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RFLP linkage map of the Ethiopian cereal tef [Eragrostis tef (Zucc) Trotter]

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Abstract Tef [*Eragrostis tef* (Zucc) Trotter] is one of the most important cereal crops in Ethiopia. It is an allotetraploid species with a genome size of 720 Mbp. In this paper we report results of genetic linkage-map construction for *E. tef* using tef and heterologous cDNA probes for the first time. One hundred and sixteen F_8 recombinant inbred lines (RILs) from the cross *E. tef* cv Kaye Murri×*Eragrostis pilosa (*accession 30–5) were used for mapping. Parental lines were digested with nine restriction enzymes and screened using 159 tef cDNA and 162 heterologous probes including the grass genome anchor probes. The polymorphism level between parental lines was 66.9%. One hundred and thirty nine polymorphic probes were hybridized against 116 RILs. Both the tef and the heterologous probes hybridized well against tef genomic DNA. The linkage map defined 1,489 cM of the tef genome comprising 149 marker loci distributed among 20 linkage groups. The average interval between markers was 9.99 cM. A fraction (14.8%) of the markers deviated significantly from the expected segregation. Such a genetic linkage map is useful for tagging economically useful genes in tef because a wide range of agronomically important traits is segregating within this population. This would enable the use of a marker assisted breeding strategy which, in turn, will enhance breeding efficiency. Alignment of the tef RFLP map with the rice RFLP map indicates that a number of syntenic chromosomal fragments exist between tef and rice in which the gene order was for the most part collinear. The comparative mapping information should enable tef scientists to take advantage of whatever genetic progress is made on the cereal model species rice.

Keywords *Eragrostis tef* · Tef · Linkage map · Synteny · Restriction fragment length polymorphisms

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Introduction

Tef [*Eragrostis tef* (Zucc) Trotter] is a small grained (2–3 mg per kernel), sexual, autogamous, allotetraploid, C4-metabolism cereal plant of medium stature and short growth-duration (Moffett and Hurcombe 1949; Jones et al. 1978; Berhe et al. 1989; Tavasoli 1986; Ayele et al. 1996). It has 40 chromosomes (2n=4*x*=40) with a relatively small genome size of about 730 Mega-base pairs (2 C DNA amount=1.5 picograms per cell nucleus) (Ayele et al. 1996). Tef is one of the more important and widely adapted staple cereal crops in Ethiopia grown annually on 1.38 million hectares of land and giving a total of about 12-million quintals of grain. The nutritive value of tef grain compares well with some of the major cereals such as wheat, barley, maize and sorghum and, in fact, is better than some of these crops in terms of mineral content, especially zinc, copper and manganese (Ketema 1993).

Because of the foregoing importance of tef to Ethiopia in particular, and potentially to the world in general, a public tef improvement programme has been undertaken in Ethiopia since 1956 (Tefera and Ayele 1995). Both the rate and amount of progress in tef research has been low due to lack of basic information on the biology of the crop. This is particularly true for information on the molecular genetic aspects of the crop. It is widely accepted that the construction of a linkage map in many species greatly increases the efficiency of genetic improvement. Therefore, development of a molecular linkage map in tef will enhance our understanding of tef

genetics and improve the efficiency of crop breeding, especially for those aspects involving quantitative traits.

DNA markers have been successfully used for genetic mapping in a wide range of species. Restriction fragment length polymorphisms (RFLPs) have been valuable markers for the construction of molecular maps in plants and allows synteny studies because of their locus specificity (Ahn and Tanksley 1993). Chromosomal RFLP linkage maps exist for cereal crops like wheat (*Triricum aestivum* L.) (Chao et al. 1989; Liu and Tsunewaki 1991; Devos et al. 1992, 1993; Xie et al. 1993; Nelson et al. 1995 a, b, c), rice (*Oryza sativa* L.) (Causse et al. 1994; Kurata et al. 1994), maize (*Zea mays* L.) (Gardiner et al. 1993), and oat (*Avena sativa* L.) (O'Donoughue et al. 1992, 1995). Though we have a genetic linkage map of tef based on AFLPs (Bai et al. 1999), a map based on RFLPs is non-existent for tef.

