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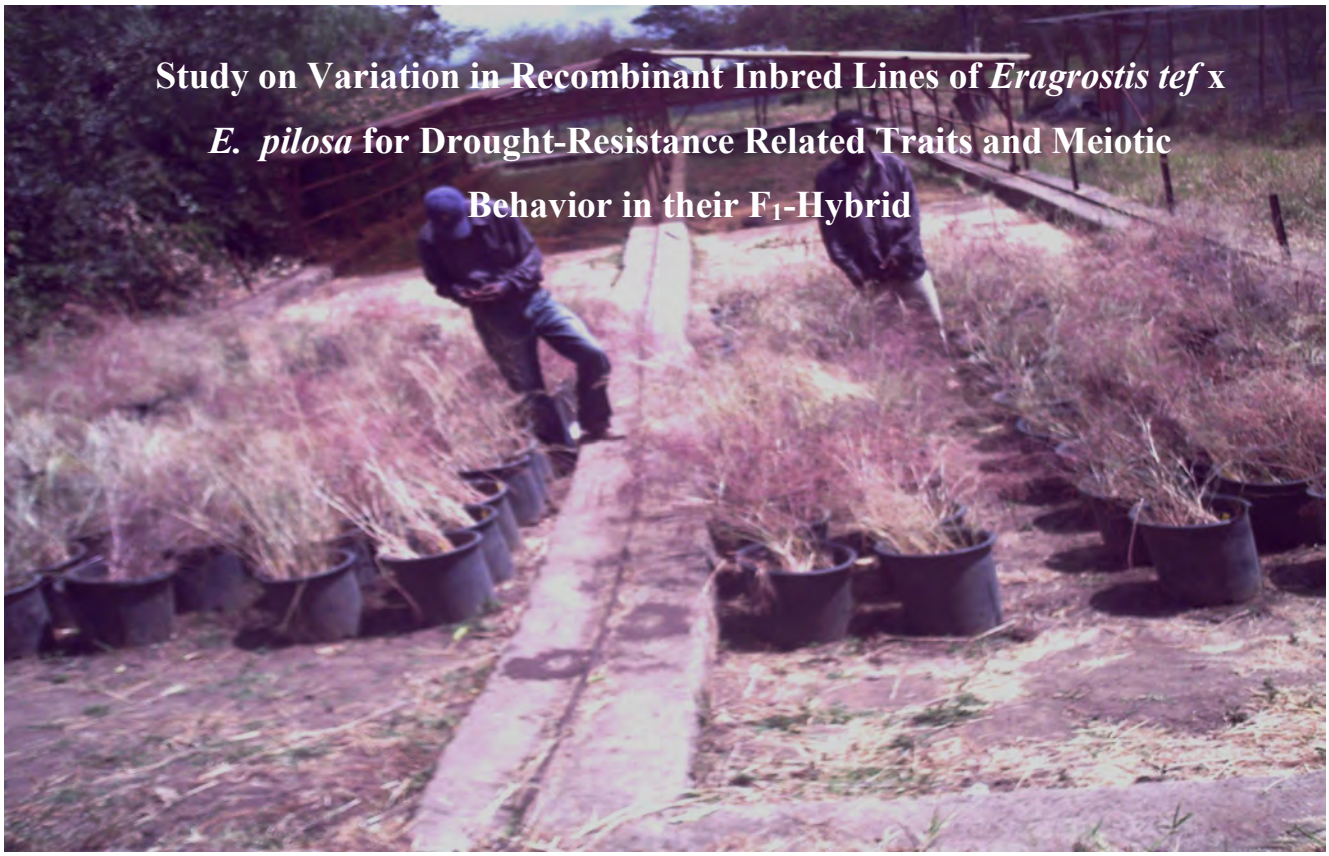

Study on Variation in Recombinant Inbred Lines of *Eragrostis tef* x *E. pilosa* for Drought-Resistance Related Traits and Meiotic Behavior in their F_1-Hybrid

By

Sintayehu Admas Mekonnen

A Thesis Submitted to Research and Graduate Studies Programmes of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Genetics)

