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Mapping QTL for traits related to summer dormancy in tall fescue

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Tall fescue is an important perennial, coolseason forage grass in the United States, and mediterranean morphotypes of the grass exhibit partial summer dormancy as drought adaptation. Tall fescue breeders in the USA are focusing on using the trait for improved persistence of tall fescue during harsh summers. The present study was conducted to identify genomic regions associated with summer-dormancy-related traits in tall fescue. In this study, a pseudo F1 bi-parental population of 195 members was developed by crossing between a Mediterranean parent, 103-2, and a Continental parent, R43-64. The population was genotyped using 2,000 molecular markers consisting SSRs and DArT. Parent-based linkages maps were produced using JOINMAP 4.0, and QTL mapping was conducted using QTL Cartographer V2.5. A total of 17 traits were used for QTL mapping in 22 and 23 linkage groups of R43-64 and 103-2 parent respectively. Chromosome 1, 2, 4, 5, 6, 7, 11, 12, 13, 17, 19 and 22 in R43 and 3, 4, 6, 7, 12, 13 14, 15, 18, 20, 22 and 23 in 103-2 parent were detected with major QTL for plant height, tiller number, new leaf, fresh biomass weight and dry biomass weight. The variability explained by these QTL ranged from 9.91 to 22.32. Markers in the flanking region of the identified QTL will be applied for marker-assisted breeding in tall fescue for integration of summer dormancy in Continental morphotypes.

Materials and Methods

A pseudo F1 bi-parental population consisting of 200 progenies was developed by crossing a Mediterranean parent, 103-2, and a Continental parent, R43-64. Plants were phenotyped under a long day of 16-hourlight condition accompanied by 34 degrees Celsius/24 degrees Celsius (day/night) temperature for summer dormancy and 10 hours light condition accompanied with 21 degrees Celsius/16 degrees Celsius (day/night) temperature for optimum growth. Data were taken on tiller number (TN), new leaf (NL), plant height (PHT) fresh weight (FW), dry weight (DW), average tiller weight (ATW) and moisture content (MST) during three different stages, i.e., be-

Schematic presentation of phenotyping procedure



fore cut back, after cut back and return to normal growth (Fig 1).

Data analysis was done using PROC UNIVARIATE procedure and PROC GLM procedure of SAS 9.3. Total of 1,968 molecular markers (SSR and DArT markers) were used for linkage mapping. Linkage maps were constructed by JOINMAP 4.0. QTL analysis was conducted using the Windows version of QTL Cartographer V2.5. The difference of the mean data between two conditions was used for QTL mapping. Epistasis analysis was done using QTL IciMapping V4.0

Introduction

Summer dormancy is a phenomenon found in Mediterranean tall fescue (*Festuca arundinacea* (Schreb.) S.J. Darbysh), which is endogenously controlled and coupled with a series of processes including growth reduction, cessation and/ or senescence under nonlimiting moisture conditions during summer (Norton et al., 2006). It is an important drought adaptation trait of cool-season perennial grasses in the Mediterranean climates to survive under harsh summer conditions (Vegis, 1964). In the Southern Great Plains, the persistence and productivity of cool-season perennial grasses is significantly affected by hot and dry summers (Malinowski et al., 2005). Thus understanding and using summer dormancy mechanism in tall fescue would help in developing suitable cultivars for the region. Marker-assisted selection (MAS) is an appealing method for selecting complex traits like summer dormancy. Identifying and using markers flanked to quantitative trait loci (QTL) associated with summer-dormancy-related traits will pose a significant advantage over phenotypic selection to the breeders. The aim of the project is to identify markers and QTL regions associated with summer-dormancy-related traits for potential use in marker-assisted breeding programs.



Fig 1. Schematic presentation of phenotypic data collection at three different stages of the experiment.

Results

All the traits showed normal distribution and transgressive segregation except AATW (Fig. 2 and Fig. 3) and were significantly correlated (Table 1).