Establishment of the relationship between the genomes of different species not only provides researchers with large pools of available markers and a tool for trans-genomic gene identification and isolation, but will also allow an enhancement of the existing knowledge of gene action and metabolic and physiological pathways via consensus maps (Devos et al. 1998). Comparative maps have been developed, and the conservation of linkage relationships have been reported for a number of grass species such as maize and sorghum (Whitkus et al. 1992; Berhan et al. 1993); rice, maize and wheat (Ahn et al. 1993); rice and wheat (Kurata et al. 1994); and rice and millet (Devos et al. 1998). Rice is a model plant for cereals due to its small genome size, its synteny with other monocots, its efficient transformation system, the availability of large-scale expressed sequence tags, and its dense molecular genetic map. This being the case, in the present study tef has been compared with rice using the grass anchor probes (Van Deynze et al. 1998) and other rice cDNA probes for the first time. As there is little information on the genetics of tef, this crop will benefit greatly from the information already available in rice and other genera through such a synteny study.

Therefore, the objectives of the present study were to (1) develop a genetic linkage map of tef, and (2) to analyze the synteny between tef and rice genomes using RFLP probes of tef, rice, and other grass species.

Materials and methods

Plant materials

The linkage map was constructed using F_8 recombinant inbred lines (RILs) from an interspecific cross between *E. tef* cv Kaye Murri and *Eragrostis pilosa* (accession 30–5) employing the single seed-descent method. Kaye Murri and 30–5 manifest extreme phenotypic characteristics for several qualitative and quantitative traits. Kaye Murri is a tall, thick-culmed, late maturing, white-seeded cultivar with red lemmas and very compact panicles. By contrast, 30–5 is a short, thin-culmed, early maturing, brown-seeded accession with yellow white lemmas and very loose panicles. A random sample of 116 RILs and the parental lines were grown in the greenhouse at Texas Tech University during the fall of 1998. For the purpose of DNA extraction, plants of each F_8-RIL and the parental lines were harvested 1 month after planting, freeze-dried and ground to powder using a mechanical mill.

DNA probes

Three sources of DNA probes were utilized: tef cDNA, grass "Anchor" probes, and rice cDNA. The grass "Anchor" probes (Van Deynze et al. 1998) were kindly provided by Dr. Susan McCouch (Cornell University). The rice cDNA probes, designated as RZ, (Causses et al. 1994) were obtained from Dr. Tanksley (Cornell University).

Tef cDNA probes were isolated from a tef cDNA library. Total cellular RNA was extracted from the mature expanded leaves of cultivar Kaye Murri according to the method of McDonals et al. (1987). The mRNA was isolated using the MESSAGEMAKER mRNA Isolation System from the Life Technology company. The cDNA library was constructed with the lambda ZipLox cDNA synthesis system (Life Technology). The estimated titer of the primary library was 2.4×10^6 pfu/ml. The DH 10B *Escherichia coli* strain was used for in vivo excision. More than 1,000 cDNA plasmid clones were rescued through an in vivo excision of part of the cDNA library, and were screened with tef genomic DNA as a probe to isolate single- and/or low-copy clones for RFLP mapping. Eventually 514 single- or low-copy cDNA clones were prepared in the form of plasmid DNA. These clones are named TCD, followed by a numerical identification.

DNA extraction and Southern-blot analysis

DNA was isolated using the potassium acetate method (Tai and Tanksley 1990). Seven micrograms of DNA per line were digested with restriction enzymes. Parental lines were surveyed with nine enzymes (*Apa*I, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Xba*I, and *Xho*I). The digested product was electrophoresed on 0.8% agarose gels using 1×TAE buffer. The DNA was then transferred to a Hybond N^+ nylon membrane (Amersham Company) in accordance with the technique Southern (1975). Probes were labeled using the random primer labeling method (Feinberg and Vogelstein 1983). DNA filters were pre-hybridized in hybridization buffer (6×SSC pH 7.0, 5×Denhardt Solution, and 0.5% SDS) containing 200 mg/ml of denatured salmon sperm DNA at 65°C for 4–6 h. This was followed by the addition of the labeled probe into the pre-hybridization mix, and overnight hybridization at 65°C. After hybridization, the filters were washed twice with 2×SSC, 0.1% SDS, and twice with 0.5×SSC, 0.1% SDS at 65°C, and exposed to X-ray film.