Addis Ababa

June 2008

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Abstract

***Tef* (*Eragrostis tef* (Zucc.)** Trotter) *is major cereal crop cultivated in Ethiopia. It occupies two million hectares of land each year. However, its productivity is constrained by drought. To curb the situation, assessment of drought-resistance related traits variability among recombinant inbred lines (RILs) is essential. Morpho-physiological drought related (pot) and meiotic behavior (laboratory) studies were conducted at Debre Zeit and Addis Ababa in 2007/08. For pot experiment, twenty-five RILs of a cross between Eragrostis tef and E. pilosa, their parental lines and standard check cultivar were laid dawn in RCB design with three replications under moisture stressed and non-stressed conditions to examine differences in some drought related traits. There was significant ($p<0.05\%$) difference among lines for all characters considered except thousand seed weight and peduncle length under non-stressed and panicle seed weight under stressed conditions. Under non-stressed condition, GCV and PCV ranged from 1.4% to 58.96% and 1.8% to 52.6%, respectively while GCV range of 3.5% to 71.9% and PCV range of 3.8% to 72.3% was observed under stressed condition. More than 45% heritability was observed for all traits except number of tillers (21.8%) at stressed condition. Generally considerably high genetic advance as % of mean was obtained for most of the traits considered in the study except for days to maturity. Under non-stressed condition, strong positive correlation was revealed for grain yield with number of tillers, excised leaf water loss and water use efficiency, and strong negative correlation with total root length, root biomass and relative growth rate. Under stressed conditions, total root length, root biomass, water use efficiency and relative growth rate were positively correlated with grain yield, while drought susceptible index and excised leaf water loss showed strong negative correlation. Moisture stress prolonged the time to anthesis in few RILs and Key Murri, but accelerated for most other RILs, E. pilosa and standard check cultivar. Drought hastened root growth in most RILs, E. pilosa and the check but not for Key Murri and some RILs. RILs with lowest drought susceptibility index showed better yield under stress except RIL-183. Seventeen RILs were identified to be resistant and eight were drought susceptible. The laboratory study was conducted to examine meiotic behavior of F_1-hybrid (**E. tef x E. pilosa**) and pollen fertility of the F_1-hybrid, parental lines and RILs. The F_1-hybrid showed regular meiotic cell division and high level of pollen fertility (94.3%). Pollen fertility test of the parental lines and selected RILs was also high (90 to 95%). The result demonstrated the presence of wide variability among RILs for drought related trait and the potential of **E. pilosa** to diversify tef germplasm for drought related traits. Therefore, drought resistant RILs were identified which can serve as breeding materials for further research work.*

Key wards: *Drought resistance, meiotic behavior, RILs*

1. Introduction

Tef (*Eragrostis tef* (Zucc.) Trotter) is one of the most important cereal crops grown in Ethiopia. The area under tef cultivation is nearly two million hectares of land each year and ranks first in cultivation and market price among other cereal crops produced in Ethiopia (CSA, 2006). Even though tef is a major stable food in the country, its national productivity is low (0.91 t/ha). This is due to low yielding potential of the landraces, susceptibility to lodging, unimproved traditional cultural practices, and drought (Seyfu Ketema, 1997; Kebebew Assefa *et al*., 1999).

Tef production is affected by many factors. Among many of tef production constraints, drought stress and poor soil fertility in the semi-arid areas and waterlogging in Vertisol area with excess rainfall are the major ones (Abuhay Takele *et al.*, 2001). Tef is considered to be well adapted to drought as well as waterlogged condition. However, considerable yield loss due to drought was reported by investigators. For example, 7.25% to 85.1% grain yield loss of tef due to drought under greenhouse (Abuhay Takele, 1997) and 69% to 77% biomass yield loss under field conditions (Abuhay Takele, 2001) were reported. Result from field experiment of Belay Shiferaw and Baker (1996a) indicated that on average 14% grain yield loss of tef was observed due to water stress. Mulu Ayele (1993) showed that grain yield loss due to drought may reach up to 40%. So, drought is considered as a critical problem in tef production.

At the National Tef Genetic Improvement Project, the breeding strategies include direct selection from the landraces and the conventional methods of breeding that involves intra and inter-specific crossing. Variation for drought-resistance related traits among tef landraces have been observed (Mulu Ayele, 1994-95; Abuhay Takele, 2001; Mulu Ayele, 2001). These investigators tried to exploit the natural variation of the traits towards stress and indicated the adaptation mechanism of tef to drought. According to their work, the adaptation mechanisms in tef were due to morphological, physiological and biochemical traits like that of most cereals. Although, selection from naturally existing genetic variability of tef has contributed to the development of improved varieties, since recently

utilizing the benefit of inter-specific crosses of tef with its wild relative species have been employed in parallel (Hailu Tefera *et al*., 2003a).

