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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SELECTED TEF (*Eragrostis tef* (Zucc.)Trotter) ACCESSIONS FROM THE GENE BANK OF ETHIOPIA (REVIEW)

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Abstract: **Tef (Eragrostis tef (zucc.) Trotter) is a major cereal crop resilient to adverse climatic and soil conditions, and possessing desirable storage properties. The crop is grown mainly for food in Ethiopia and is used as a health food alternative in the Western part of the country. Evaluating the extent of genetic diversity within and among populations is one of the first and most important steps to identify useful breeding materials and design appropriate collection and conservation strategies. Hence, this review is motivated to further dissect and characterize the genotype composition of tef accessions obtained from the gene bank at EBI (Ethiopian Biodiversity Institute) using morphological and SSR molecular markers and investigate similarity and variation both among and within tef accessions collections obtained from EBI.**

Keywords: **Phrases: Conservation, Diversity, Genotype composition, Gluten free, SSR markers.**

1. INTRODUCTION

Tef (*Eragrostis tef* (Zucc.) Trotter) belongs to family *Poaceae*, subfamily *Eragrostoideae,* tribe *Eragrosteae* and genus *Eragrostis* (Costanza, 1974). The genus *Eragrostis* constitutes about 350 species of which only tef is cultivated for human consumption (Watson & Dallwitz,1992). It is a C4, self-pollinated, and allotetraploid plant species and closely related to finger millet (*Eleusine coracana* Gaerth) as both are in the sub family *Chloridoideae* (Kebebew Assefa *et al.*, 2015).

Tef is an endemic tropical cereal crop of Ethiopia and it has been cultivated for thousands of years in high lands of Ethiopia (Viswanath, 2013), and Ethiopia is recognized as the center of origin and diversity of tef (Vavilov, 1951). Tef is a superior cereal grain crop simply produced and is considered as the noble grain of Ethiopia (Yihenew Gebresellasie, 2002). The crop is also a highly valued primarily grown for its grain that is used for making “injera” (Seyfu Ketema, 1997). Injera is a fermented pancake made from tef flour and is a sour flatbread used in Ethiopian and Eritrean cuisine that is thicker than a crepe but thinner than a pancake and has a delightfully sour taste. In Ethiopian and Eritrean cuisines, vegetable, lentil, or meat dishes are served on top of the injera, and the food is eaten with your hands, using the injera to scoop up the food (Ashley, 2020).

Tef is highly nutritious crop riched with different minerals, carbohydrates and vitamins (Bultosa *et al*., 2002; and Abebe Yewelsew *et al*., 2007) and the eight Essential Amino Acids (isoleucine, leucine, methionine, lysine, phenylalanine, threonine, tryptophan and valine). The protein digestibility is high because the main protein fractions are the most digestible types, and the absence of gluten makes it an alternative food for people suffering from celiac disease (Spaenij *et al*., 2005).

In Ethiopia, out of the total grain crop area, 80.71% (10,232,582.23 hectares) was under cereals, but tef took up 23.85% (about 3,023,283.50 hectares) as compared with maize, sorghum and wheat 16.79% (about 2,128,948.91 hectares), 14.96% (1,896,389.29 hectares) and 13.38% (1,696,907.05 hectares) of the grain crop area, respectively and the crop is considered as the leading crop in terms of area coverage (CSA, 2017/18). The crop is also grown outside Ethiopia only in few countries including Eritrea, Kenya (near Marsabit), Yemen, Malawi, and India. Tef has been introduced to South Africa and cultivated as a forage crop, and in recent years cultivated as a cereal crop in northern Kenya (Seyfu Ketema *et al*., 1997).

