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Assessment of the genetic relationship of tef (*Eragrostis tef*) genotypes using SSR markers

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ABSTRACT

Knowledge of the genetic relationships of plant genetic resources is fundamental for effective selection and conservation. Tef [*Eragrostis tef* (Zucc.) Trotter] is a gluten-free small cereal crop that exhibits considerable genetic variation. The objective of this study was to determine the genetic relationships among 60 diverse tef genotypes to select unique and genetically unrelated lines by using 10 selected diagnostic and polymorphic simple sequence repeat (SSR) DNA markers. The results indicated that the number of alleles per locus varied from 10 to 23 with a mean of 16. The polymorphic information content (PIC) ranged from 0.64 for marker CNLTS11 to 0.94 (CNLTS136A/B) with a mean of 0.84 suggesting sufficient discrimination power of the markers to discriminate the tested genotypes. The analysis of molecular variance showed that 63% and 35% of the total variability could be attributed to differences within and among tef genotypes, respectively. The high level of genetic dissimilarity within the tested tef genotypes provides an opportunity for systematic selection and conservation. Overall, the SSR analysis identified distinct genotypes such '*DZ-Cr-385*', '*222076*', and '*213237*', which are known for their early maturity and good yields under moisture stress. The analysis also identified the genotypes '*DZ-Cr-387*', '*205896*', '*205917*', and '*Dschanger*', which are consistent to their unique agronomic attributes such as late maturity, high grain yields, relatively good plant heights, and long panicles under optimum rainfall conditions. The identified and agronomically complementary tef genotypes are valuable genetic resources for further breeding.

1. Introduction

Systematic genetic characterization and well-defined genetic relationships among plant genetic resources are fundamental for effective selection and conservation. This would allow for the identification of genetically unrelated and agronomically complementary genotypes for designed crosses and improved selection for important traits. Molecular markers are more efficient in germplasm characterization than phenotypic or biochemical markers (Aremu, 2011; Jonah et al., 2011; Ranade and Yadav, 2014; Jingura and Kamusoko, 2015).

Various molecular marker systems were used effectively to assess the genetic relationships and patterns of association among plant genetic resources. These included restricted fragment length polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplification Regions (SCARS), simple sequence repeat (SSR) or microsatellites and single-nucleotide polymorphism (SNP) (Datta et al., 2011; Govindaraj et al., 2015). The simple sequence repeat (SSR) markers have been extensively used in genetic diversity analysis of different cereal crops including rice (Ishak et al., 2015; Sarma et al., 2015), wheat (Bafghi et al., 2014; Hamdalla, 2014; Drikvand et al., 2015), sorghum (Beyene et al., 2014), and barley (Hua et al., 2015). These markers are known for their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance, and good genome coverage (Gyulai et al., 2006, 2012; Senan et al., 2014; Ali et al., 2014, 2015).

Tef [*Eragrostis tef* (Zucc.) Trotter] is an allotetraploid (2n = 4x = 40, AABB) autogamous small cereal crop widely cultivated in the Horn of Africa (Eritrea and Ethiopia) for over 2000 years (Ponti, 1978). In the region, tef supports some 60 million people providing food security and rural livelihoods. Tef products such as breakfast cereal, porridge, bread, pancake, waffles, and juices are becoming popular worldwide for their gluten-free status which is helpful to people with gluten intolerance. Tef straw is also a valuable source of livestock feed. In South Africa, India, Pakistan, Uganda, Kenya, and Mozambique, tef is mainly grown as a forage or pasture crop (Assefa et al., 2011).

Ethiopia is believed to be the center of origin and diversity of tef (Vavilov, 1951). In the country, tef remains the number one crop in terms of area coverage with an estimated annual acreage of more than 3 million ha (CSA, 2014). Tef shows considerable phenotypic variation, with wide adaptation across a range of agro-ecologies (Assefa et al.,

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2015). However, tef yields are low, with a mean national yield of 1.47 t ha^{-1} (CSA, 2014). The low yield of tef is attributed to its susceptibility to lodging, frequent moisture stress, and poor agronomic management with few inputs (Ketema, 1997). Tef yield could be enhanced through selective breeding using locally adapted, farmers-preferred, genetically complementary lines. This requires a genetic diversity analysis using effective and diagnostic molecular markers, and evaluation economic agronomic traits.