Genus *Eragrostis* comprises large number wild relatives, which can provide desirable gene for tef improvement (Jones *et al*., 1978). An important prerequisite for using wild species in genetic improvement is the identification of closely related wild species as potential gene donors and finding useful genes. For example, *Eragrostis pilosa* is found to be relatively drought-resistant since it grows in environments that are far more adverse than tef environments. It is a cosmopolitan species and is sympatric with tef (Endashaw Bekele, 1986). *E. pilosa* is closely related and is believed to be the immediate ancestor of tef (Jones *et al*., 1978; Costanza *et al*., 1979; Endashaw Bekele and Lester, 1981; Tavasoli, 1986; Ingram and Doyle, 2003). This species is crossable conventionally with tef and serves as a donor species for some desirable traits for tef improvement (Endashaw Bekele, 1986; Likyelesh Gugsa and Tesfaye Mengistu, 1999; Hailu Tefera *et al*., 2003a)

Breeding of tef by crossing with *E. pilosa* is crucial for tef improvement that can adapt and perform better in prevailing water stress conditions and provide a new pool of genetic variation (Seyfu Ketema, 1997; Likyelesh Gugsa and Tesfaye Mengistu 1999; Hailu Tefera *et al*., 2003a; Yu *et al.,* 2007). National Tef Genetic Improvement Project has started to utilize the advantage of interspecific cross of tef with *E. pilosa* (Hailu Tefera *et al*., 2003a). The Project has developed recombinant inbred lines (RILs) that broaden the genetic pool of tef. Cultivated tef and the wild species, *E. pilosa*, differ greatly for most agronomic traits and the close relationship between these two species facilitate hybridization, providing a unique opportunity to develop a new pool of genetic variation (Yu *et al.,* 2007)

Inter-specific hybrid plants result in a tremendous diversity of types in the F_2 and later generations due to segregation and recombination of alleles derived from the parental species (Allard, 1960). The first concern in inter-specific breeding is whether or not a viable F_1-hybrid plant can be produced. The most characteristics feature of inter-specific hybrid is sterility in greater or lesser degree. The causes of hybrid sterility are probably

numerous among which are genetic imbalance, irregular chromosome pairing and segregation during meiosis and even where chromosome pairing appears to be fairly regular, segregation often does not fit classical patterns (Allard, 1960). The second feature is the difficulty in identifying the true hybrid progenies, which can be assessed by taking into consideration morphological markers. In tef, markers used to check hybridity include manifestation of hybrid vigor at F_1, and segregation of panicle form, lemma color, seed color, and heading and maturity durations in the later generations. Therefore, it is essential to examine meiotic stability, pollen fertility and the level of variation among the recombinant inbred lines (RILs) and their parental lines.

2. Literature review

2. 1 Taxonomic classification of tef

Tef belongs to the genus *Eragrostis*. Report by Costanza *et al*. (1979) has shown that this genus contains about 300 species, but according to Watson and Dallwitz (1992) the genus comprises 350 species. Costanza *et al.* (1979) has indicated that the genus *Eragrostis* is widely spread throughout the tropical and subtropical regions of the world. For example, 43% of the species seem to occur 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). Of the 54 *Eragrostis* species listed in Ethiopia, 14 (or 26 percent) are endemic (Cufodontis, 1974 cited in Seyfu Ketema, 1997) but Phillips (1995) estimated that only 44 *Eragrostis* species exist in Ethiopia.

2.2 Morphological description

Tef is a warm season annu al grass, characterized by a wide variation of morphological and agronomic traits (Tadesse Ebba, 1975; Endashaw Bekele, 1996; Kebebew Assefa *et al.*, 1999)

1. Stem

Tef is fine stemmed tufted annual grass with mostly erect stems, although some cultivars have bending or elbowing stems. The stems are covered with sheaths which are smooth, glabrous, open and distinctly shorter than the internodes (Seyfu Ketema, 1997).

2. Roots

The roots are shallow and develop a massive fibrous rooting system. Tadesse Ebba (1975) estimated rooting depth within the range of 4-8 cm.