2. LITERATURE REVIEW

2.1. ORIGIN AND DISTRIBUTION OF TEF

Tef (*Eragrostis tef* (Zucc.) Trotter) is a crop for which Ethiopia is the center of origin and diversity (Vavilov, 1951). Tef is endemic to Ethiopia and its major diversity is found only in this country. Tef is a fine grain that comes in a variety of colors, from white and red to dark brown. The exact date and location for the domestication of tef is unknown, it is native and an important cereal crop to Ethiopia which is believed to be originated between 4000 and 1000 BC (Ponti, 1978). However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ (Seyfu Ketema, 1997). According to Ponti (1978), tef was introduced to Ethiopia well before the Semitic invasion of 1000 to 4000 BC. It was probably cultivated in Ethiopia even before the ancient introduction of wheat and barley (Shaw, 1976).

In the genus *Eragrostis*, 43% of the species seem to have originated in Africa, 18% in South America, 12% in Asia, 10% in Australia, 9% in Central America, 6% in North America and 2% in Europe (Costanza *et al*., 1979). According to Cufodontis (1974), 54 species are found in Ethiopia, out of which 14 (or 26%) are said to be endemic.

2.2. MORPHOLOGY AND PHYSIOLOGY OF TEF

Morphology

Tef is possibly the smallest cereal grain with an average length of ~ 1 mm (Bultosa, 2007; Adebowale *et al.*, 2011). The name tef is said to have probably originated from the Amharic word “tefa”, (Seyifu Ketema, 1993) which means lost because of small seed size that is difficult to find once it is dropped. However, other more credible sources state that it was derived from the Arabic word “tahf” a name given to a similar wild plant used by Semites of south Arabia during the time of food insecurity (Costanza *et al.*, 1980). The seed sizes are quite small, ranging from 1–1.7 mm long and 0.6–1 mm diameter with l000 seed weight averaging 0.3–0.4 g and 150 grains of tef has equivalent weight with almost one seed of wheat (Arguedas *et al*., 2008). The average thousand kernel weight of 12 tef varieties tested by Bultosa (2007) was 0.264 g. The minuteness of tef grains has nutritional and technological implications. For instance, as tef grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers.

Morphologically, tef is a fine stemmed, tufted annual grass characterized by a large crown, many shoots and a shallow, diverse root system. Its inflorescence is a loose or compact panicle and is a fine grain that comes in a variety of colors, from white and red to dark brown. (Arguedas *et al*., 2008). The tef plant root system is thin and fibrous (thread-like) rarely emerging from nodes above the base, and growing 4–8 cm deep under field conditions (Tadesse Ebba, 1969). The stems are mostly erect (ascending), but creeping or bending or elbowing (geniculate) in some cultivars, and jointed with hollow internodes separated by nodes. Each culm internode, except the most basal one, bears one leaf consisting of a sheath and a blade. The Paniculate tef inflorescence ranges in form from very compact (whip-like) to very loose and open. The panicles ramify into primary, secondary and tertiary branches bearing spikelet’s. Each spikelet bears a pair of unequal sized glumes at the base and a number of florets (3–17) above. Each floret in turn comprises a tri-nerved lemma, a two-nerved bow- or boat-shaped palea, three stamens (arising from the ovary base and having very fine and slender filaments bearing length-wise opening anthers at the apex), and an ovary or a pistil. The ovary consists of two or, in a few exceptional cases; three styles each ending in a plumose (feathery) yellowish white stigma. Tef is a highly self-fertilized species with natural out-crossing of about 0.2–1% (Seyifu Ketema, 1993). Flowering, anthesis and maturity of florets and grains are basipetal (top–down) on a panicle basis and acropetal (bottom–up) on individual spikelet basis.

The color of tef can vary from white (ivory) to dark brown (black) depending on the variety. In Ethiopia, three major categories can be identified: white (nech), red (quey) and mixed (sergegna). It is also common for wholesalers to further subdivide white tef into very white (magna) and white (nech). However, given that these classifications are imprecise and subjective, what may be referred as magna by some may be considered as nech by others. White tef generally grows only in the Ethiopian highlands and require relatively good growing conditions. This, along with its higher consumer preference, may justify why white tef is the most expensive type of tef. However, in recent years, red tef, which is believed to be more nutritious, is also gaining popularity among health conscious consumers in Ethiopia (Kaleab Baye, 2014).

