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ORIGINAL PAPER

QTL mapping for yield and lodging resistance in an enhanced SSR-based map for tef

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Abstract Tef is a cereal crop of cultural and economic importance in Ethiopia. It is grown primarily for its grain though it is also an important source of fodder. Tef suffers from lodging that reduces both grain yield and quality. As a first step toward executing a marker-assisted breeding program for lodging resistance and grain yield improvement, a linkage map was constructed using 151 F_9 recombinant inbred lines obtained by single-seed-descent from a cross between *Eragrostis tef* and its wild relative *Eragrostis pilosa*. The map was primarily based on microsatellite (SSR) markers that were developed from SSR-enriched genomic libraries. The map consisted of 30 linkage groups and spanned a total length of 1,277.4 cM (78.7% of the genome) with an average distance of 5.7 cM between markers. This is the most saturated map for tef to date, and for the first time, all of the markers are PCR-based. Using agronomic data from 11 environments and marker data, it was possible to map quantitative trait loci (QTL) controlling lodging, grain yield and 15 other related traits. The positive effects of the QTL identified from the wild parent were mainly for earliness, reduced culm length and lodging resistance. In this population, it is now possible to combine lodging resistance and grain yield using a marker-assisted selection program targeting the QTL identified for both traits. The newly developed SSR markers will play a key role in germplasm organization, fingerprinting and monitoring the success of the hybridization process in intra-specific crosses lacking distinctive morphological markers.

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Introduction

Tef, *Eragrostis tef* (Zucc.) Trotter, belongs to the family Poaceae, subfamily Eragrostoideae. In Ethiopia, the flour from tef is fermented and baked to produce flat pancake-like bread known as "injera". Tef straw is the most appreciated feed for cattle (Ketema 1997). The continuous and rising demand from urban consumers and for export purposes has recently created a very profitable market for the crop. It is currently the leading cereal crop in Ethiopia, occupying 22% of the total area devoted to field crops. Tef has also crossed the Ethiopian borders and now grows in the United States, Australia and South Africa as a forage crop for its fine, highly palatable stems. In Europe, its gluten-free flour has prompted the use of tef in food products for individuals prescribed a life-long gluten-free diet due to diagnosis of celiac disease (Spaenij-Dekking et al. 2005; Hopman 2008).

Tef is an allotetraploid ($2n = 4x = 40$; Tavassoli 1986) whose origin within the large genus *Eragrostis* is unknown. Its genome size is estimated to be equal to 730 Mbp. Early studies based on morphological, anatomical, cytological and biochemical data have suggested a total of 14 wild *Eragrostis* species as potential progenitors. However, phylogenetic analysis of sequence data from the nuclear gene *waxy* and the plastid locus *rps16* (Ingram and

Doyle 2003) strongly support the widely held hypothesis of a close relationship between tef and *E. pilosa*, a wild allotetraploid.

Tef production in Ethiopia suffers from two major problems: low grain yield and stem lodging. Improvement of grain yield through phenotypic selection within existing natural variation and inter-specific hybridization derivatives has been progressing slowly. It was estimated that the average annual increase amounts to about 0.398 kg/ha/year (Teklu and Tefera 2005). Lodging has been attributed to the tall and weak stems that are unable to hold the heavy panicles, making lodging resistance a hard trait to breed for as it is negatively correlated with increase in grain yield (Hundera et al. 2000). To date, there have not been any lodging-resistant genotypes identified in the available germplasm or any success in breeding for that trait. In attempt to increase the amount of variation in cultivated tef, especially for plant height, the first inter-specific cross between *E. tef* and its wild relative *E. pilosa* was conducted by Tefera et al. (2003). *E. pilosa* has a much shorter stature and could contribute to lodging resistance. Stem lodging, however, is a complex trait affected by many stem characteristics and is hard to estimate in the field, especially since it can occur at different growth stages. Many studies have been conducted on the correlation between lodging and stem characteristics in cereal crops to indirectly select for lodging resistance. Shorter and stronger stalked wheat and rice plants led to improvement in lodging resistance and at the same time better response to nitrogen fertilization (Hedden 2003). Other stem characteristics that have been utilized for improving lodging resistance in cereals include stem diameter, culm wall thickness, length of the basal internodes, number of internodes, peduncle length and stem strength (Zuber et al. 1999; Milach et al. 2002; Flint-Garcia et al. 2003; Kelbert et al. 2004; Hai et al. 2005; Kashiwagi et al. 2008). Molecular markers have played a major role in understanding the nature of the genes underlying the dwarfed phenotype (Peng et al. 1999) and facilitated the identification and selection of the desired phenotype (Ahmad and Sorrells 2002; Ellis et al. 2002). In tef, not much is known about dwarfing genes, however, attempts to clone and sequence the orthologues of the *rht1* and *sd1* genes are in progress (J. L. Bennetzen, personal communication).

Different molecular marker techniques have been employed to study the genetic diversity available in tef (Bai et al. 1999b, 2000; Ayele and Nguyen 2000). One common feature observed in these studies was the generally low level of polymorphism detected on the marker level. Despite a 6.1% polymorphism level using amplification fragment length polymorphism (AFLP) markers, Bai et al. (1999a) constructed the first genetic map in tef using a set of F_5 recombinant inbred lines (RILs) from the intra-specific cross of the cultivars Kaye Murri and Fesho. Higher polymorphism (67%) was achieved by Zhang et al. (2001) who utilized restriction fragment length polymorphism (RFLP) markers on 116 F_8 RILs from an inter-specific cross between the cultivar Kaye Murri (*E. tef*) and its wild progenitor *E. pilosa* (accession 30-5). Utilizing 94 RILs of the same inter-specific cross, Yu et al. (2006b) constructed another map based on some of the previously mapped RFLP markers in addition to a set of tef-specific markers from expressed sequence tags (EST-SSR) developed from the parental lines of that cross. The developed EST-SSRs showed a polymorphism level of 24%. Another inter-specific cross (*E. pilosa* accession (30-5) × cultivar DZ-01-2785) was also used for linkage map development in tef (Chanyalew et al. 2005).

With the existence of mapping populations and polymorphic markers, identification of quantitative trait loci (QTL) for important agronomic traits in tef was made possible (Chanyalew et al. 2005; Yu et al. 2007). QTL for yield, lodging index, shoot biomass and other traits were identified in both studies. One of the major obstacles for further utilizing the outcomes of those studies was that most of the QTL identified were linked to either AFLP or RFLP markers, known to be very laborious and require special lab settings to reproduce.

The aim of this study was to develop PCR-based markers that would be suitable for updating the existing map based on the cross Kaye Murri × *E. pilosa* accession (30-5) and identify QTL for grain yield and lodging resistance and related traits. This was achieved by: (1) developing a set of SSR markers through library enrichment methods; (2) screening heterologous markers from pearl millet, finger millet and rice for polymorphism to insure anchor-markers are included on the map for comparative mapping purposes; (3) QTL mapping of grain yield, lodging resistance and related traits on the newly developed map to identify putative QTL that can improve the cultivated tef.