3. Panicle

Tef is self-pollinated chasmogamous annual cereal. The degree of outcrossing in tef is low, i.e. 0.2 to 1% (Seyfu Ketema, 1997). It has a panicle type of inflorescence showing different forms ranging from very loose, loose, semi-loose, semi-compact to compact, appearing like a spike. Its spikelets have 2-12 florets. Each floret has lemma, palea, three stamens, an ovary and mostly two, in exceptional cases three, feathery stigmas (Seyfu Ketema, 2001). Flowering in tef starts from the top of the inflorescence and proceeds towards the base (basepetally). While in spikelets, it starts from the bottom and proceeds towards the top (acropetally) (Seyfu Ketema, 2001).

4. Seed

Tef has very small seed size having 0.9-1.7 mm in length and 0.7-1.0 mm in diameter. Its shape is generally ovoid or oblong to ellipsoidal and opaque or lustrous (Tadesse Ebba, 1975). It has very variable seed coat color. Endashaw Bekele (1996) classified tef seed color into three, i.e. white, brown and grayish-orange. Tadesse Ebba (1975) observed brown, vinaceous, yellowish white, orange white, or intermediates of these colors.

2.3 Use of tef

Tef flour is used by Ethiopians to make an unleavened sourdough bread called "injera.". It is also used as porridge or ingredient of home-brewed alcoholic drinks. The straw is utilized for feed for livestock. The straw has high feeding quality as compared to that of other cereal species. The straw is also used for reinforcing mud for plastering wooden walls of building. Tef has a high economic value as its grain can be kept for many years without being seriously damaged by common storage insect pests (Tadesse Ebba, 1969; Endashaw Bekele *et al*., 1995; Seyfu ketema, 1997).

Tef is recently becoming an export crop. The crop is exported to the Middle East, North America and many European countries, mainly for Ethiopians or of Ethiopian origin that have migrated to these countries. Outside Ethiopia, there is a growing interest in using tef. For example, small-scale commercial production of tef has begun in a few areas of the

wheat belts of the USA, Canada and Australia (Mulu Ayele *et al*., 2001). Tef has been introduced to South Africa and is cultivated as a forage crop and in recent years, it has been cultivated as a cereal crop in Northern Kenya (Hailu Tefera and Seyfu Ketema, 2001).

The nutritive value of tef grain compares well with some of the major cereals such as wheat, barley, maize and sorghum (Seyfu Ketema, 1993). Tef contains more calcium, copper, zinc, aluminium and barium than winter wheat, barley and sorghum (Asrat Wondimu and Frew Tekabe, 2001). Since the plant is also free of gluten, many people who develop severe allergies to wheat gluten can safely consume bread or other products made from tef. So, it is advocated as a health food in the USA and Europe (Mulu Ayele *et al*., 2001).

2.4 Origin and domestication

Based on the occurrence of wild relatives and the diverse genetic variation of tef in Ethiopia, Vavilov (1951) has indicated Ethiopia as the center of origin and diversity of tef. Seyfu Ketema (1997) believed that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ. According to Ponti (1978, cited in Seyfu Ketema, 1997) tef was domesticated in Ethiopia 1000–4000 BC.

2.5 Polyploid origin of tef

Despite considerable economic importance of tef for Ethiopia, there have been no detailed cytogenetic studies of tef. This is due to the small size of tef chromosomes and the crossing technique to create hybrids for further cytogenetic studies, is very tedious (Likyelesh Gugsa *et al*., 2001). Mulu Ayele *et al.* (1996) reported the size of chromosome to be in the range between 0.8-2.9 µm. Tef is an allotetraploid plant with chromosome number of 40 (2n=4x=40) and the basic chromosome number of the genus *Eragrostis* is x=10 (Tavassoli, 1986). The polyploidy origin of tef within the large genus *Eragrostis* (350 species, Chlorideae) are still unknown. Many investigators have tried to identify the

putative diploid progenitors of tef (Jones *et al*., 1978; Costanza *et al*., 1979; Tavasoli, 1986; Ingram and Doyle, 2003), and none of them came up with conclusive results and it is awaiting further research work. However, there are many reports suggesting the potential progenitors of tef based on several lines of evidence. These are reviewed below.