Several studies have been conducted to examine the diversity in tef germplasm using morphological and agronomic traits (Assefa et al., 2000, 2001a, 2001b, 2002a, 2002b, 2003b; Adnew et al., 2005; Admas and Belay, 2011; Ayalew et al., 2011; Shiferaw et al., 2012; Plaza-Wuthrich et al., 2013). However, few attempts have been undertaken to study the diversity using DNA markers. Ayele et al. (1999) by using AFLP markers, and Bai et al. (2000) by using RAPD markers reported genetic similarity coefficients of 85–90% and 84–96%, which indicated a high level of genetic similarity of the tested genotypes. Previous research (Assefa et al., 2003a; Zeid et al., 2012) has shown the usefulness of SSR markers in genetic diversity analysis and in establishing genetic relationship among tef germplasm. Therefore, the objectives of this study were to determine the genetic relationship present among 60 diverse tef genotypes and to select unique and genetically unrelated lines using 10 known diagnostic and polymorphic simple sequence repeat (SSR) DNA markers.

2. Materials and methods

2.1. Plant materials

Sixty diverse tef genotypes were used for this study. Table 1 presents the names and origins of the genotypes. Thirty-three genotypes were improved varieties that were released by six agricultural research centers (MoA, 2014), 18 were landraces identified by Ebba (1975), and nine were new accessions collected from the Tigray region of northern Ethiopia. The lines identified by Ebba (1975) were originally collected from diverse tef growing zones in Ethiopia including Shoa (8), Gojam (3), Keffa (2), Welega (3), and Hararge (2) (Table 1). Photos of the popular landrace varieties '*Dschanger*' and '*Kaye-Murri*' are shown in Fig. 1.

Table 1
List of the 60 tef [*Eragrostis tef* (Zucc.) Trotter] genotypes used in the present study.

Populations	Collection zones or sources	Name of genotypes
Landraces	Shoa	'*Ada*','*Rosea*', '*Fesho*', '*Kaye-Agachew*', '*Purpurea*', '*Manya*', '*Enatite*', '*Rubicunda*'
	Welega	'*Gea-Lamie*', '*Alba*', '*Kaye-Murri*',
	Hararge	'*Gorradie*', '*Denkeye*'
	Gojam	'*Dabbi*', '*Curati*', '*Jano*'
	Keffa	'*Shawa-Gemerra*', '*Dschanger*'
New accessions	West Tigray	'*9446*'
	South Tigray	'*213237*'
	South east Tigray	'*205921*', '*205896*', '*205917*'
	Central Tigray	'*222076*', '*9415*', '*Zezew*',' *Zagure*'
	DZARC	'*DZ-Cr-387*', '*DZ-01-2675*', '*DZ-Cr-44*', '*DZ-01-99*', '*DZ-Cr-37*', '*DZ-Cr-409*', '*DZ-01-899*', '*Ho-Cr-136*', '*DZ-01-1681*', '*DZ-Cr-358*', '*DZ-Cr-82*', '*DZ-01-1281*', '*DZ-01-196*', '*DZ-Cr-354*', '*DZ-01-974*', '*DZ-01-787*', '*DZ-Cr-385*', '*DZ-Cr-255*', '*DZ-01-1285*'
Improved varieties	HARC	'*DZ-01-2053*', '*DZ-01-1278*', '*Acc.205953*'
	MARC	'*DZ-Cr-387-RIL127*'
	SARC	'*DZ-01-2054*', '*DZ-01-146*', '*DZ-01-1821*', '*DZ-Cr-387RIL273*'
	ADARC	'*DZ-01-1868*', '*DZ-01-2423*', '*DZ-01-3186*'
	BARC	'*DZ-01-1880*', '*23-Tafi-Adi-72*'
	ARARC	'*PGRC/E 205396*'

DZARC = Debre-Zeit Agricultural Research Centre, HARC = Holleta Agricultural Research Centre, MARC = Melkassa Agricultural Research Centre, SARC = Sirinka Agricultural Research Centre, ADARC = Adet Agricultural Research Centre, BARC = Bako Agricultural Research Centre, ARARC = Areka Agricultural Research Centre.