Materials and methods

Plant material and statistical analysis of phenotypic data

The 151 F_9 RILs used in this study were derived by single-seed-descent from the inter-specific cross between *E. tef* (cultivar Kaye Murri) and *E. pilosa* (accession 30-5) made in 1995 at the Debre Zeit Agricultural Research Center in Ethiopia (Tefera et al. 2003). Kaye Murri is tall, thick culmed and late maturing, with compact and long panicles, red lemmas and white grains. The accession 30-5 of *E. pilosa*, on the other hand, is a weedy plant with a short,

thin culm, early maturing and a very lax panicle, having grayish lemmas and dark brown grains. The RILs were grown in 1 m long rows with 1 m between rows in a randomized complete block design and phenotyped in 1999 in three locations with four replications each and in 2000 in eight locations with two replications each (Tefera et al. 2003; Yu et al. 2006b).

Nineteen traits were measured and scored on the RILs including grain yield (GY) and yield-related traits, panicle weight (PW), panicle seed weight (PSW), panicle length (PL), 100 seed weight (100SW), days to heading (DH), days to maturity (DM) and shoot biomass (SB). In addition, lodging was measured as lodging index (LI), assessed according to Caldicott and Nuttall (1979) and lodging-related traits; culm diameter at the first internode (CD1) and the second internode (CD2), culm length (CL), peduncle length (PdL), number of internodes (NI), length of first internode (IL1) and of second internode (IL2), crown diameter (CrD) were scored and analyzed. The crushing strength at the first (Crush1) and second (Crush2) internode were measured as the force required to crush the stem at the specified internode in pounds. All traits were measured on the main shoots of ten randomly selected plants, except DH, DM, GY, SB and LI that were recorded on 1 m × 1 m plot basis (Tefera et al. 2003).

Phenotypic data were subjected to analysis of variance (ANOVA) to verify the significance of variance component of the RILs for each trait (SAS institute, Cary, NC, USA, version 9.2). Broad-sense heritability (H^2) on a line mean basis and plot basis was calculated according to Holland et al. (2003). Best linear unbiased predictions (BLUPs) for each line across all locations/years were calculated using ASReml v2 software (Gilmour et al. 2006). A mixed linear model was fit with random effects for line, year, location, location within year and replication within location/year. Model solutions gave BLUPs for each line which was used for further analysis and QTL modeling. Pearson's correlations for all combinations of traits on the basis of RIL means across environments were also calculated.

SSR development

Genomic DNA was extracted from leaves of the cultivar Kaye Murri according to Tai and Tanksley (1990). DNA (250 ng) was simultaneously digested with *Mse*I and ligated to *Mse*I AFLP adaptor (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′). AFLP fragment production and enrichment employing 3′-biotinylated $(AG)_{17}$ and $(AC)_{17}$ probes were performed according to Zane et al. (2002). Fragments enriched for both (AG) and (AC) repeats were cloned using the TOPO-TA cloning kit (Invitrogen). Recombinant clones plated out on LB-agar plates containing 50 μg/ml kanamycin were directly amplified using forward and reverse vector primers, and 10 μL of the PCR products was screened on a 1% agarose gel for the presence of inserts. PCR products from 2,496 (AG) and 672 (AC) enriched clones were sequenced from one direction on an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA), using BigDye™ terminator chemistry (Applied Biosystems). Sequences were aligned and redundant sequences identified using the ChromasPro 1.41 software (http://www.technelysium.com.au/ChromasPro.html).

Sequences were searched for repeat motifs of a minimum of five units of di-, tri- or tetra-nucleotide repeats using the SSRIT (Simple Sequence Repeat Identification Tool) available from GRAMENE (http://www.gramene.org/). Primers flanking the repeat motifs were designed using the software Primer 3 (Rozen and Skaletsky 2000). The optimum conditions for selecting the flanking primers were a length of 20 bp, a GC content of 50% and when possible, a melting temperature (T_m) of 60°C with no more than 2°C difference in T_m between primers of each pair and a product size within the range of 100–300 bp. Possible hairpins, complementarity and potential self-annealing sites for the designed primers were double checked using the program Oligonucleotide Properties Calculator, version 3.10 (http://www.basic.northwestern.edu/biotools/oligocalc.html.

Genotyping

For the new developed SSR markers, 542 primer pairs were tested for amplification and production of the expected-size fragments in Kaye Murri, along with polymorphism compared to *E. pilosa*. PCR reactions were conducted in 10μL volumes containing 20 ng DNA, 1× PCR buffer, 0.2 mM of each dNTP, 0.2 Units *Taq* DNA polymerase (Promega) and 10 μM of each individual primer pair. Cycling protocol consisted of an initial denaturation at 95°C for 4 min, followed by 30 cycles of 95, 54 and 72°C, for 45, 60 and 60 s, respectively, and a final elongation time of 7 min using a PTC-225 thermocycler (MJ Research, Waltham, MA, USA). Electrophoretic size separation of SSR loci was performed using 6% denaturing polyacrylamide gels (PAGE) visualized with silver staining (Bassam et al. 1991). Thirty-one SSR markers (UGEP), three ESTs (INF/LFM) from the finger millet linkage map (Dida et al. 2007) and 38 markers from the linkage map study on tef (Yu et al. 2006b), including eight rice ESTs (RM), two CISP markers (PRSC/SRSC), eight wheat ESTs (3 CNL, 3 KSUM and 2 DupW), 15 tef EST-SSRs (CNLT), two tef IFLP (TCD) and three ISSR markers, also tested on the entire population of 151 RILs, and results were visualized on 4% PAGE as described above. Two-hundred twenty-three expressed sequenced tags (ESTs) from pearl millet (CD) were

screened for amplification and polymorphism between the parental lines using single-stranded conformational polymorphism (SSCP-SNP) as described by Bertin et al. (2005). Moreover, 146 conserved orthologous set (COS) markers that are functional in rice wheat and brachypodium (John Innes Center, UK), were screened on SSCP-SNP gels. Data from 16 RFLP markers used by Zhang et al. (2001) from oat (CDO), rice (RZ) and tef (TCD-RFLP) probes, in addition to a maize dwarfing gene-RFLP marker (D8) and a lignin biosynthesis gene-RFLP marker (phenylalanine ammonia-lyase; PAL) from rice, published by Kantety et al. (2001) and also included in the map of Yu et al. (2006b), were rescored for all 151 RILs from available X-ray films. In total, 945 newly tested PCR-based markers were screened on the two parental lines, and those showing polymorphism were further screened on the RILs.

Linkage map and QTL analysis

Markers showing scorable polymorphism between the parental lines were evaluated on the 151 RILs. χ^2 tests were performed to determine the loci that deviated significantly ($P < 0.01$) from the expected 1:1 segregation ratio. The genetic map was constructed using the program JoinMap 3.0 (Van Ooijen and Voorrips 2001). The Kosambi (1944) mapping function was used to convert recombination frequency to genetic map distance in centiMorgen (cM). All linkage groups were determined using log of odds (LOD) scores equal to or greater than 4. Genome map size was estimated according to Fishman et al. (2001). For QTL analysis, the software QTL Cartographer 2.5 (Wang et al. 2007) was used, and composite interval mapping (CIM) using forward and backward regression with a scanning interval of two cM was employed. Significant thresholds for declaring a QTL were estimated using permutation tests (1,000 runs) for each of the 19 studied traits using the calculated BLUP values.