2.5.1 Morphological evidences

Jones *et al*. (1978) studied the relationships among 41 *Eragrostis* species based on morphological characterization and cytological analysis. The authors suggested that *E. pilosa* was closely related to *E. tef*. Constanza *et al*. (1979) examined morphological similarities among 36 accession of tef, two *E. pilosa* and one *E. aethiopica* accessions. The result indicated that there was far more similarity between *E. pilosa* and tef than between tef and *E. aethiopica.* Endahaw Bekele (1986) examined the relationship among 11 cultivars of tef and 14 wild *Eragrostis* species based on 51 morphological characteristics and seed and pollen morphology. Data from the morphological analysis indicated that *E. pilosa, E. aethiopica* and possibly *E. curvula* and *E. cilianensis* were closely related to tef. And the author concluded that these species are involved in the origin of tef.

2.5.2 Biochemical evidence

Endashaw Bekele and Lester (1981) conducted biochemical studies on 11 cultivars of tef and 14 accessions of wild *Eragrostis* species using chromatography of leaf phenolics and electrophoresis of seed proteins to see evolutionary relationships among the species. The result generally supported the origin of tef from wild *E. pilosa.* They also suggested the affinities of *E. aethiopica* and *E. barrelieri* to tef and perhaps also to *E. curvula* and *E. cilianensis.*

2.5.3 Evidence from cytological examination

Tavassoli (1986) examined 37 *Eragrostis* species cytologically to see genomic affinity among them. The karyotype analysis indicated that six wild *Eragrostis* species were closely related to tef. These are: *E. aethiopica (2x), E. barrelieri (6x), E. cilianensis (2x), E. mexican (6x), E. minor* (2x) and *E. pilosa* (4x) are close relatives of tef.

2.5.4 Evidence from molecular analysis

Various investigators have tried to see the wild ancestors of tef and considerable evidences have been released from molecular analysis. For example, cluster analysis of RFLP result indicated a closer affinity between tef and *E. pilosa* than between tef and *E. curvula* (Bai *et al*., 1999). Mulu Ayele *et al*. (1999) investigated the close relationship among tef and *E. pilosa* accessions using radiolabelled and silver stained amplified fragment length polymorphism (AFLP). Tef cultivars (Keyi Murri, Alba, Fesho and Jea Lamie) and 14 accessions of *E. pilosa* collected from different countries of the world were used in the study.

The report from Ingram and Doyle (2003) indicated the relationship of tef and wild *Eragrostis* species using sequence data from the nuclear gene waxy and the plastid locus *rps*^{16}. The phylogenic analysis of sequence data from the nuclear gene waxy and the plastid locus *rps*^{16} confirmed the widely held hypothesis of a close relationship between tef and *E. pilosa.* And also *E. heteromera,* another previously proposed progenitor of tef, is shown by the waxy data have a genome closely related to one of the tef genomes. The data did not support other taxon included in the study as close relative of tef.

Tileye Feyissa (1999) examined the degree of similarity and variation among 35 cultivars of tef and two lines of *E. pilosa* using chloroplast DNA sequence analysis of the intergenic spacer region between *trn*T(UGU) and *trnL*(UAA)5'exon. The result indicated that 33 of the tef cultivars and one line of *E. pilosa* showed no variation. Two cultivars of

tef and one line of *E. pilosa* showed deletion of one base. This work confirmed the close relationship between tef and E. *pilosa.*

Analysis of data from all the morphological, biochemical, molecular and cytological analysis have shown that *E. pilosa* (2n=4x=40) was the closest relative of tef. It is now believed that, *E. pilosa* is the immediate wild progenitor to tef. And it is postulated that tef is evolved from *E. pilosa* through human selection under cultivation (Likyelesh Gugsa *et al*., 2001)

2.6 Genetic studies in tef

2.6.1 Wide sexual hybridization in tef

Wide crossing like inter-specific crossing (crossing between two species belonging to the same genus) and inter-generic crossing (crossing between two species from different genera), have been employed by breeders in many crop plants for germplasm improvement and to determine the evolutionary relationship among species. Conventional crossing is also applicable to study the mode of inheritance of traits, the number of gene involved and their action and interaction, the presence of maternal effect and linkage (Allard, 1960; Jones *et al*., 1978; Agrawal, 1998; Tareke Berhe, 1981).

