QTL Analysis of Moderate Drought Response in a Rye Recombinant Inbred Line (RIL) Population Shows Co-Localization of QTLs for Morphological and Physiological Traits

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The study aimed to identify the loci responsible for some morphological traits, induced by drought in a recombinant inbred line (RIL) population of rye and to find out their connections with loci for other traits, using high density, consensus, genetic map. Twenty-five QTLs engaged in the response to drought (drought index, DI) of four morphological traits were detected. Thirteen QTLs induced by drought stress co-localized with QTLs for morphology, leaf rolling, pre-harvest sprouting (PHS), alpha-amylase activity and heading earliness. XrPt507374 from 3R was found to be the most validated marker, as the nearest one to the LOD peak of QTLs for five different traits: TGW-DI, plant height, spike length, spike compactness and PHS. Three genes were considered putative candidate genes for drought response on the basis of sequence alignment of DArTs linked to QTLs from 5R: DEAD-box ATP-dependent RNA helicase, thaumatin-like protein precursor (TLP) and transcription initiation factor TFIID.

Keywords: abiotic stress, genetic map, *Secale cereale* L., yield

INTRODUCTION

Drought is an environmental phenomenon that affects plant growth, development, and finally commercially available yield of cultivated plants. Many studies have focused on the drought resistance mechanisms in the most cultivated cereal crops (for review see Todaka et al., 2015). As rye is not so widespread over the world as bread wheat, maize or rice, it has not been the subject of as much intensive research as the other species. Rye is known for its outstanding drought tolerance and other positive attributes, such as excellent cold hardiness, tolerance to acidity of soils, and strong disease resistance. Thus, rye seems to be a good resource of beneficial traits for other crops. Better knowledge of the rye genome and genes responsible for drought tolerance could increase the efficiency of utilizing rye genes in wheat breeding and facilitate rye improvement itself.

Our research is a step toward increasing the knowledge about genetic base of rye response to water-deficit conditions. Till now, very few studies concerning rye reaction to drought have been published (Hübner et al., 2013; Haffke et al., 2015). Very little is known about QTLs for rye yield stability in response to drought conditions. All QTL analyses were conducted using rye chromosomes in wheat genetic background (Ehdaie et al., 2003; Farshadfar et al., 2012, 2013; Hoffman, 2008), and there is a lack of classical QTL analysis using the standard mapping population in the current scientific reports.

The aims of our study were: (1) to assess the differences of some morphological traits in control and provoked drought stress conditions,

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(2) to identify QTLs responsible for reaction to drought, (3) to detect co-localization of QTLs for drought response and for other morphological and physiological traits, and (4) to point out the most important markers correlated to DI. The recombinant inbred line (RIL) population and high density, consensus, genetic map of rye were used for these purposes.

MATERIALS AND METHODS

The experiment was carried out using two inbred lines of rye (S120 and S76) and 60 recombinant inbred lines of population RIL-M (S$_7$), which originated from the cross between S120 and S76. RIL-M is the mapping population, consisting of 143 lines, used to obtain high-density genetic map of rye (Milczarski et al., 2011) and for analysis of QTLs controlling some morphological traits (Myśków et al., 2014), earliness, and pre-harvest sprouting (Myśków et al., 2012).

Plant material used for QTL analysis was grown in a greenhouse of the West Pomeranian University of Technology in Szczecin (53.45°N, 14.53°E). Each inbred line was represented by two variants (control and stress conditions). There were seven plants of each line in every variant, grown together in one bucket of 10 dm^3 volume, filled with an equal mass (14.4 kg) of soil and sand mixture (1:1 v/v). Seeds were sown in January; initially plants were grown at the temperature of 15°C for the first three weeks, followed by a gradually decreasing temperature until close to atmospheric conditions. Further vegetation proceeded under natural conditions of the winter–spring period (February–September), with natural daylight duration.