Table 1. Correlation among the phenotypic traits measured at three different stages during the experiment																	
	Ν	ATN	ANL	APHT	AFW	ADW	AMST	AATW	RTN	RNL	RPHT	RFW	RDW	RMST	RATW	BFW	BDW
ATN	765																
ANL	765	0.59053**															
APHT	765	-0.27017**	-0.19575**														
AFW	761	-0.02083	0.01646	0.03961													
ADW	763	0.07081	0.09999**	0.24477**	0.05658												
AMST	760	-0.05151	-0.09467**	0.15159**	0.08542*	-0.1074**											
AATW	760	-0.15375**	0.10181**	-0.13795**	0.46551**	0.24145**	-0.25972**										
RTN	762	0.84286**	0.49101**	-0.26935**	-0.03221	0.09933**	-0.01499	-0.19463**									
RNL	762	0.48244**	0.30238**	-0.25157**	-0.03294	0.02304	-0.07993*	0.09022*	0.5952**								
RPHT	762	-0.28537**	-0.27098**	0.46662**	0.00258	0.04733	0.09539**	-0.19532**	-0.28075**	-0.25654**							
RFW	710	-0.13417**	0.12885**	-0.15215**	0.06664	0.26994**	-0.32904**	0.88886**	-0.18109**	0.12931**	-0.15883**						
RDW	710	0.07421*	0.1256**	-0.01735	0.01792	-0.01267	-0.15312**	0.29762**	0.04132	0.10968**	0.1978**	0.4839**					
RMST	710	0.00027	-0.06243	0.24302**	0.0014	0.09498*	0.37088**	-0.31907**	0.00446	-0.08834*	0.10407**	-0.3271**	-0.38853**				
RATW	710	-0.16569**	0.1121**	*0.18306**	0.06584	0.25625**	-0.35192**	0.91224**	-0.20861**	0.12181**	-0.23103**	0.97313**	0.3316**	-0.35705**			
BFW	755	0.24694**	0.10733**	0.41181**	0.03532	0.42299**	0.10543**	-0.1693**	0.26979**	-0.03052	0.28082**	-0.14963**	0.14926**	0.16653**	-0.21765**		
BDW	757	0.21595**	0.17804**	0.21663**	0.04656	0.46259**	0.06287*	0.17959**	0.22532**	0.06909	0.18149**	0.24956**	0.32285**	-0.02003	0.18795**	0.84547**	
BMST	754	0.03257	-0.0906*	0.30711**	0.01513*	-0.06215	0.24239**	-0.44212**	0.05132	-0.11975**	0.12837**	-0.53134**	-0.27627**	0.24413**	-0.52912**	0.1806**	-0.30794**

The linkage maps pertinent to the Continental parent (R43-64) comprised 817 markers. Twenty-two chromosomal groups have been identified covering a total and average size of 1956.30 and 88.92 cM respectively. Average marker interval of the groups was 2.39.

The Mediterranean parental linkage maps accommodated 395 markers. Twenty-three chromosomal groups were identified covering a total size of 1534.56 cM. The average chromosomal group size was 69.75 cM with a marker interval of 4.32 cM. QTL were identified in 11 linkage groups of male parental maps and 13 groups in female parental maps for various measured traits (Table 2 and 3). QTL identified in the male parental maps had the LOD range of 2.18-4.79 with explained phenotypic variability (EPV) of 10.03 to 22.32. All the traits except ATN were found associated with at least one QTL distributed in chromosomal group 1, 2, 4, 5, 6, 7, 11, 12, 13, 17, 19 and 22 (Table 2).

Table 3. Significant QTL along with marker intervals, LOD value and explained phenotypic var	riati
on various female (103-2) linkage groups.	