Results

SSR development

The percentage of clones harboring SSR motifs was calculated to be 79% of the 2,496 sequenced clones for the (AG/CT) enriched library while for the (AC/GT) enriched library the enrichment rate was much lower (35%) for the 864 sequenced clones. Primer pairs were designed for 26% of the sequences harboring the AG/CT repeat motifs. The number of redundant sequences was estimated to be 67%, and 7% of the sequences were not suitable for primer design. For the AC/GT repeat motif harboring sequences, 15% were used for primer design, 76% were redundant and 9% were not suitable for primers design. For both repeat motifs, 542 primer pairs were designed, and sequences of high quality for 535 were submitted to Genbank (GQ274326-GQ274860). Almost all motifs flanked by the primer pairs were either simple or complex di-nucleotide repeats matching the probes used for enrichment, except 13 motifs (2.4%) that were mostly tri-nucleotide repeats.

Genotyping

We screened 542 SSR primer pairs on the two parental lines, of which 496 amplified successfully in both lines (Supplementary Table 1), and 46 gave weak products or failed to amplify. Primers generally amplified 1–3 fragments, with a few cases (six markers) amplifying more than three. Polymorphism between the two parental lines was observed for 262 markers, and the polymorphism level was correlated with the length of the di-nucleotide repeat motif. Sixty-four percent of the monomorphic fragments had dinucleotide repeat sequences equal to or less than ten repeats. The number of polymorphic markers from other grass species tested on the parental lines was very limited. The 31 finger millet SSRs screened yielded three polymorphic markers, and out of 233 pearl millet markers, 13 were polymorphic. In addition, only two COS markers were polymorphic between the parental lines out of the 146 markers screened.

The linkage map

We were able to score 424 loci from 341 markers on the mapping population consisting of 151 F_9 RILs. Segregation distortion from the expected 1:1 using χ^2 test ($P < 0.01$) was observed in 33.5% of the loci, and distortion was in favor of the *E. tef* allele in 67% of the cases. Thus, 282 loci were available for map construction after excluding distorted loci from the analysis. The map comprised 252 loci distributed across 30 linkage groups and 30 loci remained unlinked (Fig. 1). The individual linkage groups (LG) varied in length from 6.8 to 116.1 cM. The map covered an estimated 78.7% of the genome with an average distance of 5.7 cM between markers. Of the 252 loci present on the map, 35 (14%) were shared with the map of Yu et al. (2006b).

Trait analysis

The cultivated parent Kaye Murri was significantly better than the wild parent in all traits except LI. The mean value for LI for *E. pilosa* (79.12) was higher than that for Kaye Murri (67.21) across environments (Table 1). Significant differences between RILs were observed for all traits. Transgressive segregation in either parental direction was

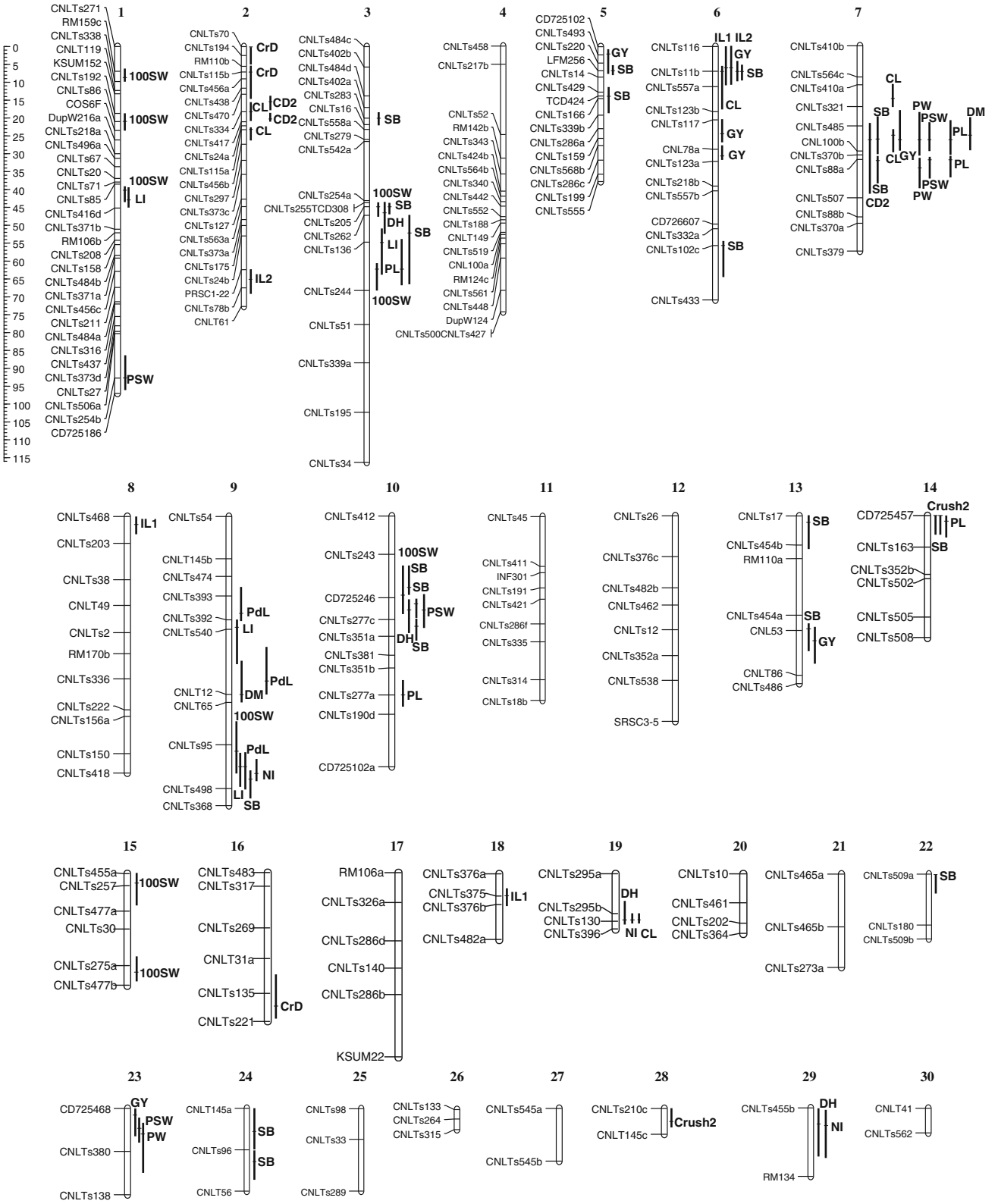

Fig. 1 Genetic linkage map of tef showing 83 QTL identified for 17 traits. Marker names are positioned equivalent to their cM distances on the *left of the bars*. Intervals for each QTL are indicated by the length of the *vertical closed bars* to the *right* of the linkage groups, and the name of the trait underlying each QTL is given to the *right* of the linkage map

Table 1 Mean phenotypic values of parental lines, and min, max, mean phenotypic values and standard deviation of RILs for the different traits