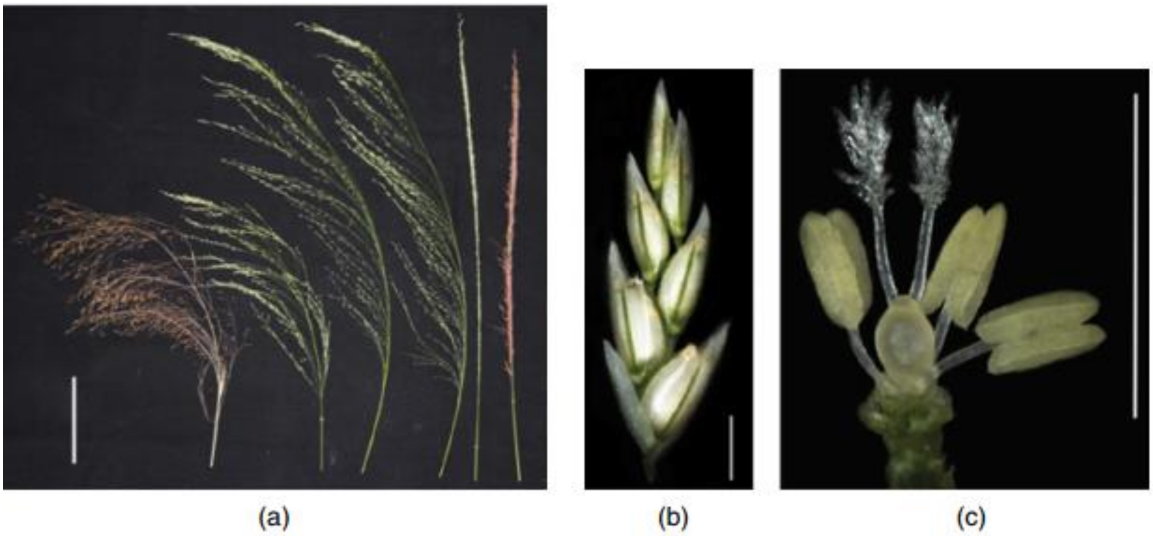

Figure 1: The infloresence and flower of tef (a) panicles of tef differ in color and size (scale bar = 10 cm); (b) spike of tef showing individual spikelet (scale bar = 1mm); (c) structure of tef flower indicating three stamens and a pair of hairy stigmas (scale bar = 1mm). Source: (Kebebew Assefa *et al.*, 2017).

Physiology

Tef is an herbaceous annual plant requiring 60–140 days to attain physiological maturity (Kebebew Assefa *et al.*, 2001). It is a C4 plant having 4-carbon compounds (malic or aspartic acid) as the first photosynthesis product. It also requires high optimum temperature for photosynthesis, and while the characteristic carbon dioxide compensation points are in the range of 14–20 ll/l for C3 and 5 ll/l or less for C4 plants, for tef it is 6 ± 1 ll/l at 38 ºC. In addition, the leaf anatomy of tef is Kranz type (wreath-like arrangement) having vascular bundles surrounded by bundle sheath cells in a circular manner (Abuhay Takele *et al.*, 2001). On the other hand, tef is also relatively waterlogging tolerant as compared to the other small cereals such as wheat and barley (Seyifu Ketema, 1993).

3. IMPORTANCE OF TEF TO ETHIOPIAN ECONOMY

Tef is largely produced for market because of the high price it fetches and absence of alternative cash crops (such as coffee, tea or cotton) (Asmiro Abeje *et al.*, 2019). Tef is the second most important cash crop after coffee and generating around 500 million USA dollar or (15,000,000,000 ETB) incomes per year for local farmers (Minten. *et al.*, 2013). Tef is the most value-added crops compared to other cereal crops. Following the imposing ban on raw tef grain export; selling of processed form of tef product is started to rise at national and global level as well as benefited many stakeholders involved in the process. Currently, Ethiopian pancake “injera” is found for sale in domestic and international market. It contributes to job creation for many people of the country (Asmiro Abeje *et al.*, 2019), and plus due to the number of benefits (gluten free and high nutrition values); the demand for Ethiopian tef is exponentially increased worldwide. Therefore, it is suitable to prepare food products for people who suffer from celiac disease.