	•		U				
		Traits	QTL			additive	phenotypic
	LG	name	range	Flanking marker		value	variation
Qsdatn.nf-3f	3	AIN	29-35	nffg1/0_224, nffg2/3_194	2.23	1.04	10.31
Qsdbmst.nf-3f	3	BMST	42-50	nffg273_194, nffa155_211	2.4	-3.28	11.01
Qsdanl.nf-4f	4a	ANL	50-58	nffa788_298, nffg335_177	2.2	1.1	10.05
Qsdrdw.nf-4f	4a	RDW	15-25	loPt_558615, loPt_355986	2.35	0.046	10.76
Qsdanl.nf-4bf	4b	ANL	12-30	nffg391_147, nffa295_239	2.18	-0.89	9.91
Qsdrtn.nf-5f	5	RTN	19-40	loPt_560563, loPt_555824	3.11	-1.56	14.22
Qsdbfw.nf-5f	5	BFW	4-30	nffa877_277, loPt_557383	2.35	-0.37	10.8
Qsdadw.nf-6f	6	ADW	0.01-21	loPt_562378, nffg171_143	3.86	-0.08	17.83
Qsdrpht.nf-7f	7	RPHT	65-85	nffa773_126, nffa206_321	3.07	1.09	14.34
Qsdbdw.nf-7f	7	BDW	90-94	nffa206_321, nffg106_176	2.5	0.19	11.51
Qsdapht.nf-12f	12	APHT	2-14	nffg332_230, loPt_556156	2.4	2.2	10.82
Qsdadw.nf-12f	12	ADW	6-23	nffg332_230, loPt_562496	3.45	0.08	15.88
Qsdatn.nf-13f	13	ATN	67-80	nfmf088_168, loPt_561075	2.2	-1.18	10.06
Qsdrtn.nf-13f	13	RTN	69-80	nfmf088_168, loPt_561075	3.5	-1.27	16.28
Qsdrpht.nf-20f	20	RPHT	18-46	nffa802_496, loPt_561092	2.45	1.28	11.2
Qsdamst.nf-22f	22	AMST	0.5-10	nffa677_316, nffs205_564	2.68	-8.32	12.33
Qsdafw.nf-23f	23	AFW	16.1-16.7	nffa635_433, nffa671_230	4.67	-4.1	21.44
Qsdrfw.nf-23f	23	RFW	2-14	nffa360_464, nffa709_441	2.7	-0.19	12.45
Qsdaatw.nf-23f	23	AATW	16.1-16.7	nffa635_433, nffa671_230	4.6	-0.78	21.27
Qsdratw.nf-14bf	14b	RATW	0.1-5	nffa787_580, nffa635_431	2.3	-0.033	10.31
Qsdatn.nf-14cf	14c	ATN	7-16	nffg295_173, nffa800_181	3.65	-1.62	16.84
Qsdrtn.nf-14cf	14c	RTN	11-18	nfmf050_181, nfmf228_287	3.47	-1.61	16.1
Qsdrfw.nf-14cf	14c	RFW	13-16	nffa635_388, nffa800_181	3.52	0.28	16.2
Qsdratw.nf-14cf	14c	RATW	13-16	nffa635_388, nffa800_181	2.4	0.04	10.98
Qsdafw.nf-22m	22	AFW	2-8	nffg301_188, nffg301_190	2.9	-2.08	13.33
Qsdaatw.nf-22m	22	AATW	0.02-8	nffg301_188, nffg301_190	2.81	-0.38	13.02

Conclusion

QTL on chromosome 6 in male parental map and chromosome 14c in female parental map have high potential and should be targeted for marker-assisted breeding for screening summer dormancy in the hybrid population.

Fig 2. Phenotypic distribution of various traits under optimum growing condition.