Trait	Abbreviation	Parental lines		RIL population		
		Kaye Murri	*E. pilosa*	Maximum	Minimum	Mean (SD)
Yield and yield-related traits						
Grain yield/plot (g)	GY	299.43	172.39	433.07	61.31	188.55 ± 67.50
Panicle weight/plant[a] (g)	PW	0.71	0.22	0.74	0.17	0.35 ± 0.09
Panicle seed weight/plant[a] (g)	PSW	0.42	0.11	0.45	0.07	0.20 ± 0.07
Panicle length[a] (g)	PL	28.89	21.71	32.76	17.41	23.94 ± 2.69
100 seed weight[a] (g)	100SW	26.75	17.79	27.29	11.64	17.91 ± 3.49
Shoot biomass/plot (g)	SB	2,021.32	1,147.65	1,972.10	625.10	1,129.74 ± 256.63
Days to heading (days)	DH	48.57	33.54	39.58	26.50	32.56 ± 2.53
Days to maturity (days)	DM	99.36	81.82	91.50	72.41	83.13 ± 4.17
Lodging and lodging-related traits						
Lodging index	LI	67.21	79.12	85.43	52.83	71.65 ± 6.45
Culm length[a] (cm)	CL	54.54	38.16	52.39	34.47	43.50 ± 3.56
Peduncle length[a] (cm)	PdL	20.72	17.33	22.42	14.17	19.10 ± 1.40
Number of internodes[a]	NI	3.47	2.94	3.49	2.49	3.02 ± 0.22
Length of first internode[a] (cm)	IL1	8.29	5.99	9.06	5.57	6.88 ± 0.71
Length of second internode[a] (cm)	IL2	12.03	9.38	12.81	8.30	10.37 ± 0.90
Culm diameter at first internode[a] (cm)	CD1	1.56	0.98	1.40	0.98	1.17 ± 0.09
Culm diameter at second internode[a] (cm)	CD2	2.27	1.40	1.41	0.95	1.15 ± 0.09
Crown diameter[a] (cm)		1.95	1.21	1.80	1.12	1.53 ± 0.14
Crushing strength at first internode[a] (lb)	Crush1	9.49	3.59	8.22	1.98	4.76 ± 1.13
Crushing strength at second internode[a] (lb)	Crush2	9.64	3.24	8.53	1.17	4.07 ± 1.22

[a] Measurements were based on the mean of ten randomly selected plants/plot

observed on the RILs for all traits except for DH, DM, SB, CL, CD1, CD2, CrD and crushing strength at the first and second internodes (Table 1). For these traits, RILs with higher values than the parent Kaye Murri were not observed. The phenotypic correlations between the traits are presented in Table 2. Grain yield had significant positive correlations with all traits except CrD. The correlations were especially high with yield-related traits. Lodging index, on the other hand, showed generally weaker correlations with all traits, and the highest correlations were observed for GY ($r = 0.54$), 100SW ($r = 0.46$) and SB ($r = 0.45$). There were no correlations between LI and either CD or crushing strength measured on both the first or second internodes. Also a weak correlation was observed between LI and CL ($r = 0.29$), while LI was negatively correlated with PdL ($r = -0.35$) (Table 2).

QTL analysis

Detailed results of composite interval mapping QTL analysis based on the phenotypic BLUPs for 17 of the 19 traits (no QTL were detected for CD1 and Crush1) are presented in Table 3. A total of 83 QTL were identified on the map distributed among 20 out of the 30 linkage groups. Thresholds for declaring a QTL estimated using permutation tests were LOD = 2.5 except for GY and LI, where the values were 3.1 and 3.2, respectively. Linkage group LG7 (57.4 cM) harbored the largest number of QTL for eight different traits (Fig. 1). Two major clusters of QTL were observed on LG6 and LG7. On LG6, at the CNLTs11b locus, QTL for GY, SB and plant height-related traits (IL1, IL2 and CL) were detected. On LG7, QTL for eight traits (GY, PW, PSW, PL, DM, SB, CL and CD2) were co-located with the peak of the QTLs at 5 cM between the markers CNLTs485 and CNL100b. The amount of phenotypic variance explained by each of the 83 QTL ranged from 4.8 for SB and NI to 33.0 for NI (Table 3). Both parents of the mapping population contributed desirable alleles for the 17 traits, and 36% of those alleles were contributed by the wild parent *E. pilosa* (Table 3). The positions of the QTL for GY on LG13 and for PdL on LG9 were shared between our study and that of Yu et al. (2007).

QTL for yield and related traits

GY

Three of the seven QTL for GY were located on LG6 and the rest on LGs 5, 7 and 13, respectively. The contribution

Table 2 Correlation among the 19 traits studied in the Kaye Murri × *E. pilosa* RIL population

	GY	PW	PSW	PL	100SW	HD	DM	SB	LI	CL	PdL	NI	IL1	IL2	CD1	CD2	CrD	Crush1
GY																		
PW	0.61**																	
PSW	0.68**	0.97**																
PL	0.48**	0.83**	0.78**															
100SW	0.56**	0.47**	0.50**	0.39**														
HD	0.43**	0.58**	0.59**	0.62**	0.28**													
DM	0.458**	0.52**	0.53**	0.53**	0.23**	0.64**												
SB	0.89**	0.61**	0.64**	0.60**	0.59**	0.54**	0.53**											
LI	0.54**	0.24**	0.29**	0.13	0.46**	0.1	0.01	0.45**										
CL	0.54**	0.59**	0.58**	0.6**	0.43**	0.47**	0.5**	0.63**	0.29**									
PdL	−0.33**	−0.12	−0.15*	−0.06	−0.3**	−0.18**	−0.03	−0.34**	−0.35**	0.21**								
NI	0.57**	0.56**	0.56**	0.49**	0.41**	0.61**	0.52**	0.65**	0.33**	0.60**	−0.44**							
IL1	0.34**	0.27**	0.26**	0.35**	0.31**	0.13	0.28**	0.41**	0.15*	0.66**	0.19**	0.14*						
IL2	0.47**	0.39**	0.39**	0.47**	0.45**	0.21**	0.27**	0.53**	0.30**	0.76**	0.16*	0.20**	0.78**					
CD1	0.37**	0.79**	0.74**	0.73**	0.3**	0.55**	0.44**	0.43**	−0.04	0.63**	0.14*	0.45**	0.35**	0.39**				
CD2	0.42**	0.82**	0.77**	0.76**	0.35**	0.57**	0.47**	0.5**	0.02	0.68**	0.07	0.52**	0.38**	0.43**	0.95**			
CrD	−0.01	0.55**	0.47**	0.51**	−0.0	0.31**	0.27**	0.10	−0.23**	0.44**	0.28**	0.24**	0.21**	0.14*	0.73**	0.71**		
Crush1	0.37**	0.5**	0.48**	0.54**	0.32**	0.41**	0.42**	0.42**	−0.06	0.45**	−0.07	0.41**	0.25**	0.38**	0.58**	0.60**	0.36**	
Crush2	0.45**	0.53**	0.51**	0.54**	0.4**	0.46**	0.43**	0.51**	0.04	0.49**	−0.15*	0.49**	0.23**	0.38**	0.56**	0.59**	0.3**	0.87**

* Significant at $P < 0.05$, ** Significant at $P < 0.01$

Table 3 Genomic regions significantly associated with QTL for yield and lodging and related traits based on composite interval mapping using BLUPs of 11 environments