Data analysis and map construction

Plants containing alleles from parental lines were scored as '1' or '3', and heterozygous and ambiguous genotypes were designated as '–'. A chi-square test was performed to analyze the segregation pattern of markers for goodness-of-fit to the theoretical ratio. Data were analyzed using the computer program MAPMAKER v2.0 (Lander et al. 1987) on a Power Macintosh computer. The map was constructed with 'three point' and 'first order' commands with a LOD value of at least 3.0, and a theta set at 0.4. The 'try' command was used to place unassigned markers to a possible linkage group. Map distances were calculated using the Kosambi function (Kosambi 1944).

Results

Parental polymorphsim

All the 159 tef cDNA clones tested for polymorphism hybridized to digested tef genomic DNA; 151 revealed

Table 1 Summary of the parental survey for RFLPs

Clone source[a]	Tested	Non-signal or non-distinct bands	Distinct bands	Polymorphism	
				No.	%
Tef cDNA	159	8	151	105	69.5
Rice cDNA	116	20	96	62	64.6
Barley cDNA	16	3	13	9	69.2
Oat cDNA	29	5	24	14	58.3
Total	320	36	284	190	66.9

[a] The wheat clone is not included, since only one clone, WG110, was used

Table 2 Distribution of markers mapped on the linkage groups

Linkage group	Type of marker loci		Total no.	Map distance
	Tef cDNA	Markers previously mapped in rice		
A	21	7	28	346.91
B	17	9	26	312.94
C	10	12	22	239.46
D	6	4	10	127.05
E	4	2	6	84.86
F	5	1	6	69.15
G	2	4	6	68.14
H	4	3	7	55.90
I	3	1	4	41.14
J	2	1	3	25.88
K	2	4	6	18.69
L	2	0	2	14.84
M	1	1	2	12.50
N	1	1	2	12.11
O	1	4	5	11.97
P	1	2	3	11.66
Q	2	0	2	11.16
R	3	1	4	9.83
S	2	1	3	8.51
T	1	1	2	6.60
Total	90	59	149	1489.3

distinct bands and 105 (69.5%) detected polymorphism between the cv Kaye Murri and accession 30–5 with at least one of the nine restriction enzymes employed (Table 1). Of the 161 heterologous probes, 36 (22.3%) yielded no, or poor, hybridization signals and were not scored; out of the remaining 133 probes 85 (63.9%) detected polymophic bands between the two parents (Table 1). No significant difference was found between these two sources of probes for polymorphism level. The overall level of polymorphism was 66.9%. Most of the probes produced hybridization patterns with three to six bands against the tef DNA blots, with an average number of 3.6 RFLP fragments per probe.

Segregation of markers

One hundred and thirty nine restriction enzyme/probe combinations that detected polymorphism on the parental lines were screened against 116 RILs. Twenty seven markers (19.4%) detected two to four segregating loci. Therefore, this brought the total number of markers analyzed for mapping to 169, with an average of 1.2 polymorphic loci per probe. The different loci detected by one probe were indicated by small letters at the end of the probe name (a, b, c, and d). Most of the multi-locus probes showed a similar hybridization intensity for all bands. Based on all 116 RILs of the mapping population and all the marker loci, the overall level of heterozygosity was 2.5%. This value was close to the expected 1.56% in an F_8 generation.

Out of the 169 segregating RFLP loci, 157 (92.9%) segregated as co-dominant markers and 12 (7.1%) showed a dominant inheritance pattern. The chi-square test indicated that the majority of marker loci (85.2%) fitted the expected segregation ratio, whereas 25 (14.8%) segregated with significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) distortion from the expected ratio. Moreover, 72% (18) of the marker loci with the distorted segregation ratios deviated in favor of the cv Kaye Murri alleles.