The crossability among species, the meiotic chromosome pairing and fertility in F_1-hybrids are good indicators of relationship among species. Closely related species will show high level of crossability, more regular meiotic division and high pollen fertility in their F_1-hybrids. In most crosses, the meiotic abnormalities were observed in their hybrid at different stages of meiosis. These include segregational abnormality, chromosome stickiness, cytoplasmic connections among cells, cytomixis, irregular spindle formation, laggard chromosomes, bridge formation, fragmentation of the whole chromosome, and uniparental chromosome elimination (Jones *et al*., 1978; Allard, 1960). Meiotic irregularity results in low fertility and seed setting of the hybrids. These were demonstrated for various crops. For example, Kifle Dagne (1994) in *Guizotia* species,

Caetano-Pereira and Pagliarini (2001) in maize and Adamowski *et al*., (2008) in *Brachiaria sp*.

Although the crossing technique is tedious in tef, conventional crossings have been employed for germplasm development. Likyelesh Gugsa and Tesfaye Mengiste (1997) studied the crossability of two tef verities (DZ-01-196 and Key Murri) with four wild *Eragrostis* species (*E. pilosa, E. aethiopica, E. cilianensis and E. minor*) for potential use in tef breeding strategy. Among the crosses made, Key Murri *x E. pilosa* and DZ-01-196 *x E. pilosa* produced seed. Seed set of 70-75% was observed when *E. pilosa* was used as pollen source but seed percentage reduced to 10-20%, when it was used as female parent. The rest *Eragrostis* species failed to set seed. The authors concluded that *E. pilosa* can serve as a donor plant of desirable traits into cultivated tef through sexual crossing. Tavasoli (1986) conducted meiosis study in tef and intra-specific hybrids, where regular meiosis with 20 bivalents was observed. There is no available information regarding meiotic studies for interspecific progenies of cross between tef and a wild relative.

2.6.2 Inheritance of some qualitative traits in tef

The inheritance of qualitative characters is relatively simple since these are governed by a few genes (major genes). Major genes have large effect and are relatively insensitive to environmental change. Individual variations can be put into discrete classes and also simple and easily done. The effect of these genes can be separated by usual Mendelian techniques (Allard, 1960; Agrawal, 1998). Tareke Berhe (1981) studied the genetics of three qualitative traits (i.e. lemma color, seed color and panicle form) in tef. Four cultivars with contrasting characteristics were used. According to the result, four pairs of genes were identified controlling lemma color inheritance. These genes have shown dominant, complementary and epistatic gene action and interaction. The inheritance of seed color was controlled by a pair of duplicate genes with simple dominance and additive gene effects. Panicle form was controlled by a pair of duplicate genes for looseness and by another pair of genes for unilateral versus multilateral branching. Here, inactive and suppressor gene effect were observed. The authors also observed no

maternal effect and linkage among these three traits. Their inheritances were independent of each other.

2.6.3 Quantitative traits inheritance in tef

Quantitative traits are characterized by continuous distribution. The inheritances of such traits are controlled by many genes. Each gene has minor, similar and additive effect. Non-heritable agencies like environment also influence the phenotypic expression of these characters. Most of the agronomic traits are quantitative in nature. Their inheritance pattern is complex. The effect of these individual genes cannot be separately measured and rather are considered in mass. The genetic behavior of quantitative characters in a population is studied through well-designed experiments and working out certain statistics, like means, variances, covariances, regression, correlation and partitioning of the variance. These statistical analyses will help the plant breeder to identify superior genotypes among related individuals and families. The action of the genes controlling quantitative characters can be described by the use of gene model. The genetic contribution to the variation can be estimated using models that explain data adequately by using six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2). These models include additive, dominance, additive x additive, additive x dominance and dominance x dominance (Allard, 1960, Singh and Chaundry, 1977; Dabholkar, 1992; Agrawal, 1998).

Hailu Tefera and Peat (1997) used these models to see the inheritance of quantitative traits in tef. The result from generation means, additive ([a]), dominance ([d]), additive x additive [(aa]), additive x dominance ([ad]) and dominance x dominance ([dd]) were effective to explain variability in days to heading, days to maturity, plant height, culm diameter, panicle length, tiller number, plant and panicle yield per panicle and harvest index weights among generations. Analysis from generation variances indicated that large amount of additive (V_A) and dominance (V_D) variance were estimated for days to heading and days to maturity. The variability observed in plant height, culm and panicle length, and culm diameters were largely due to genetic basis than environmental effect and the dominance variances were higher than the additive ones. In yield, the variation

was due to both genetic and environmental factors. The dominance variances were found to be higher than the additive variance. The authors also estimated the heritability of quantitative traits. Relatively high narrow-sense heritability values for days to heading (0.48 to 0.74), days to maturity (0.32 to 0.69), and panicle length (0.40 to 0.68) were estimated. Yield per panicle (0.23), panicle weight (0.31 to 0.39) and plant weight (0.23 to 0.37) also showed moderate amount of heritability with less variation across locations.