Trait	Linkage group	Position	Marker/interval	LOD	Additive effect[a]	R^2 (%)
GY	5	0.0431	CNLTs220	3.6	−15.36	6.8
	6	0.0691	CNLTs11b	3.4	−13.70	5.6
	6	0.2451	CNLTs117	4.7	−18.44	10.6
	6	0.3071	CNL78a	3.3	−17.47	9.5
	7	0.2641	CNLTs485−CNL100b	3.5	−15.51	7.1
	13	0.3351	CNL53	3.8	−16.19	8.0
	23	0.0201	CD725468	7.2	−23.84	17.1
H^2 [b]			0.39 (0.95)			
PW	7	0.2641	CNLTs485−CNL100b	2.8	−0.02	7.4
	7	0.3381	CNLTs88a	3.5	−0.02	9.6
	23	0.0801	CNLTs380	4.0	−0.03	13.6
H^2			0.23 (0.96)			
PSW	1	0.9281	CNLTs254b	3.7	−0.02	8.7
	7	0.2641	CNLTs485−CNL100b	4.7	−0.02	12.4
	7	0.3181	CNLTs88a	4.3	−0.02	10.2
	10	0.2641	CD725246−CNLTs277c	2.5	−0.02	6.7
	23	0.0601	CD725468−CNLTs380	6.5	−0.03	21.1
H^2			0.25 (0.96)			
PL	3	0.6061	CNLTs136	2.9	−0.75	8.7
	7	0.2641	CNLTs485−CNL100b	4.2	−0.79	9.7
	7	0.3181	CNLTs88a	4.3	−0.77	9.2
	10	0.4911	CNLTs277a	2.5	−0.59	5.2
	14	0.0201	CD725457	2.8	−0.68	7.1
H^2			0.40 (0.98)			
100SW	1	0.0891	RM159c−CNLTs338	2.9	−0.80	6.9
	1	0.2351	CNLTs86−Cos6f−DupW216a	4.0	−0.86	7.7
	1	0.4051	CNLTs85−CNLTs416d	2.7	−0.87	8.1
	3	0.4471	CNLTs255/TCD308−CNLTs205−CNLTs262	2.6	−0.72	5.6
	3	0.6061	CNLTs136−CNLTs244	3.4	−0.93	9.3
	9	0.6411	CNLTs95	4.2	−1.06	11.8
	10	0.2055	CD725246	3.6	−0.90	8.2
	15	0.0201	CNLTs455a−CNLTs257	2.8	−0.78	6.6
	15	0.2861	CNLTs275a	3.4	0.97	8.2
H^2			0.30 (0.99)			
DH	3	0.4671	CNLTs262	3.6	−0.63	6.8
	10	0.2641	CNLTs277c	6.5	−0.94	15.3
	19	0.1351	CNLTs295b−CNLTs130	5.2	−0.78	10.4
	29	0.0401	CNLTs455b	4.3	−0.84	12.6
H^2			0.44 (0.94)			
DM	7	0.2441	CNLTs485−CNL100b	3.2	−1.17	8.1
	9	0.4841	CNLT12−CNLT65	3.1	1.09	6.6
H^2			0.34 (0.90)			
SB	3	0.2001	CNLTs283	2.7	−56.48	4.8
	3	0.4471	CNLTs205	3.8	−64.28	6.8
	3	0.5111	CNLTs262−CNLTs136	3.1	−63.14	6.6
	5	0.0671	LFM256	3.2	−61.10	5.9
	5	0.1381	TCD424	2.6	−53.78	4.8
	6	0.0691	CNLTs11b	2.8	−59.65	5.8

Table 3 continued

Trait	Linkage group	Position	Marker/interval	LOD	Additive effect[a]	R^2 (%)
	6	0.5561	CNLTs102c	3.3	−58.67	5.8
	7	0.2641	CNLTs485−CNL100b	3.2	−65.32	6.5
	7	0.3181	CNLTs88a	2.8	−56.41	5.0
	9	0.7211	CNLTs498	4.0	−77.65	9.3
	10	0.1861	CNLTs243−CD726246	11.7	−135.46	30.4
	10	0.2441	CD725246−CNLTs277c	12.4	−139.30	32.1
	10	0.3041	CNLTs277c−CNLTs351a	8.9	−115.13	22.3
	13	0.0201	CNLTs17−CNLTs454b	3.6	−65.73	7.2
	13	0.3131	CNL53	3.6	−65.62	7.2
	14	0.0001	CD725457	3.0	−57.48	5.4
	22	0.0001	CNLTs509a	3.9	−65.64	7.1
	24	0.0601	CNLT145a−CNLTs96	2.8	62.14	6.6
	24	0.1541	CNLTs96	2.7	64.54	7.0
H^2			0.30 (0.98)			
LI	1	0.4249	CNLTs85	3.3	−1.55	12.0
	3	0.5461	CNLTs136	4.3	−1.41	9.8
	9	0.3011	CNLTs540	5.5	−1.64	13.7
	9	0.6811	CNLTs95−CNLTs498	3.5	−1.69	13.4
H^2			0.14 (0.89)			
CL	2	0.1821	CNLTs334	4.5	−1.00	9.9
	2	0.2321	CNLTs115a	2.6	−0.81	6.2
	6	0.0691	CNLTs11b	4.8	−1.06	11.1
	7	0.1491	CNLTs410a−CNLTs321	3.1	−0.91	8.2
	7	0.2441	CNLTs485−CNL100b	4.3	−1.04	11.0
	19	0.1351	CNLTs295b−CNLTs130	5.1	−1.03	10.1
H^2			0.26 (0.97)			
PdL	9	0.2561	CNLTs393−CNLTs392	2.8	0.34	8.4
	9	0.4271	CNLT12	7.1	0.55	22.0
	9	0.6811	CNLTs95	4.6	0.47	14.6
H^2			0.16 (0.94)			
NI	9	0.7011	CNLTs95−CNLTs498	13.0	−0.11	33.0
	19	0.1351	CNLTs295b−CNLTs130	3.3	−0.04	4.8
	29	0.0601	CNLTs455b	4.2	−0.06	9.6
H^2			0.24 (0.98)			
IL1	6	0.0601	CNLTs11b	3.8	−0.16	8.7
	8	0.0201	CNLTs468	3.9	0.18	11.4
	18	0.0641	CNLTs375	3.7	−0.15	8.1
H^2			0.11 (0.95)			
IL2	2	0.5891	CNLTs24b−PRSC1-22	3.3	−0.23	8.4
	6	0.0601	CNLTs11b	2.6	−0.18	5.5
H^2			0.22 (0.96)			
CD2	2	0.2021	CNLTs334−CNLTs417	2.9	−0.02	7.3
	2	0.1527	CNLTs470−CNLTs334	2.7	−0.02	8.1
	7	0.2641	CNLTs485−CNL100b	2.7	−0.02	7.8
H^2			0.18 (0.89)			
CrD	2	0.0001	CNLTs70−CNLTs194−RM110b	2.6	−0.03	7.3
	2	0.0711	RM110b−CNLTs115b−CNLTs456a−CNLTs438−CNLTs470	2.7	−0.03	7.4
	16	0.3901	CNLTs135	2.6	−0.03	9.1
H^2			0.30 (0.45)			

Table 3 continued

Trait	Linkage group	Position	Marker/interval	LOD	Additive effect[a]	R^2 (%)
Crush2	14	0.0001	CD725457	3.7	−0.37	8.1
	28	0.0401	CNLTs210c	2.8	−0.34	7.5
H^2			0.86 (0.92)			

[a] Positive values of the additive effect indicate that alleles from *E. pilosa* increased the phenotypic value of the trait

[b] Broad sense heritability estimates are based on plot basis, and the numbers in parenthesis are based on line mean basis

to the phenotypic variation for each QTL varied between 5.6 and 17.1%, with the position CD25468 on LG23 showing the highest contribution at a LOD = 7.3. Based on the single location analysis, the same QTL was detected in nine of the 11 locations with 8.8–20% of the phenotypic variance explained by this QTL in each location (data not shown).