Map construction

A tef RFLP linkage map was constructed with 116 F_8 RILs (Fig. 1). Twenty linkage groups covering a total distance of 1,489.3 cM and 149 loci were established. The linkage groups were named A, B, C, etc. and ar-

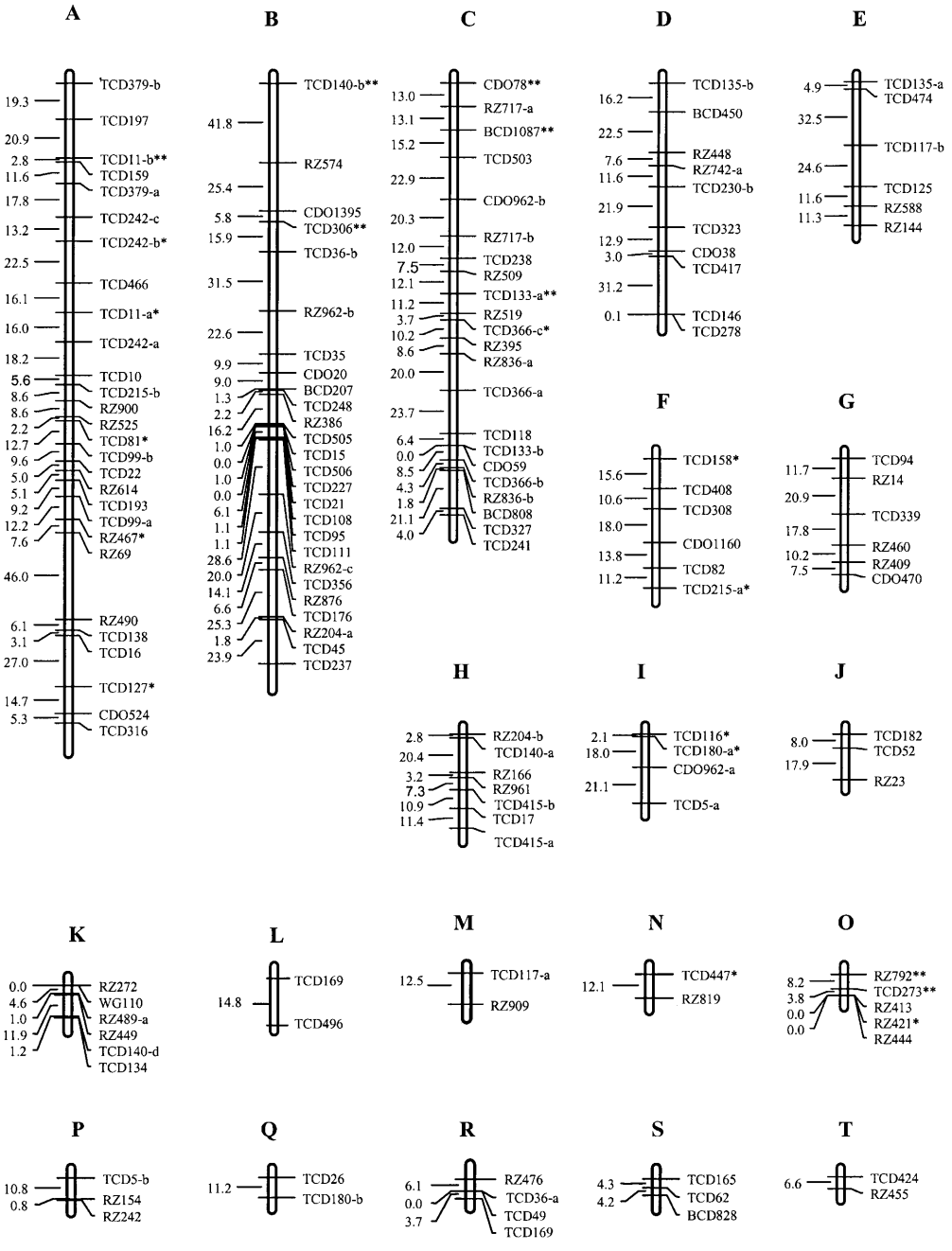

Fig. 1 RFLP linkage map of tef derived from recombinant inbred lines of 116 progenies (*E. tef* cv Kaye Murri×*E. pilosa*). Linkage groups are denoted as *A* to *T*. Map distance and marker names are shown to the left and right of each group, respectively. Tef cDNA clones are denoted by *TCD*; rice, barley, and oat cDNA clones are designated as *RZ*, *BCD* and *CDO*, respectively. Markers with the above names followed by the suffix *a*, *b*, *c*, and *d* represent the duplicated loci or multiple loci detected by one probe. Markers with an *asterisk* (*) or a *double asterisk* (**) indicate a significant ($P \leq 0.05$) and a highly significant ($P \leq 0.01$) distorted segregation

ranged in descending order based on genetic distance. The map was composed of 90 tef cDNAs (TCD), 43 rice cDNAs (RZ), ten oat cDNAs (CDO), five barley cDNAs (BCD), and one wheat clone (WG110). Twenty marker loci were unlinked. The linkage groups varied in length from 6.6 to 346.9 cM and the genetic distance between marker-loci pairs ranged from 0.0 to 46.1 cM with an average distance of 9.99 cM. Marker distribution throughout the genome was 2–28 markers per linkage group (Table 2).