Two irrigation regimes were applied via a drip system: well-watered control and water-limited variant, referred to as drought. Irrigation was maintained twice a day, every day for the control, to the first signs of leaking (0.26–0.33 dm^3 of water, depending on the temperatures). For the drought treatment, the watering was limited for four weeks. The period of drought coincided with the shooting, heading and the beginning of flowering.

After the harvest, the mean values of grain number per spike (GNPS), grain weight per spike (GWPS), thousand grain weight (TGW, estimated on the basis of GNPS and GWPS values), and spike number per plant (SNPP) were calculated for the control variant and for the variant subjected to drought. The difference between the averages, expressed in % of the control variant, represented a parameter named the drought index (DI). DI values were the base for the assessment of correlation coefficients and QTL analysis. Correlation

coefficients and significance of differences between rye lines were established by using the STATISTICA v.10.0 software (http://www.statsoft.com).

QTL analysis was performed using the consensus map of rye (Milczarski et al., 2011) and CIM method with Windows QTL Cartographer (WQTLCart) version 2.51 (Wang et al., 2007). Significant thresholds for declaring the presence of a QTL were estimated from 1,000 permutations of the data at $P = 0.05$.

Known sequences of DArTs (Gawroński et al., 2016) were subjected to alignment to NCBI database (https://www.ncbi.nlm.nih.gov/), using Megablast algorithm. Hits exceeding a score of 200 or/and identity of 95% and those with known (predicted) function were chosen.

RESULTS AND DISCUSSION

All the analyzed features exhibited Gaussian distribution, and the progeny lines showed transgression of all trait values. Although parents do not differ significantly (Table 1), our earlier experience with this mapping population suggests that QTL detection is possible because of transgression (Myśków et al., 2012). This was confirmed by analysis of variance for two groups of RILs (control genotypes, C, and genotypes under drought, D). One sample t-test showed significant differences for all traits and t-test for dependent samples (C vs D) revealed significant differences for all traits except for SNPP (Table 2).

We can observe an interesting phenomenon of developing more grains of smaller mass in plants undergoing stress. This rule has concerned parental lines as well as all inbred lines of the mapping population (Table 1). Both TGW and GWPS as well as the mean SNPP were lower. A similar rule was observed, for example, for wheat (Ehdaie et al., 2008) and for a rye inbred line in another experiment (Czyczyło-Mysza and Myśków, 2017). It seems that plants in response to adverse environmental conditions, occurring before flowering, produce a larger number of grains at the expense of their quality (weight) in order to increase the chances of survival of a possibly large number of offspring.

The drought indexes of GNPS and GWPS were significantly negatively correlated (-0.66, $P < 0.01$), and this was also reflected in the results of QTL analysis (QTL coincidence). TGW-DI and GWPS-DI were significantly correlated (0.73, $P < 0.01$). A lower, but statistically significant correlation was noted for GNPS-DI and SNPP-DI (0.33, $P < 0.01$), which also resulted in coincidence of some QTLs (Fig. 1).

CIM procedure revealed 25 QTLs engaged in the response to drought (DI) of four morphological

TABLE 1. Characteristics of grain parameters of control (C) and stressed plants (D) of mapping population and parental lines. GNPS – grain number per spike, GWPS – grain weight per spike [g], TGW – thousand grain weight [g], SNPP – spike number per plant; a,b,c,d – homogeneous groups established according to Duncan's test at probability level $P = 0.01$. s.e. – standard error; N – number of analyzed spikes.

genotype	factor	GNPS						GWPS					TGW				SNPP	N
		mean	s.e.	a	b	c	d	mean	s.e.	a	b	c	mean	s.e.	a	b	mean	
S120	C	19.13	1.51	*	*			0.47	0.04	*	*		25.03	0.96	*		2.29	16
	D	20.57	1.53		*	*		0.46	0.03	*	*		22.72	1.31	*	*	2	14
S76	C	15.59	1.52	*				0.38	0.05	*			23.53	1.07	*	*	2.83	17
	D	16.05	1.39	*				0.36	0.03	*			22.87	0.67	*	*	2.71	19
RILs	C	24.31	0.29		*	*		0.61	0.01			*	25.04	0.17	*		2.64	1048
	D	26.09	0.28			*		0.51	0.01		*	*	19.90	0.19		*	2.55	1014