PW, PSW and PL

The QTL for these three traits were co-located at two positions on LG7. Also co-location of QTL for PSW and PL and for PW and PSW were detected on LG10 and 23, respectively. Two more QTL for PL were observed on LG3 and LG14 and one for PSW on LG1. The amount of phenotypic variance explained by these QTL ranged between 7.4 and 13.6% for PW, 6.7–21% for PSW and 5.2–9.7% for PL. The interval CD25468–CNLTs380 on LG23 contributed the highest percentage of the variances explained for PW (13.6%) and PSW (21.1%) at LOD values of 4 and 6.5, respectively.

100SW

Nine QTL for 100SW were detected on five linkage groups, two of which were the only QTL detected on LG15. On LG1, 3 and 9 QTL for 100SW co-located with QTL for LI while on LG3, 9 and 10 they co-located with QTL for SB. The QTL explained between 5.6 and 11.8% of the phenotypic variance, and all nine QTL together explained 72% of the variance. Desirable increasing effects of one QTL on LG15 at the position CNLTs275a were contributed by *E. pilosa* while all other QTL including the one on the same LG15 were contributed by Kaye Murri.

SB

Shoot biomass had the largest number (19) of QTL detected in this study. QTL were distributed across ten linkage groups, and the amount of variation explained by the QTL ranged between 4.8% on LG3 and 32% on LG10. On LG24, the only QTL detected were for SB, and the increasing effect of both QTL was attributed to the *E. pilosa* parent. Co-location of QTL for SB with those of GY was detected on LG 6, 7 and 13 and with LI on LG3. The higher value allele came from Kaye Murri at each of these QTL locations for each trait.

DH and DM

Four QTL for DH were detected on four different linkage groups that explained 6.8–12.6% of the phenotypic variance. The two QTL for DM were located on different linkage groups than those for DH, and both QTL together explained 14.6% of the variance observed. All alleles for early DH were contributed by the wild parent *E. pilosa*, and one of the two alleles for early maturity date was contributed by the cultivar Kaye Murri (Table 3).

QTL for lodging and culm-related traits

LI

Lodging index was used in this study as a measure to circumvent the problems related to scoring spots varying in their lodging score within a single plot. Four QTL for LI were observed that together explained 49% of the variance, two on LG9 and one on each of LG1 and LG3, respectively. The two QTL on LG9 each explained more than 13% of the phenotypic variation. QTL for LI co-located with QTL of 100SW on LG1, 3 and 9 with the higher value alleles coming from Kaye Murri. However, no LI QTL co-located with QTL for GY or other related traits (Fig. 1).

CD1 and CD2

No QTL were detected for CD1, while for CD2 two QTL were detected very close to each other on the same LG2. Together, the two QTL explained 15% of the phenotypic variance.

CL and NI

The two traits were highly correlated ($r = 0.60$) and are known to contribute to a taller stature in grasses. Six QTL

were detected for CL and three for NI. Only one QTL on LG9 detected for NI co-located with a QTL for LI. This QTL explained 33% of the variance with a LOD score of 13. The same QTL position was detected in six of the 11 environments. QTL for CL co-located with those for SB on LG6, while on LG19 clustering of QTL for CL, NI and DH was observed (Fig. 1). The positive effects of the QTL for both traits were from the cultivated parent, Kaye Murri.

IL1, IL2 and PdL

One QTL for each of IL1 and IL2 was located on LG6 close to QTL for CL, SB and GY. LG8 and LG18 had a single QTL for IL1 with no other QTL on the two linkage groups. A second QTL for IL2 was located on LG2 where no other QTL was detected. The maximum phenotypic variance was 11.4% for IL1 on LG8 with positive effects attributed to the wild *E. pilosa*, while R^2 for IL2 was 8.4% on LG2, and its positive effects were due to the cultivated tef allele. The three QTL for PdL were detected on LG9 two of which closely positioned near QTL for LI. The three loci explained 8–22% of the variation with positive effects of the trait contributed by *E. pilosa*.

CrD and Crush2

Two QTL for CrD on LG2 explained 7% of the variance each, while the third detected on LG16 explained 9% of the variance. None of the CrD QTL was positioned close to any other QTL, and positive effects came form Kaye Murri. The QTL for Crush2 were detected on LG14 and 28; the former co-located with QTL for SB, and the latter was the only QTL detected on that linkage group. The two QTL together explained 15.5% of the phenotypic variance, and positive effects were from Kaye Murri.

Discussion

SSR markers' development

The techniques used to identify SSR markers have improved dramatically in the past decade and are becoming highly successful in identifying the fragments harboring the repeats for sequencing. The technique described by Zane et al. (2002) has been used on a few crop species including white clover (Zhang et al. 2008) and faba bean (Zeid et al. 2009), besides medicinal and industrial crops (Huang et al. 2009; Pamidimarri et al. 2009). To our knowledge, this is the first time the method was used on any of the cereal crops. The enrichment rate of the tef libraries (79%) was much higher than reported in any of the previously mentioned studies for the (AG) repeat using the same technique. For the (AC) repeat, the enrichment rate of 35% observed in this study was comparable to 39% in case of *Jatropha curcas* (Pamidimarri et al. 2009). Compared to previous studies for microsatellite development with various protocols including library enrichment methods as in rice (Panaud et al. 1996; Chen et al. 1997), wheat (Bryan et al. 1997; Song et al. 2005), sorghum (Brown et al. 1996), tall fescue (Saha et al. 2006) and maize (Sharopova et al. 2002), the protocol used in this study was less laborious and extremely efficient for microsatellite marker development.

The (AG) and (AC) probes are the most widely used dinucleotide probes in microsatellite development studies in grasses. The higher enrichment for the (AG) enriched library as compared to (AC) library might be an indication of the abundance of the (AG) repeat in the tef genome as compared to the (AC) repeat. Dinucleotide repeats were the most frequent in 3,603 EST sequences from *E. tef* with the (AG) repeats representing 43% of all dinucleotides (Yu et al. 2006a). In perennial ryegrass, however, the (AC) repeats were much more abundant (33%) as compared to 16% for the (AG) from a library enriched for multiple types of repeats (Jones et al. 2001). The potentially high abundance and the level of polymorphism for dinucleotide-repeat markers have been elaborated in rice (Temnykh et al. 2001). Furthermore, their study indicated that the highest rate of successful amplification of microsatellite sequences in rice was for the dinucleotide (AG) and (AC) repeats as compared to the more abundant and variable, but poorly amplifying (AT) repeats. Our results are in general agreement with those from rice, where 91% of the markers designed for both the (AG) and the (AC) amplified well in tef.

One of the major limitations to the usefulness of the sequences obtained from our enriched libraries was the redundancy of the sequences of the repeat containing clones. The redundancy rate reached 76%; however, this was observed for a large number of the sequences obtained and was not restricted to only a few, indicating that the amplification of a few multicopy sequences in the original genome that were over-represented and carried over during the enrichment process (Zane et al. 2002) cannot account for this redundancy. However, this redundancy may be partly due to the fact that a size-selected library derived from digestion with a single enzyme represents only a subset of the DNA fragments potentially available in a genome as hypothesized by Rafalski et al. (1996).

