain-growing areas worldwide are transient, unpredictable, and imprecisely measured. These features result in the difficulties in breeding efforts for drought tolerance. Moreover, plant drought tolerance is a complex trait controlled by many genes and a large “genotype × environment” interaction. For AL, FL and PED we detected some consistent QTLs in two environmental conditions, suggesting the presence of genes related to stability of these traits under drought stress conditions. The magnitudes and signs of the additive effects reflect no genotype-environment interaction observed in these traits, as for the allele coming from a parent resulted in the same behavior in two environments. Although stable QTLs for these traits were identified, a more enriched molecular map as well as a more deep phenotypic characterization are essential to validate the QTL.

Table 6. MQM analysis for the traits under rainfed and irrigated conditions for RILs population of durum wheat.

Environment	Traits	Chromo-	Locus	LOD	Expl./ var. (%)	Donor	Additive effects
Rainfed	PED	5B	Xgwm234	5.13	16.5	Zardak	1.484
	AL	6B	Xbarc68	5.3	16.5	249	-0.640
	FL	7A	Xgwm233	3.18	10.6	249	-0.019
Irrigated	PED	6B	BF483631	3.26	10.9	249	-1.033
	FL	7A	Xgwm6	4	13.2	249	-0.020

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