Due to its high demand for consumption, tef is being distributed to other areas. As reported by FAO (2015) while the bulk of the grains move through Addis Ababa, some urban consumption centers, such as Mekele and Dessie, get their supplies directly from production areas. Other urban centers, such as Harar and Dire Dawa are supplied from Adama (East Shewa).

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The demand for the crop is consistently high over time as compared to other grain crops in Ethiopia, because of its unique taste highly preferred by consumers (Engdawork Tadesse, 2009). Compared to other staples, the price of tef has increased at a faster rate in recent years; hence, the price gap between tef and other staples is widening. In particular, the price gap between tef and maize has widened considerably since 2008 (Fufa Hundera *et al*, 2011). Farmers growing tef have benefited in recent years, as the relative price of tef (which they sell) has increased. Although tef has been consumed in the Horn of Africa for millennia, it has not been known to the outside world until very recently. With increased globalization and labor migration, tef began to be exported to the Middle East, North America and Europe to satisfy the demand of Ethiopians abroad (Mulat Demeke *et al.*, 2013). Furthermore, because of its nutritional properties high in fiber, calcium, iron and increasing demands in European and North American markets (FAO, 2015).

As illustrated in (Fig.2), the exports of injera in 2015 were estimated at around 10 million US dollar. Then main “injera” international market outlets were North America; Middle East and Europe. The largest share (approximately 2.5 million US dollars) of “injera” exports has gone into North America in the 2015 year. The reason could be driven by the presence of millions of Ethiopian immigrants who remain attached to this native cooking culture. However; in the future; the demand for “injera” might be exponentially increased due to its high nutritional values and being gluten free.

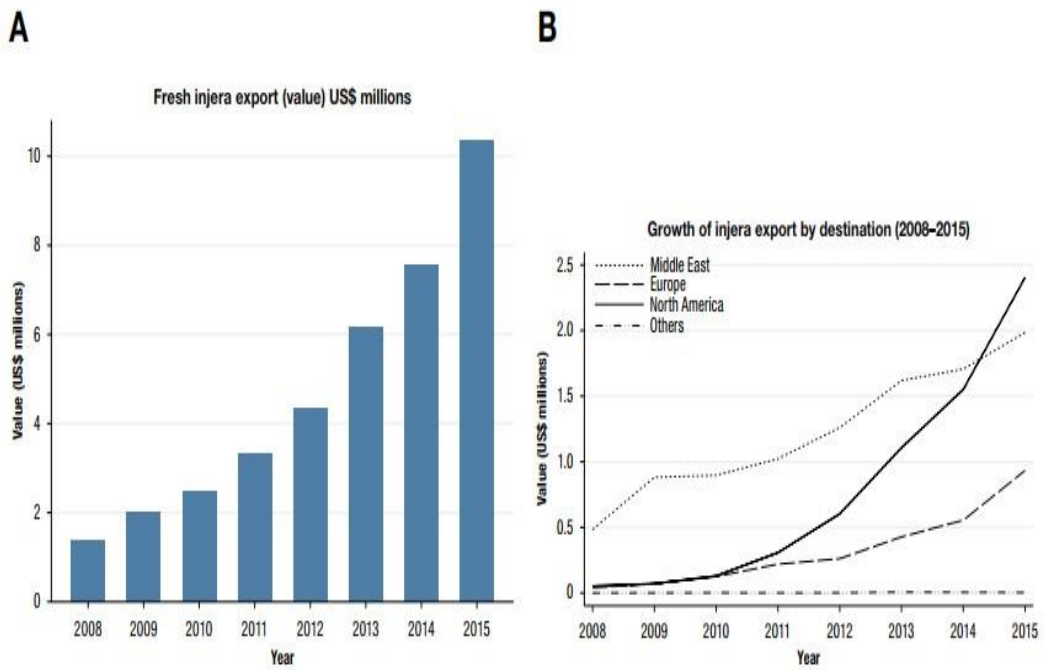

Figure 2: Injera exports from Ethiopia by value 2008- 2015. Source: (Asmiro Abeje *et al.*, 2019).