Out of the 496 primers amplifying in both parental lines, 262 (52.8%) were polymorphic. This polymorphism level is comparable to 50% polymorphism from RFLP markers (Zhang et al. 2001) and twice as much as EST-SSRs (Yu et al. 2006b) for the same parents. Temnykh et al. (2001) tested a set of rice SSR markers on a divergent collection of

parental lines and reported polymorphism rates of 80.7 and 73.9% for primers flanking the (AG) and (AC) repeats, respectively. Here, 54% polymorphism was observed for primers flanking the (AG) repeat and 42% of those flanking the (AC) repeat with two *Eragrostis* species. The majority (96%) of the 410 markers from different grass species including conserved orthologous sequences between wheat, rice and Brachypodium were monomorphic between the two parental lines, limiting the initiation of comparative mapping studies in tef. This low level of polymorphism, in addition to that observed using tef-specific EST-SSR markers (Yu et al. 2006a), supports the assumption of a close relationship between the two species (Ingram and Doyle 2003) and elaborates the importance of the polymorphic SSR markers developed here for tef breeding. Our preliminary results on a small subset of the newly developed SSRs (including a few that were monomorphic in this study) have indicated their usefulness in detecting polymorphism among different tef accessions (data not shown) suggesting that those markers will become very useful in the tef germplasm fingerprinting and organization.

The linkage map

Significant segregation distortion was observed in this study for 33.5% of the marker loci with skewing toward the cultivated tef line in 67% of these loci. Similar and higher levels of distortion were reported in other studies based on inter-specific crosses (DeVicente and Tanksley 1993; Jenczewski et al. 1997; Ky et al. 2000; Moncada et al. 2001; Thomson et al. 2003) and also wide intra-specific crosses as indicated by Xu et al. (1997). Based on results from those studies and others, the type of marker does not seem to be the cause of this distortion, while the parents of the population studied can play a major role. Xu et al. (1997) compared segregation distortion in 56 populations of different species and showed that recombinant inbred populations had significantly higher frequencies of distorted markers than doubled haploid and backcross populations. They attributed this distortion to the cumulative effect of genetic (G) and environmental (E) factors on multiple generations and the G × E interaction that becomes more pronounced with the progression of selfing of the RILs. Also the selective advantage of the plants with cultivar-like traits during establishment of RILs might have played a role in favoring those alleles as previously observed in rice (Cai and Morishima 2002). Another source of distortion is the double-dose fragments that are likely to be common in a polyploid with low polymorphism like tef, and they are very difficult to detect (Sorrells 1992). When distorted loci were included in the map, the majority of these loci formed clusters at the terminals of a few linkage groups (data not shown). Since the cause of this distortion in our population was not clear, we were obliged to exclude the significantly ($P < 0.01$) distorted loci from building the map.

The linkage map presented here was based on 252 loci estimated to cover 78.7% of the genome, which is 10% less than the map of Zhang et al. (2001) and almost equivalent to the genome coverage by Yu et al. (2006b) using the same estimation method. Nevertheless, our map was solely based on PCR-based markers with an average distance of 5.7 cM between loci, utilizing 151 RILs of the same cross used in both previous studies rendering it the most updated and reliable map for tef. It was not possible to combine any of the RFLP markers used by Zhang et al. (2001) because the data were no longer available (H. T. Nguyen, personal communication). In addition, only 35 of the loci present on the map of Yu et al. (2006b) were shared in the current map, while the rest were discarded either due to quality of the amplified products, especially ISSR markers or due to the high distortion level observed in those markers, making it difficult to link both maps. Besides tef-specific markers, the map comprised 31 markers from other grass species that could be useful for comparative mapping as shown by Zhang et al. (2001) using RFLP markers. However, the recent work by Sim et al. (2009) and Zeid et al. (2010) has indicated that sequencing the amplified products form such PCR-based markers is essential before homology of the fragments is assumed.

Phenotypic traits and QTL analysis

Higher-yielding tef varieties that do not lodge have been the target of breeders in Ethiopia for decades. Utilizing RILs from the inter-specific cross between tef and its wild relative *E. pilosa* are valuable for determining the prospect of introgressing desirable alleles from *E. pilosa*. This cross is one of the very rare inter-specific crosses available in tef, aiming to provide novel variation that is usually lacking in tef lines. Kaye Murri, the tef parent used in the cross studied here, is generally favored by breeders because the cultivar has stiff culms contributing to better standing-ability and has better crossing-ability than other tef lines. Its white colored seeds and compact panicle (most improved tef lines are of an open panicle type) provided morphological traits that facilitated the validation of the difficult hybridization process in the absence of molecular markers. Because of these merits, many of the successful tef varieties from intra-specific crosses, for example DZ-Cr37, DZ-Cr82 and DZ-Cr255, have Kaye Murri in their pedigree, rendering QTL detected in this study candidates for improvement in many other crosses. A similar strategy for broadening the genetic diversity of cultivated crops and identification of QTL for beneficial traits was successfully adopted in rice (Septiningsih et al. 2003) and barley (Li et al. 2006).

Useful transgressive segregants for many traits including GY, DH, DM, PL, LI and CL were observed in the population of RILs studied here (Table 1), indicating the presence of desirable alleles in *E. pilosa*. While RILs with shorter culm length (34.4 ± 3.56 cm) than the shortest parent (*E. pilosa*) were identified from this cross, shorter tef genotypes were described in previous studies (Assefa et al. 2000). Thus, it might also be possible to obtain shorter culmed plants from intra-specific crosses and avoid the negative effects of linkage drag from an inter-specific cross. The same observations apply to other traits like PL and PdL, where useful variation for those traits in tef genotypes are greater than that observed in the RIL population studied here (Assefa et al. 2000, 2001, 2002). Transgressive segregants for DH (26.5 ± 2.53 days) and DM (72.4 ± 4.17 days), on the other hand, were earlier than the earliest tef germplasm (DH = 36.7 ± 0.4 days, DM = 83.1 ± 0.75 days) reported by (Assefa et al. 2001), suggesting that earliness was probably the most important trait the parent *E. pilosa* (30-5) contributed in this cross.

We identified seven QTL for grain yield on five different linkage groups that together explained 64.7% of the observed variance. The QTL on LG23 explained 17% of the variance at LOD = 7, and the same QTL was detected in nine of the 11 environments (data not shown), suggesting that it is a very promising QTL to target. One QTL on LG13 that explained 8% of the variance was reported to be linked to the same locus (CNL53) on the map of Yu et al. (2007) and explained 11% of the variance based on the mean value of the environments in that study. GY showed a significant positive correlation with PW, PSW and PL (Table 2), and QTL for those traits co-located with QTL for GY on LG7 and 23. Similar clustering of GY-QTL and those traits was observed by Chanyalew et al.(2005) and Yu et al. (2007) in tef. Independent QTL for GY were detected on LG5, 6 and 13. At the same time, QTL for PW, PSW and PL that did not co-localized with GY were detected on LG1, 3, 10 and 14. Thus, targeting QTL for GY, in addition to QTL for GY component traits (PW, PSW and PL), could result in improvement in grain yield in tef, especially where the heritability of component traits is high (Table 3). These observations are in general agreement with the result of Tefera et al. (2003) and Chanyalew et al. (2005) on tef yield improvement strategies.