The duplicate or multiple polymorphic loci detected revealed different distribution patterns on the map. Linkage groups B and H shared the ‘parallel’ duplicated RFLP loci detected by TCD140 and RZ204. Many other duplications involving just one probe were identified in pairs of linkage groups, i.e., probe TCD215 in groups A and F, TCD140 in groups B and K, TCD36 in groups B and R, CDO962 in groups C and I, TCD135 in groups D and E, TCD117 in groups E and M, and TCD180 in groups I and Q. In addition, several intra-chromosomal duplications were identified, where duplicated and/or triplicated loci were found on a given linkage group at different locations. For example, linkage group A contains duplicated and/or triplicated loci detected by TCD99, TCD242 and TCD379. Similar cases were found on group B, group C and group H.

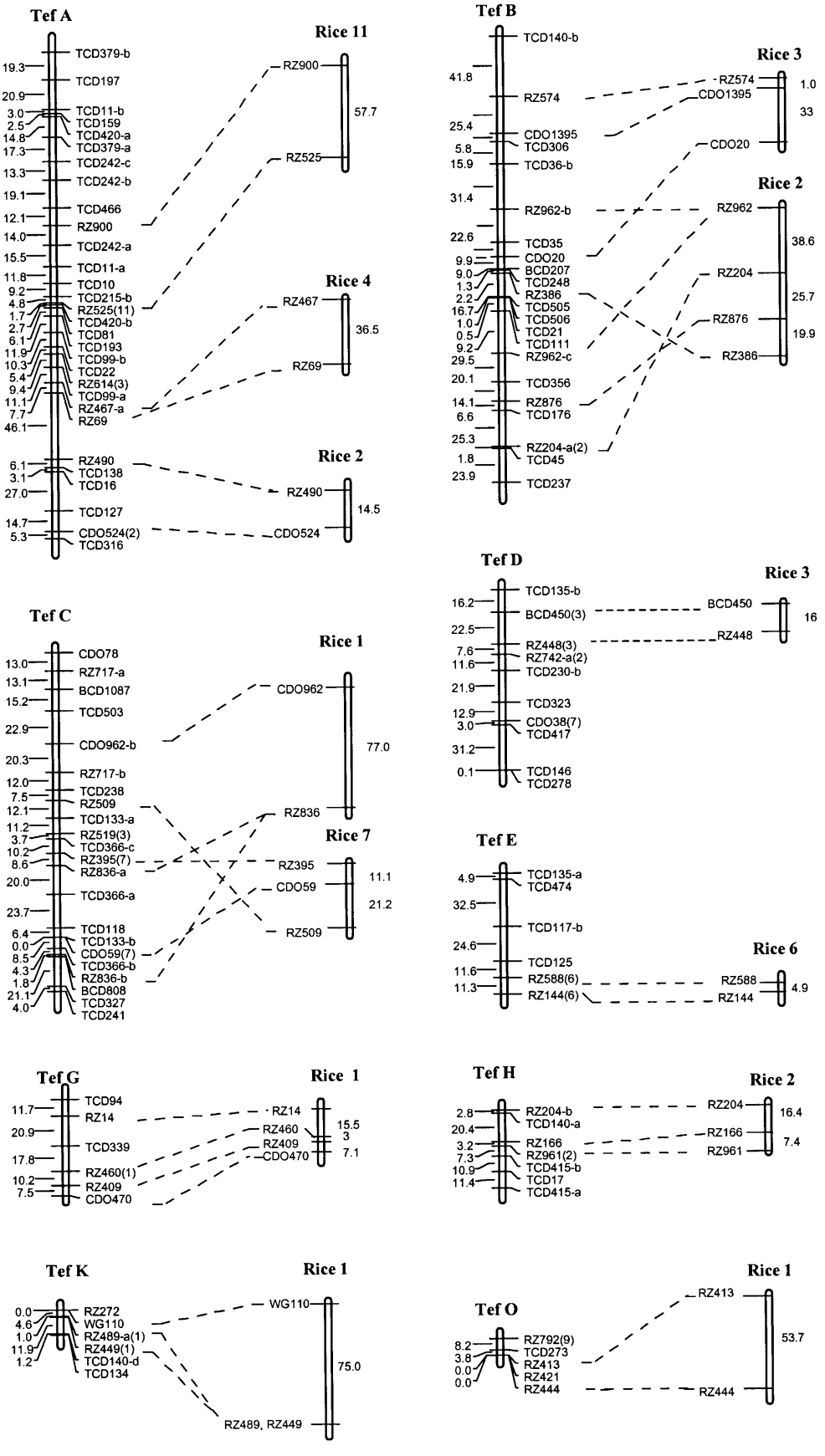

Fig. 2 Alignment of the RFLP linkage maps between tef and rice. *Vertical lines* represent the linkage maps of tef and rice. *Numbers* in parentheses after markers on a tef linkage group indicate the rice chromosome to which markers have been mapped. *Dashed horizontal lines* connect corresponding marker loci on the respective linkage maps

Comparison of the tef and rice RFLP maps

For the purpose of comparison of linkage in tef and rice, reference information about rice was obtained primarily from the map of Causse et al. (1994). Alignment of the tef RFLP map with the rice RFLP map indicated that a number of syntenic chromosomal fragments exist between tef and rice (Fig. 2). It is noteworthy that among these syntenic regions the genetic distance spanned by the same loci differed between the two genomes. For example, tef linkage group G contained four collinear marker loci from rice chromosome 1. These four loci spanned 56.4 cM in tef and 25.6 cM in rice. In total, 12 conserved linkage segments with two or more loci were identified between the tef and rice genomes and they covered 428 cM and 403 cM of the tef and rice genomes, respectively. Some tef linkage groups consisted of two or more different rice chromosome fragments. Out of six conserved linkage segments with three or more loci, four showed a completely conserved order with the rice chromosomes. Results of the distribution of conserved fragments across the various tef linkage groups are presented as follows:

Linkage group A

Seven marker loci previously mapped on rice chromosomes were mapped to this linkage group, of which two map to rice chromosome 2, two to rice chromosome 4, two to rice chromosome 11, and one to rice chromosome 3.

Linkage group B

Nine markers from rice were mapped onto linkage group B. Five of these nine loci were contiguous in this linkage group and correspond to rice chromosome 2. Comparison of the order of these loci showed inversion or duplication (RZ204 and RZ386 were inverted, and RZ962 was duplicated in the tef genome). Three of these nine loci are also contiguous in this linkage group and correspond to rice chromosome 3. The order of these markers is the same in both tef and rice genomes. The remaining marker (BCD207) corresponds to rice chromosome 10.

Linkage group C

Twelve marker loci from rice were mapped to this linkage group. However, these marker loci appear to have the least conserved linkage relationship between tef and rice. RZ509, RZ395 and CDO59 correspond to rice chromosome 7, but RZ509 and RZ395 were inverted. CDO962 and RZ836 correspond to rice chromosome 1; however, RZ836 is duplicated in the tef genome. The remaining five loci (RZ519, RZ717a and b, CDO78, BCD1087 and BCD808) were from rice chromosomes 3, 4, 6, 9 and 11, respectively.

Linkage group D

Linkage group D contains four loci of which two map to rice chromosome 3, one maps to rice chromosome 2, and one to rice chromosome 7.

Linkage group E

Only two markers from rice chromosome 6 map to linkage group E.

Linkage group G

Linkage group G comprises four markers from rice chromosome 1. These marker loci were located in the same linear order in both the tef and rice genomes.

Linkage group H

Three markers from rice were mapped to this linkage group. All of them were located to rice chromosome 2 and with the same order.

Linkage group K

This group contains four markers of which three markers from rice chromosome 1 were mapped to this linkage group with collinearity to rice and one mapped to rice chromosome 7.

Linkage group O

Linkage group O contains four markers of which two map to rice chromosome 1, one maps to chromosome 9, and one to chromosome 10, respectively.