In general, cereal production and marketing constitute the single largest sub-sector in Ethiopian economy. It accounts for roughly 60% of rural employment, 80% of total cultivated land, more than 40% of a typical household’s food expenditure, and more than 60% of total caloric intake (These numbers are taken from various CSA publications). The contribution of cereals to national income is also large. According to available estimates, cereal production represents about 30% of gross domestic product (GDP). This calculation follows from the fact that agriculture is 48% of the nation’s GDP (World Bank, 2007), and that cereals’ contribution to agricultural GDP is 65 %.

4. OVER VIEW OF TEF RESEARCH

Scientific research to improve tef started in the late 1950s at Jimma Junior College of Agriculture. The research was focused largely on the development of improved varieties through direct selection from farmers’ varieties. In 1960 tef improvement research was transferred to the Debre Zeit Agricultural Research Center. Since then the breeding work has passed through three main phases. During the period from 1960 to 1974, the work focused largely on mass selection of superior genotypes from farmers’ varieties. Due to lack of knowledge on the floral biology of tef, crossing techniques were unavailable in these years.

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The second phase covers the years from 1975 to 1995 when the supposed impediment to crossbreed tef was finally resolved. The early and short pollination period that lies between about 6:45 and 7:50 am was discovered by Tareke Berhe towards the end of 1974 (Tareke Berhe, 1975). This discovery opened the possibility of developing varieties through trait recombination. This conventional plant breeding procedure has led to a remarkable yield increase of cereal crops in the world. Nonetheless, in tef this technique has been little applied, and only about 400 crosses have been made since 1975. The tiny floret poses a problem in making 158 crosses (Hailu Tefera *et al.*, 2003). Presumably, this floral problem has made it less attractive to crop breeders, which in turn has prevented accumulation of knowledge about its genetics and breeding.

In the third phase, which began in 1995, molecular breeding approaches were considered to augment the conventional breeding to characterize tef diversity at the DNA level, to develop a genetic linkage map and to identify quantitative trait loci.

5. GENETIC VARIABILITY IN TEF

For sustainable and stable food production, maintaining genetic diversity within and between crop types is increasingly being realized as the most appropriate and indispensable action. This is further emphasized by unpredictable human food needs, changes in taste, technological demand, and the biotic and abiotic production constraints that change with the environments (Seyfu Ketema,1997).

The molecular characterization within and between plant populations is routinely performed using various techniques such as morphological, biochemical characterization and DNA marker analysis, as they can exhibit similar modes of inheritance. DNA markers are the most widely used type of marker predominantly due to their abundance (Paterson, 1996). The first investigations of the genetic relationship among accessions *of E. tef, E. pilosa and E. curvula* by molecular markers were made using AFLP (Mulu Ayele *et al.*, 1999, Bai *et al.*, 1999) and RAPD markers (Bai *et al.*, 2000). In general, analyses revealed a relatively low level (18%) of DNA polymorphism and genetic variations within *E. tef*, and high similarity between *E. tef* and *E. pilosa* as compared to *E. curvula.* However, inter-simple sequence repeats (ISSR) analysis showed a higher diversity among tef cultivars; with Jaccard similarity coefficients ranging from 26% to 86% (Kebebew Assefa *et al.*, 2003).

SNPs are also the most abundant molecular markers and are able to detect variation at a single base change in a DNA sequence. In recent years, analyses of SNPs using next-generation sequencing protocols have commonly been used in genetic and genomic studies such as genome-wide association studies, population genomic analysis, construction of genetic linkage maps, reconstruction of phylogenetic relationships, and identification of quantitative trait loci. Investigation from the close relationship between tef and its putative wild *Eragrostis* Progenitors using SNP markers revealed that 92% of wild *Eragrostis* species such as *E. pilosa*, *E. aethiopica*, *E. obtusa*, *E. ferruginea*, *E. lugens* and *E.lehmanniana* sequences was represented in the tef reference genome and the wild *Eragrostis* species were more diverse than the tef cultivars and could therefore potentially be used to enrich the tef gene pool (Dejene Girma *et al.*, 2018).