TABLE 2. Analysis of variance for two groups of RILs (control genotypes and genotypes under drought), using the t-test for dependent samples. GNPS – grain number per spike, GWPS – grain weight per spike [g], TGW – thousand grain weight [g], SNPP – spike number per plant.

trait	mean difference	s.d.	t	df	P	95% confidence interval	
						lower	upper
GNPS	-1.70*	4.14	-3.18	59	0.00	-2.77	-0.63
GWPS	0.09*	0.17	4.29	59	0.00	0.05	0.14
TGW	4.96*	4.09	9.40	59	0.00	3.91	6.02
SNPP	0.09[ns]	0.71	0.98	59	0.33	-0.09	0.27

* difference significant at $P = 0.01$; [ns] not significant

traits in RIL-M population (Fig. 1, Table 3). QTLs for DI were distributed over all rye chromosomes, in 21 intervals. The number of QTLs varied from two on chromosomes 6R and 7R to five on chromosomes 1R and 3R. One QTL was detected for TGW-DI, 5 for GWPS-DI, 6 for SNPP-DI, and 13 for GNPS-DI. Three QTLs for GNPS-DI coincided with GWPS-DI and one GNPS-DI with SNPP-DI (Fig. 1). QTLs parameters (a, r^2) are presumably overestimated (Table 3) because of a relatively small number of RILs used for QTL analysis and due to WQTLCart algorithm specificity.

Up until now, in order to locate QTLs controlling agro-physiological criteria of drought tolerance in rye, wheat-rye disomic addition lines were used. This research revealed QTLs for agro-physiological indicators of drought tolerance on chromosomes 6R, 3R, 4R (Farshadfar et al., 2013) and 5R (Farshadfar et al., 2012). Also the wheat lines with 1RS translocations were more tolerant to drought (Ehdaie et al., 2003; Hoffman, 2008)

suggesting that the short arm of rye chromosome 1R carries genes controlling tolerance to environmental stresses. Our results also showed that chromosomes 5R and 1RS contain loci active in response to drought. There are four QTLs for GNPS-DI with high values of additive effect and variance explained on 5R and one on 1RS. However, it seems that even more interesting genomic regions for agronomic practice are these controlling GWPS (1RL, 3RS, 3RL, 4R) and TGW (3R). The coincidence of QTLs for different traits may indicate higher importance of particular genomic regions in determination of plant resistance to drought stress.