Both 100SW and SB showed a positive significant correlation with almost all traits studied except CrD and PdL, and the majority of the positive alleles for both traits were contributed by the cultivated parent Kaye Murri. The co-location of QTL for both traits on LG3 and 10 suggested either linkage or pleiotropic effects indicating that selection for a single allele can bring about improvement in both traits. The three QTL for SB on LG10 together explained almost 85% of the variation; however, this is probably an over-estimation of the true effects. Changing the scan interval for declaring a QTL position to be 5.0 cM (i.e. the test positions for a QTL were spaced 5 cM instead of 2 cM) resulted in a single QTL that explained 17% of the variance. This QTL for SB could be very useful if validated in crosses involving Kaye Murri as a parent since it co-localizes with QTL for 100SW and PSW, all of which had their positive effects from Kaye Murri (Fig. 1). A QTL for DH with negative effects contributed by Kaye Murri was also associated with these QTL which may suggest delay in heading date (Fig. 1). This observation is in agreement with Tefera et al. (2003) and Chanyalew et al. (2005), who reported that longer duration plants accumulated more biomass in tef.

The co-location of the QTL for SB with those for GY on LG5, 6, 7 and 13 and with those for yield-related traits on LG7, 10 and 14 observed in this study and in previous studies by Chanyalew et al. (2005) and Yu et al. (2007) suggested either linkage or pleiotropic effects between genes for these traits. Previous studies have indicated a positive correlation with direct effects of SB on GY in tef, suggesting a common physiological basis for both traits (Teklu and Tefera 2005). However, given that tef breeders have always selected for dual purpose lines that combine high grain and straw yields, the latter being used for animal feed and local construction purposes (Tefera et al. 2008) could also explain the correlation.

Early heading and maturity are typical traits of the wild *E. pilosa* species and parent in this study. It is not surprising to observe that positive alleles at all four DH-QTL and one of the two QTL for DM were contributed by the wild parent. Especially interesting are the DH-QTL on LG19 and 29 (Fig. 1), where QTL for earliness in heading explained 10–13% of the variance co-located with those of shorter stature (CL and NI) with positive effects for those traits also contributed by the wild parent. Early heading and maturing tef cultivars are crucial for regions with a short rainy season known as "Belg" in Ethiopia, where only tef is usually grown on an average amount of rainfall of less than 300 mm in a period of 90 days (Rosell and Holmer 2007).

Because of the high correlation between GY and LI, it has always been feared that improving lodging resistance could reduce yield in tef (Tefera et al. 2003; Yu et al. 2007). Lodging index measured here was significantly correlated with GY ($r = 0.54$) and 100SW ($r = 0.46$) and to a much lesser extent with PW and PSW, but showed no correlation to PL (Table 2). Except for the clustering of the QTL for LI and 100SW on LG1, 3 and 9 and PL on LG3, none of the QTL for other traits co-located with LI (Fig. 1). Thus, the results presented here suggested that selection for the markers linked to QTL for GY and related traits should not have a negative effect on lodging in the progeny from

this cross. These results are in partial agreement with the results of Chanyalew et al. (2005) and Yu et al. (2007), where one of the three LI-QTL were associated with PL in the former and two of the five LI-QTL were associated with GY in the latter.

Of the four QTL for LI, only one coincided with a QTL for NI on LG9. In addition, moderately significant and positive correlations were observed between LI and plant height-related components namely CL ($r = 0.29$), NI ($r = 0.33$) and IL2 ($r = 0.30$). These results suggested that selecting for lodging resistance indirectly via these morphological traits may not be feasible in tef as previously indicated by Hundera et al. (2000) based on path-coefficient analysis on 30 tef genotypes. These results contradict reports from other grasses, where QTL for lodging and those for plant height coincided in the same region on the linkage map (Tinker et al. 1996; Keller et al. 1999; Börner et al. 2002; Inoue et al. 2004). Breeding for lodging resistance, however, can be improved in this population by targeting the four QTL identified here for LI, having their positive alleles from the *E. pilosa* parent, using marker-assisted selection strategies. The same strategy could be used for the QTL for CL, IL1, IL2 and NI that explained 14–56% of the variance. Because the wild parent *E. pilosa* was the major contributor of useful alleles for plant height reduction in this study, some of the RILs tested here could be used as breeding material for reducing stature in tef. Peduncle length is another component to plant height besides CL and PL. Selection for shorter PdL has been beneficial in oats leading to reduction in lodging and improvement in yield in some cultivars (Milach et al. 2002). For wheat cultivars in China, it was observed that in a period of 50 years both plant height and peduncle length have decreased, while harvest index has increased in the same period (Jiang et al. 2003). Furthermore, QTL for PdL in barley were suggested to be a composite trait contributed by both plant height and heading date (Rao et al. 2007). In this study, two of the three QTL identified for PdL coincided with QTL for LI on LG9. The alleles for the increase in PdL were contributed by the wild parent; however, the correlation between PdL and LI was negative ($r = -0.35$), suggesting that increased peduncle length should contribute to better lodging resistance. While these results are hard to interpret, it is important to further investigate this trait, especially since it represents about 20% of the plant height on the average in both parents. No QTL for CD1 were detected, while three QTL for CD2 that explained 22% of the phenotypic variance and their alleles contributed by Kaye Murri were detected on LG2 and 7. These QTL, however, coincided with QTL for CL, and considering the high correlation between the two traits ($r = 0.68$), increasing culm diameter would be associated with an increase in culm length. Another interesting culm-related trait is the culm crushing strength at the second internode. Two QTL for this trait were observed on LG14 and 28 and explaining together 15% of the variance. Since the alleles for this trait were contributed by the cultivated parent showing stiffer culms, those alleles should be useful for stem strength improvement, although there was no correlation observed between crushing strength and LI. It appears that the mechanism of lodging in tef could be similar to that of lowland rice (Ishimaru et al. 2008), where stem bending due to the increase in panicle weight during maturation occurs causing the plants to lodge, but stem breakage is not usually observed. While reduction in plant height has played the major role in improving lodging in many modern rice cultivars and other cereal crops, other stem characters have also been beneficial. Thus, reducing plant height, increasing stem strength and the weight of the lower stem have all been important targets for increasing lodging resistance in rice (Kashiwagi et al. 2005) and should be further explored in tef.

In conclusion, the SSR markers developed in this study will be useful for application of MAS strategy for tef. In addition, these markers will facilitate fingerprinting and organization of the thousands of tef germplasm accessions, many of which lack passport data. We are currently saturating the map with more markers and verifying the QTL described here in other populations. At the same time, we are studying the amount of allelic variation available in the cultivated tef germplasm for the most important QTL detected in this study. Lodging in tef is a challenging trait to measure and improve based solely on field trials. In addition, the weak correlation between culm-related traits and LI makes it difficult to indirectly assess lodging through stem morphological traits. More reliable measures such as crushing strength of the culms or stem strength measured using prostrate testers that have been successful in other crop species should be further explored in tef. The QTL identified in this study for both yield and lodging will be useful for improving tef in breeding programs that depend solely on phenotypic selection. Through rounds of backcrossing, it should be possible to introgress the QTL identified here for reduced lodging in tef without strongly affecting grain yield. Although this would be hard to accomplish considering the amount of effort needed to execute the crosses in the extremely small florets of tef, it has only become possible to consider this approach through the availability of the markers described in this study. A further benefit of the breeder-friendly markers developed here will be in the identification of tef hybrids from crosses in the breeding program. This is of great value when crossing parental lines, where distinctive morphological markers are lacking, and it would otherwise be impossible to confirm a successful cross to be used in the breeding program.

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