Linkage group P

Only two markers from rice were mapped to linkage group P. One corresponds to rice chromosome 1 and the other corresponds to rice chromosome 6.

Discussion

In this study, the level of polymorphism (66.9%) was much higher than that of our earlier report using RAPDs and AFLPs (Bai et al. 1999). This discrepancy may be attributed to the fact that we used inter-specific crosses and relatively more restriction enzymes in the present study. Compared with other cereal species, this polymorphism level appears to be relatively low. For example, a polymorphism level of around 80% is common in rice

(McCouch et al. 1988) and foxtail millet (Wang et al. 1998). Regarding the distorted segregation marker loci, this study revealed 14.8% of marker loci with skewed segregation ratios. Higher frequencies of skewed markers were reported in other studies on inter-specific crosses such as tomato (Zamir and Tadmor 1986), potato (Bonierbale et al. 1988), oat (O'Donoughue et al. 1992), rice (Xu et al. 1997), and durum wheat (Blanco A, et al. 1998). Factors such as the gametic or zygotic selection, and genetic drift has been considered as important causes for this kind of deviation.

The current map contains 149 markers spread across 20 linkage groups. The 20 linkage groups appear to be commensurate with the haploid chromosome number of tef (2n=4*x*=40). This should serve as a good entry point for further mapping, and the tagging of economically useful traits such as flowering time, plant height, yield and yield components, and lodging resistance and its components. The parents of the current mapping population differ for these and many other agronomic traits, including drought resistance mechanisms such as root depth and osmotic adjustment.

RFLP maps can provide unique insights into the structure and evolution of an organisms genome. The fact that nearly 20% of the probes in this study hybridized to duplicate loci, and that most of the probes detected at least two bands in each parent, demonstrates the characteristics of tef as a tetraploid. Polyploid histories of many contemporary diploid crop species such as maize (Helentjaris et al. 1988), sorghum (Whitkus et al. 1992), soybean (Shoemaker et al. 1996) etc., were also revealed by the conserved linkage relationships among duplicated genome regions. Duplicated loci were interspersed among all loci within most of the linkage groups. In addition, some of the duplicated loci were shared between pairs of linkage groups suggesting an inter-chromosomal homology in tef, which might correspond to the genomes of the unknown putative diploid ancestors of tef. The intra-chromosomal duplication suggests the presence of tandem duplications in the tef genome.

RFLP maps have been developed for many species of plants. Comparison of these maps revealed syntenic linkage relationships among genomes within the *Poaceae* (Whitkus et al. 1992; Ahn et al. 1993; Kurata et al. 1994; Devos et al. 1998) and the *Solanaceae* (Bonierbale et al. 1988; Tanksley et al. 1988, 1992; Gebhardt et al. 1991). The high degree of linkage conservation within the *Poaceae* is extensive enough that clones can be readily cross-hybridized to other grass species, and linkage blocks (segments) of the rice genome can be assembled to reconstruct the genomes of other species (Bennetzen and Freeling 1993, 1997; Moore et al. 1995; Devos and Gale 1997; Gale and Devos 1998). Tef is less familiar to the scientific community than other grass species. However, making strong inferences about genome structure and evolution requires the study of species with less information. Tef as a potential important grain crop is a chloridoid that would be a useful point of comparison with the major cereal crops (Kellogg 1998).

Even though the tef genome differs from the rice genome in many aspects such as ploidy level, genome size and ploid characteristics, cDNA clones previously mapped to rice chromosomes were transferable to the tef genome and 12 conserved linkage fragments were identified between the two genomes. Some linked comparative markers in tef showed the same linear order with rice chromosomes. Several tef linkage groups were composed of discrete segments from two or more different rice chromosomes. These phenomena may suggest the occurrence of inter-chromosomal translocation events during the speciation of tef. Similar discoveries were also reported in the studies of comparative mapping analysis for rice and wheat (Ahn et al. 1993; Kurata et al. 1994) and barley and rice (Saghai Maroof et al. 1996). The duplicated loci of so many probes will definitely confound the comparison between the rice map and the tef map. It is acknowledged that, compared to many other comparative maps, this study included a relatively small number of heterologous markers so that the result is only preliminary. However, synteny between tef and rice indeed exists and no similar work has yet been reported for tef.

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