6. TOOLS USED TO STUDY GENETIC DIVERSITY

A molecular marker can be defined as a genomic locus, detected through probe or specific starters (primer) which, in virtue of its presence, distinguishes unequivocally the chromosomic trait which it represents as well as the flanking regions at the 3’ and 5’ extremity (Barcaccia *et al*., 2000).

Molecular tools give valuable data on diversity through their ability to detect variation at the DNA level (Aremu, 2011; Jonah *et al*., 2011) and show polymorphism, which may arise due to change in nucleotides or mutation in the genome loci (Hartl and Clark, 1997). Molecular markers can also identify genetic differences between individual organisms or species (Collard *et al*., 2005), and are more efficient in germplasm characterization than morphological or biochemical markers (Ranade and Yadav, 2014; Jingura and Kamusoko, 2015). Not only to study molecular variation, they are also used in many different areas such as genetic mapping, paternal tests, detect mutant genes which are connected to hereditary diseases, cultivars identification, marker assisted breeding of crops, population history, epidemiology, food safety, and population studies (Hartl and Jones, 2005).

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Molecular markers may or may not correlate with phenotypic expression of a genomic trait. They offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Additionally, they are not confounded by environmental, pleiotropic and epistatic effects (Linda *et al.*, 2009).

Various molecular marker systems such as restricted fragment length polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplification, simple sequence repeat (SSR) or microsatellites and single-nucleotide polymorphism (SNP) were used effectively to assess the genetic relationships and patterns of association among plant genetic resources (Datta *et al*., 2011; Govindaraj *et al*., 2015). Collard *et al.* (2005) showed that genetic marker used to identify genetic variation among individual species or organisms.

In general, molecular markers may be broadly divided into three classes based on the method of their detection: hybridization based, polymerase chain reaction (PCR) based, and DNA sequence based, among them SSRs are highly variable, found in great abundance, and evenly distributed throughout the genome and common in eukaryotes, their number of repeated units varying widely among crop species, are also hyper variable and multi allelic nature. The repeated sequence is often simple, consisting of di-, tri-, and tetra nucleotide repeats (Armour *et al*., 1999).

7. MODERN GENETIC MARKERS

7.1. SIMPLE SEQUENCE REPEAT MARKER

Simple Sequence Repeat markers (SSR) are tandemly repeated short DNA sequences that are favored as molecular-genetic markers due to their high polymorphism index, the variation in the number of tandem repeated unit results in highly polymorphic banding patterns (Junjian *et al*. 2002). Microsatellites are ideal genetic markers for detecting differences between and within species. These are heritable, useful to monitor gene flow, excellent for parentage determination and ideally suitable for analysis via multiplexing with highly reproducible profiles (Farooq and Azam, 2002). They have been extensively used in genetic diversity analysis of different cereal crops including rice (*Oryza sativa*) (Ishak *et al*., 2015), wheat (*Triticum sativum*) (Drikvand *et al*., 2015), sorghum (*Sorghum bicolor*) (Beyene Amelework *et al*., 2014), and barley *(Hordeum vulgare)* (Hua *et al*., 2015). These markers are known for their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance, and good genome coverage (Ali *et al*., 2014, 2015).

Advantages of SSR markers are: the method is relatively simple and can be automated, most of the markers are mono-locus and show Mendelian inheritance, SSR markers are highly informative, high number of public SSR primer pairs are available, effective cost per genotype and primer (similar to that for RAPD), SSR are used for plant breeding, and population genetics as forensics, paternity analysis and gene mapping (Coates and Byrne, 2005), require little amount of DNA, which does not have to be of high quality the simple interpretation of results (de Vicente and Fulton, 2003).

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