Table 2. Significant QTL along with marker intervals, LOD value and explained phenotypic									
variation on vario	us mai	Traits		e groups.		additive	phenotypic		
QTL name	LG	name	range	Flanking marker	LOD	value	variation		
Qsdrpht.nf-1m	1	RPHT	27-29.2	nffa803_179, nffa803_182	4	1.07	18.36		
Qsdamst.nf-1m	1	AMST	0.01-10	nffs027_138, nffa448_166	3.1	10.51	14.24		
Qsdbmst.nf-2m	2	BMST	24-36	nfmf032_262, nffg009_222	3.07	-3.02	14.11		
Qsdamst.nf-4m	4	AMST	15-33	nffa387_193, nffa689_529	3	6.71	13.84		
Qsdrmst.nf-4m	4	RMST	27-35	nffa387_193, nffa689_529	2.65	6.24	12.57		
Qsdrtn.nf-5m	5	RTN	19-22	nfmf038_163, nffa722_287	3.06	0.98	14.15		
Qsdapht.nf-5m	5	APHT	11.4-18.1	nffg110_168, nfmf038_163	4.29	2.18	19.81		
Qsdrtn.nf-6m	6	RTN	4-10.5	nffg273_205, nffg170_226	3.5	-1.11	16.17		
Qsdanl.nf-6m	6	ANL	6-10.5	nffg273_205, nffg170_226	3.4	-1.12	15.69		
Qsdbfw.nf-6m	6	BFW	2-11	nffg273_205, nffg170_226	2.45	-0.38	11.12		
Qsdrfw.nf-6m	6	RFW	30-36	nffg264_211, nffa709_464	4.2	-0.12	19.43		
Qsdrdw.nf-6m	6	RDW	30-51	nffg264_211, nfmf100_135	4.01	-0.06	18.52		
Qsdadw.nf-7m	7	ADW	92.9-95.1	nffa610_194, nffa610_194	3.83	0.08	17.77		
Qsdrfw.nf-11m	11	RFW	114-130	nffg260_212, nffg222_358	2.25	0.14	10.29		
Qsdratw.nf-11m	11	RATW	56-69	nffa826_164, nffa321_109	2.64	-0.03	12.15		
Qsdrnl.nf-12m	12	RNL	123-141.5	nffa069_281, nffa475_103	2.68	-1.23	11.57		
Qsdadw.nf-12m	12	ADW	118-119.5	nffg673_596, nfmf087_159	3.04	-0.05	14.1		
Qsdbmst.nf-12m	12	BMST	12-22	nffg084_181, loPt_561700	3.75	-3.41	17.25		
Qsdbdw.nf-13m	13	BDW	24-55	nffa059_153, nffg366	4.79	-0.2	22.32		
Qsdratw.nf-13m	13	RATW	55-71	nffg366_192, nffg167_108	3.5	-0.03	16.09		
Qsdbmst.nf-17m	17	BMST	6-14	loPt_555144, loPt_561133	3.06	4.56	14.05		
Qsdrtn.nf-19m	19	RTN	62-73	nffa635_439, nffa635_412	2.93	-1.03	13.43		
Qsdatn.nf-22m	22	ATN	10-14	nffg301_190, nffg301_172	2.18	1.39	10.03		
Qsdanl.nf-22m	22	ANL	15-19.5	nffa050_213, nffa147_221	3.75	1.99	17.14		
Qsdrpht.nf-22m	22	RPHT	8-18	nffg301_190, nffa291_527	2.48	-2.43	11.46		
Qsdafw.nf-22m	22	AFW	2-8	nffg301_188, nffg301_190	2.9	-2.08	13.33		
Qsdaatw.nf-22m	22	AATW	0.02-8	nffg301_188, nffg301_190	2.81	-0.38	13.02		

Significant epistatic QTL were found for APHT, RNL, RPHT and BDW in the male (R-43) parental maps (Fig 4) and for AFW, ADW, AMST, AATW and RTN in the female parental maps (Fig 5).



Fig 4. Epistatic interaction of various traits among the chromosomes of detected QTL on male (R43-64) parental linkage maps



Norton, M., F. Volaire and F. Lelievre. 2006. Summer dormancy in Festuca arundinacea Schreb.; the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. Crop and Pasture Science 57: 1267-1277.

Vegis, A. 1964. Dormancy in higher plants. Annual review of plant physiology 15: 185-224. Volaire, F. and M. Norton. 2006. Summer dormancy in perennial temperate grasses. Ann. Bot. 98: 927-933.

Malinowski, D., D. Belesky and G. Lewis. 2005. Abiotic stresses in endophytic grasses. Neotyphodium in cool-season grasses: 187-199.

Fig 3. Phenotypic distribution of various traits under summer dormant growing condition.

The LOD and EPV range of the QTL in female parental maps were 1.88 to 4.67 and 8.47 to 21.44 respectively. All traits but RMST and RNL were found associated with at least one QTL distributed in chromosomal groups 3, 4a, 4b, 5, 6, 7, 12, 13, 14b, 14c, 20, 22 and 23 (Table 3).