Our previous research made it possible to compare QTLs for DI with QTLs for other traits mapped in the same population, such as plant height (Ph), spike length (Sl), spike compactness (Ct), pre-harvest sprouting (PHS), alpha-amylase activity (AA), heading earliness (HE) (Myśków et al., 2012, 2014) and leaf rolling (RL, Myśków et al., 2018). This comparison (Fig. 1) showed
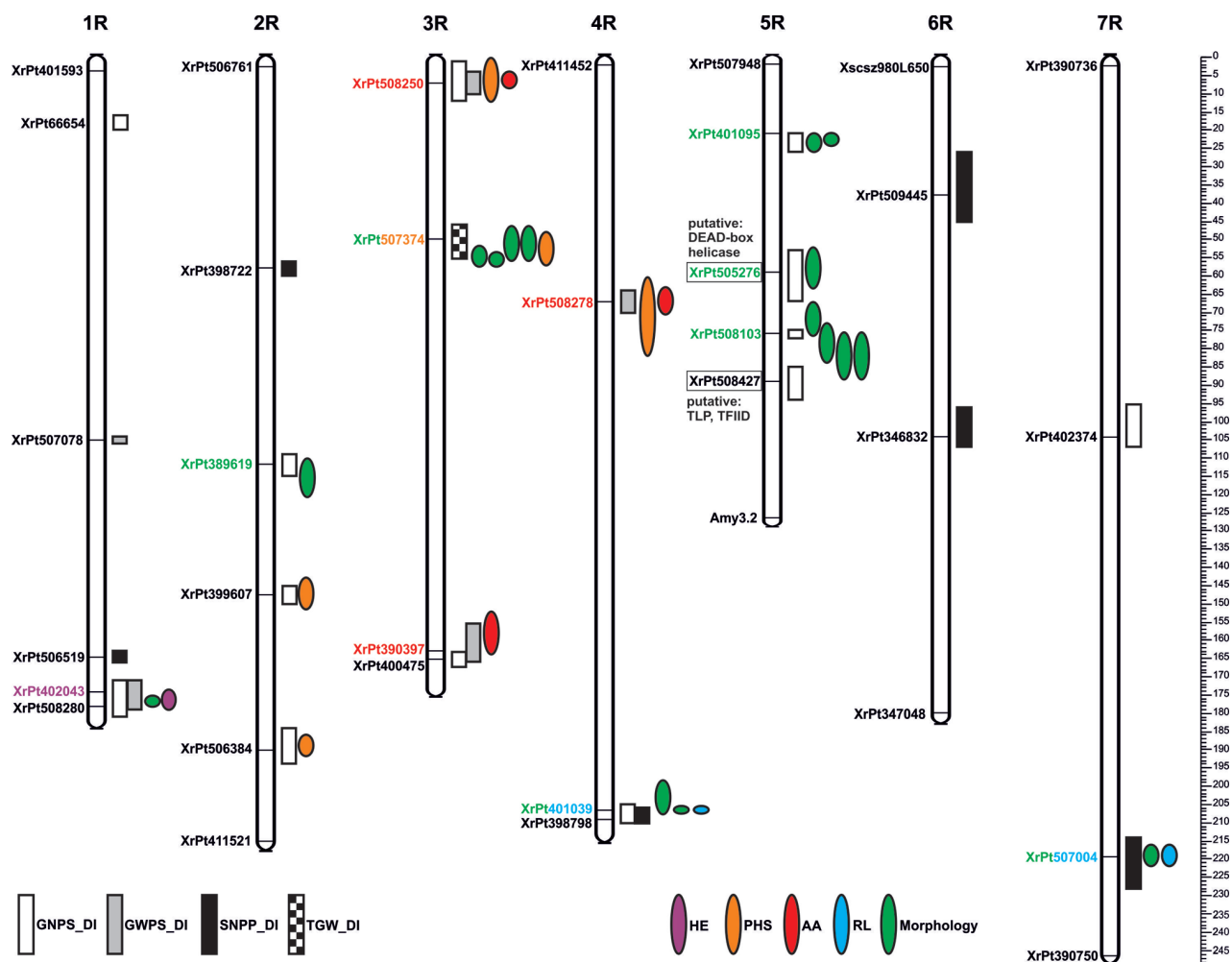
Fig. 1. QTLs for drought index (DI) of grain number per spike (GNPS), grain weight per spike (GWPS), spike number per plant (SNPP) and thousand grain weight (TGW) detected in rye RIL population S120×S76 (RIL-M), and their coincidence with QTLs for morphology (Ph, plant height; Sl, spike length; Ct, spike compactness); PHS, pre-harvest sprouting; AA, alpha-amylase activity; HE, heading earliness, published before (Myśków et al., 2012; Myśków et al., 2014) and RL, rolled leaves (Myśków et al., 2018). Markers nearest the peaks of at least two coinciding QTLs are colored. Putative candidate genes are framed.

overlapping of some QTLs for DI with QTLs both for morphology and PHS, AA, RL and HE in one case. In a few cases, some markers were common for different QTLs. The compatibility concerned 13 QTLs and 11 markers (Table 4); one of them was DArT, XrPt507374 from 3R, nearest to the LOD peak of 5 QTLs: TGW-DI, Ph, Sl, Ct, and PHS, which reaffirms the validity of this QTL.