2.2. DNA sampling, SSR markers, and PCR amplification

The 60 tef genotypes were grown in the glasshouse at the University of KwaZulu-Natal. Leaf samples were taken from 3-week-old seedlings. All samples were used in bulked amplification using DNA from 10 individual leaf samples. For each, 2 μL of bulked sample was used in the polymerase chain reaction (PCR).

The SSR analysis was done at the INCOTEC-PROTEIOS laboratory in South Africa (Incotec, SAPty. Ltd. South Africa). Ten polymorphic SSR markers were used in this study (Table 2). The primer sequences used for polymerase chain reaction (PCR) amplification were selected from published SSR-based map of tef (Zeid et al., 2011). PCR was done for all of the 10 primers. PCR products were fluorescently labeled and separated by capillary electrophoresis using an ABI 3130 automatic sequencer (Applied Bio systems, Johannesburg, South Africa).

2.3. Data analysis

Genotypic data were subjected to various measures of the genetic relationships within and among the tef genotypes using GenAlex version 6.5 (Peakall and Smouse, 2007). Genetic parameters such as total number of alleles per locus (Na), number of effective alleles per locus (Ne), Shannon's Information Index (I), and gene diversity were determined according to the protocol described by Nei and Li (1979). The F-statistics such as genetic differentiation (F_{ST}), fixation index or inbreeding coefficient (F_{IS}), and overall fixation index (F_{IT}) were calculated according to Wright's original derivation (Wright, 1951). Based on Euclidian distances, analysis of molecular variance (AMOVA) was conducted to partition the total genetic variation within and among genotypes. Among other genetic parameters, gene flow (N_m) was calculated using the following: $Nm = ¼*[(1 - F_{ST})/F_{ST})]$ where F_{ST} is genetic differentiation (Slatkin and Barton, 1989). Polymorphic information content (PIC) was estimated using the formula: $PIC = 1 - \sum P_i^2$ where P_i is the frequency of the i-th allele.

Cluster analysis was carried out by using neighbor-joining (NJ) algorithm using the un-weighted pair group method (UWPGM) in DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2006). A dendrogram was then generated using the dissimilarity matrix. Bootstrap analysis was performed for node construction using 1000 bootstrap values.

3. Results and discussion

3.1. Genetic polymorphism of SSR markers

Table 3 summarizes the genetic diversity parameters of the 10 SSR markers. The difference between the longest and shortest amplified fragment size ranged from 158 to 334 bp. The highest variation in fragment size was observed for primer CNLTS455A/B (220–299 bp) and the lowest was for primer CNLTS33 (235–257 bp). All the 10 SSR primer pairs were polymorphic and a total of 164 alleles were detected. The number of alleles per locus ranged from 10 for SSR markers CNLTS11 and CNLTS380 to 23 for markers CNLTS136A/B and CNLTS42, with a mean of 16.4 alleles per locus. This was in agreement with an average 8–23 alleles per locus reported by Zeid et al. (2012). The number of effective alleles (N_e) ranged from 3.21 for marker CNLTS11 to 10.95 for marker CNLTS136A/B, with a mean of 7.14 (Table 3).

The observed heterozygosity (H_o) varied from 0.38 for marker CNLTS538 to 1.00 for CNLTS136A/B, with a mean H_o of 0.67. Gene diversity (H_e) of the markers ranged from 0.67 (CNLTS11) to 0.94 (CNLTS136A/B), with a mean of 0.86. Similarly, Zeid et al. (2012) reported H_e ranged from 0.75 to 0.91 when using these markers. The high heterozygosity and low genetic fixation in the current study signals high genetic variability within the presently sampled tef genotypes.