Despite the low significance of QTLs, it was possible to detect putative candidate genes for morphological response to moderate drought. Out of 24 DArTs located on genetic map of rye in regions identified by CIM analysis as QTL peaks, eight of known sequences (Gawroński et al., 2016) were subjected to alignment to NCBI database. Two

DArTs from 5R (XrPt505276 and XrPt508427) showed high similarities to sequences deposited in NCBI (Table 5). The presence of maternal alleles of these markers coincided with differences in drought index of GNPS: -4.6% and +2.6%, respectively (to avoid overestimation of QTL parameters, specific to WQTLCart algorithm, these values were assessed based on raw data, as the difference between the mean values of DI of maternal and paternal allele). A similar or even stronger effect was observed for GWPS_DI (-8.3% and -3.7%, respectively) and TGW_DI (-4.3% and -5.0%, respectively), for which QTLs were not detected.

XrPt505276 sequence (450 nt) was most similar to *Aegilops tauschii* predicted DEAD-box

TABLE 3. QTLs for drought index of grain number per spike (GNPS_DI), grain weight per spike (GWPS_DI), thousand grain weight (TGW_DI) and spike number per plant (SNPP_DI), detected in rye RIL population S120×S76.

chromo-some	trait	QTL peak		interval [cM]		logarithm of odds	additive effect	variance explained
		nearest marker	position [cM]	from	to	LOD	a	r^2
1R	GNPS_DI	XrPt66654	17	16	20	1.77	-14.15	10.90
1R	GWPS_DI	XrPt507078	105	104	106	3.37	19.12	21.84
1R	SNPP_DI	XrPt506519	164	163	166	2.36	13.13	19.83
1R	GWPS_DI	XrPt402043	175	171	179	2.60	-11.24	15.30
	GNPS_DI	XrPt508280	180	171	181	1.94	-8.74	11.39
2R	SNPP_DI	XrPt398722	58	56	60	1.95	13.29	14.57
2R	GNPS_DI	XrPt389619	112	109	115	2.20	-9.79	13.93
2R	GNPS_DI	XrPt399607	147	145	150	2.05	-37.96	37.53
2R	GNPS_DI	XrPt506384	192	184	194	3.39	17.50	26.75
3R	GNPS_DI	XrPt508250	7	1	12	1.99	-41.49	13.90
	GWPS_DI	XrPt508250	7	4	10	4.50	29.29	28.40
3R	TGW_DI	XrPt507374	50	46	55	3.52	12.27	31.84
3R	GWPS_DI	XrPt390397	163	155	166	2.30	-19.11	16.32
	GNPS_DI	XrPt400475	165	163	167	2.52	14.49	17.77
4R	GWPS_DI	XrPt508278	67	64	70	3.46	20.42	26.03
4R	GNPS_DI	XrPt401039	207	205	210	2.73	12.76	15.45
	SNPP_DI	XrPt398798	209	206	210	1.86	-9.46	10.12
5R	GNPS_DI	XrPt401095	21	21	26	2.20	16.77	16.96
5R	GNPS_DI	XrPt505276	59	53	61	1.85	-36.55	31.41
5R	GNPS_DI	XrPt508103	76	75	77	3.79	-36.72	38.01
5R	GNPS_DI	XrPt508427	89	85	94	2.15	19.47	22.09
6R	SNPP_DI	XrPt509445	38	26	45	2.09	8.88	13.58
6R	SNPP_DI	XrPt346832	104	96	107	2.19	-9.22	12.99
7R	GNPS_DI	XrPt402374	104	95	107	2.39	13.13	17.68
7R	SNPP_DI	XrPt507004	219	214	228	1.85	-8.38	9.90

ATP-dependent RNA helicase 7 with the score of 412 (Table 5). XrPt508427 sequence (550 nt) was similar to *Secale cereale* thaumatin-like protein precursors and to *A. tauschii* predicted transcription initiation factor TFIID subunit 5, with the same score of 150 (Table 5). All these genes were proven to be engaged in plant response to oxidative stress. Genes in the family of DEAD-box ATP-dependent RNA helicases are known to be involved in stress resistance of different plants, i.e., *A. thaliana* (Kim et al., 2008), rice (Macovei et al., 2012) or tomato (Zhu et al., 2015). The thaumatin-like protein confers tolerance to a fungal pathogen and abiotic stress, including drought (Jung et al., 2005; Misra et al., 2016; Muoki et al., 2012; Singh et al., 2013).

TABLE 4. Coincidence of QTLs for drought index (DI) and some morphological, biochemical and physiological traits in rye RIL population S120×S76 published before (Myśków et al., 2012, 2014). GNPS – grain number per spike, GWPS – grain weight per spike, SNPP – spike number per plant, TGW – thousand grain weight, Ph – plant height, Sl – spike length, Ct – spike compactness, PHS – pre-harvest sprouting, AA – alpha-amylase activity, HE – heading earliness, RL – leaf rolling.

chromo-some	trait	QTL peak		interval [cM]		logarithm of odds	additive effect	variance explained
		nearest marker	position [cM]	from	to	LOD	a	r²
1R	GWPS_DI	XrPt402043	175	171	179	2.6	-11.24	15.3
1R	HE10	XrPt402043	175	172	179	2.45	0.4	5.22
1R	GWPS (Kps08)	XrPt509418	176	175	180	2.47	3.5	7.89
1R	GNPS_DI	XrPt508280	180	171	181	1.94	-8.74	11.39
2R	GNPS_DI	XrPt389619	112	109	115	2.2	-9.79	13.93
2R	GNPS (Kw10)	XrPt389619	113	110	121	2.96	-0.09	9.26
2R	GNPS_DI	XrPt399607	147	145	150	2.05	-37.96	37.53
2R	PHS08I	XrPt389683	151	143	151	2.9	-9	9.6
2R	PHS08I	XrPt344812	187	186	191	4.42	11.16	15.51
2R	GNPS_DI	XrPt506384	192	184	194	3.39	17.5	26.75
3R	PHS09I	XrPt399786	3	0	13	4.84	-14.31	24.27
3R	GNPS_DI	XrPt508250	7	1	12	1.99	-41.49	13.9
3R	GWPS_DI	XrPt508250	7	4	10	4.5	29.29	28.4
3R	AA08	XrPt508250	7	4	8	2.37	-2	8.04
3R	Sl10	XrPt507374	50	46	56	2.72	4.3	10.56
3R	TGW_DI	XrPt507374	50	46	55	3.52	12.27	31.84
3R	Ct10	XrPt507374	52	46	56	3.32	0.11	13.4
3R	PHS07I	XrPt507374	55	48	57	3.12	-8.87	17.19
3R	Ph09	XrPt507374	56	52	57	5.09	-4.19	14.5
3R	Ph10	XrPt507374	56	54	57	5.36	-5	15.56
3R	AA09	XrPt390397	159	151	164	3.93	-1.08	18.18
3R	GWPS_DI	XrPt390397	163	155	166	2.3	-19.11	16.32
3R	GNPS_DI	XrPt400475	165	163	167	2.52	14.49	17.77
4R	PHS09I	XrPt506460	60	60	83	2.91	-7.34	8.5
4R	AA08	XrPt508278	66	63	70	4.95	1.82	20.6
4R	GWPS_DI	XrPt508278	67	64	70	3.46	20.42	26.03
4R	GWPS (Kw08)	XrPt390758	207	198	208	4.49	-0.13	13.07
4R	GNPS (Kps08)	XrPt401039	207	206	208	4.47	-3.94	12.54
4R	RL10-2	XrPt401039	207	205	208	3.36	-0.36	10.77
4R	GNPS_DI	XrPt401039	207	205	210	2.73	12.76	15.45
4R	SNPP_DI	XrPt398798	209	206	210	1.86	-9.46	10.12

chromo-some	trait	QTL peak		interval [cM]		logarithm of odds	additive effect	variance explained
		nearest marker	position [cM]	from	to	LOD	a	r²
5R	GNPS_DI	XrPt401095	21	21	26	2.2	16.77	16.96
5R	GNPS (Kps08)	XrPt401095	22	21	24	4.43	-9.12	14.9
5R	GWPS (Kw08)	XrPt401095	22	21	26	3.13	-0.25	11.14
5R	GNPS_DI	XrPt505276	59	53	61	1.85	-36.55	31.41
5R	Sl08	XrPt505276	61	52	64	3.52	0.43	11.52
5R	Sl09	XrPt507127	71	67	77	2.3	-0.39	10.94
5R	Ct09	XrPt399477	74	73	84	3.51	-0.1	10.98
5R	GNPS_DI	XrPt508103	76	75	77	3.79	-36.72	38.01
5R	GNPS (Kps08)	XrPt508103	78	75	89	2.31	2.83	6.53
5R	GWPS (Kw08)	XrPt508103	78	75	89	2.51	0.09	7.32
7R	SNPP_DI	XrPt507004	219	214	228	1.85	-8.38	9.9
7R	Ph08	XrPt507004	220	216	222	2.76	4.15	8.31
7R	RL12	XrPt507004	221	210	221	4.24	-0.27	13.52

TABLE 5. Sequences from NCBI producing most significant alignments to DArTs localized nearest the peaks of QTLs for drought index.

	Description	Max score	Total score	Query cover	Expected value	Identity
XrPt505276, query length 450	*: *Aegilops tauschii* subsp. *tauschii* DEAD-box ATP-dependent RNA helicase 7 (LOC109748078), mRNA, XM_020307134.1	412	524	66%	3.00E-111	98%
	*: *Brachypodium distachyon* DEAD-box ATP-dependent RNA helicase 7 (LOC100837934), mRNA, XM_003578410.3	340	423	66%	1.00E-89	93%
	*: *Oryza sativa* Japonica Group DEAD-box ATP-dependent RNA helicase 7 (LOC4347610), mRNA, XM_015756807.1	296	296	52%	3.00E-76	89%
	*: *Oryza brachyantha* DEAD-box ATP-dependent RNA helicase 7 (LOC102713299), mRNA, XM_006660793.2	285	285	52%	7.00E-73	89%
	*: *Setaria italica* DEAD-box ATP-dependent RNA helicase 7 (LOC101776813), mRNA, XM_004957334.2	230	230	52%	3.00E-56	84%
XrPt508427, query length 550	*: *Aegilops tauschii* subsp. *tauschii* transcription initiation factor TFIID subunit 5 (LOC109778135), transcript variant X1, mRNA, XM_020336692.1	150	150	14%	3.00E-32	100%
	Secale cereale thaumatin-like protein 1/2/3 precursor (TLP2) mRNA, complete cds, AF096927.1, AF099671.1, AF099670.1	150	150	16%	3.00E-32	97%

* – predicted

The transcription initiation factor TFIID is involved in the regulation of gene expression and adaptation to osmotic stress (Gao et al., 2006; Kebede et al., 2015).

Our results shed light on the network of QTLs controlling reaction to water deficit and other morphological and physiological traits. The detection of predicted candidate genes is an additional value of this study, however the expression analysis is needful to verify and confirm these candidate genes' role in water-deficit stress response in rye.

AUTHORS' CONTRIBUTIONS

BM conceived, designed and performed the experiment, analyzed QTL, drafted the paper. IC-M made the statistical analysis, wrote the Introduction. SSo made the measurements and statistical analysis. SSt critically revised manuscript. The authors declare no conflict of interest.

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