2.7 Drought resistance in crop plants

Drought is defined by various disciplines in different ways. From meteorological point of view, it is defined as the absence of rainfall for a prolonged period of time, long enough to cause moisture-depletion in soil and water deficit with a decrease of water potential in plant tissues (Tardieu, 1996). But, agriculturalists define it as the inadequacy of water availability, including precipitation and soil-moisture storage capacity, in quantity and distribution during the life cycle of a crop plant, which restricts the expression of full genetic potential of the plant (Turner, 1986). It limits the agricultural production by preventing the crop plants from expressing their full genetic potential (Mitra, 2001). It strongly affects crop yield in several regions of the world.

2.7.1 Mechanism of resistance

Crop growth and crop water status in response to soil water deficit play an important role in tolerance to drought and in yield stability. Crops growing under drought conditions adapt in a variety of ways. The adaptation variability is due to the difference in drought resistance mechanisms. Drought resistance means the ability of a genotype to be more productive with a given amount of soil moisture than another genotype (Blum, 1988). Three mechanisms are involved in drought resistance. These are drought escape, drought avoidance and drought tolerance. Crops use more than one mechanism at a time to resist drought (Blum *et al*., 1982; Turner, 1986).

1. Drought escape

Early maturing crops varieties often escape drought because they complete the critical stages of crop growth prior to the setting in of drought condition. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water-deficit) and remobilization of pre-anthesis assimilates to grain (Blum, 1988; Belay Simane, 1993).

2. Drought avoidance

The crops avoid water stress by extracting water from deep soil layers with deep and dense root system or by reducing evapotranspiration without affecting yields. Mechanisms for improving water uptake, storing in plant cell and reducing water loss confer drought avoidance. Drought avoidance is performed by maintenance of turgor through increased rooting depth, efficient root system and increased hydraulic conductance and by reduction of water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evaporation surface (Levitt, 1972; Blum, 1988; Ramirez-Vallejo and Kelly, 1998).

3. Drought tolerance

Drought tolerance is the ability to withstand water-deficit with low tissue water potential. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in cell), increase in elasticity in cell and decrease in cell size and desiccation tolerance by protoplasmic resistance (Levitt, 1972; Blum, 1988).

Generally, these three types of drought resistance mechanisms are conferred by various morphological, physiological and biochemical characters. Physiological traits include maintenance of relatively higher leaf-water potential, osmotic adjustment, tolerance to stress in plant or organ growth rate, plant recovery on rehydration, tolerance in photosynthetic system or its components, tolerance in enzymatic activities, tolerance in

translocation, stability of cellular membranes (Passioura, 1986; Blum, 1988; Price *et al*., 2002). Biochemical traits such as, accumulation of proline, prolamine, polyamine, trehalose, carbohydrate and increased nitrate reductase activity (Nguyen and Babu, 1997; Blum, 2005). Morphological traits such as, root growth (number, length, thickness), stem thickness, leaf size, leaf area per plant, leaf orientation, tiller survival and organ pubescence (Passioura, 1986; Price *et al*., 2002).

In cereal crops, a lot of drought-resistance related traits have been identified and have been utilized to screen cereal crops under stressed condition. These are discussed below.

1. Osmotic adjustment (OA)

Under drought condition, drought resistant plants accumulate osmotically active compounds (amino acids, sugars and ions) inside their cell to reduce osmotic potential of the cell as a result of which water present in inter-cellular space then flows towards the inside of the cells. This process is called Osmotic adjustment (OA) (Blum, 1988). OA is recognized as one of the most important adaptive mechanisms to water deficits in many crops and is considered as a major component of drought tolerance mechanisms. It maintains turgor at lower water status, which, in turn, enables plants to maintain processes such as cell enlargement and stomatal opening (Blum, 2005). OA is a major cellular stress adaptive response in certain crop plants that enhances dehydration avoidance and supports yield under stress (Blum, 1988).

Mulu Ayele, *et al* (2001) utilized OA to characterize the variation of 54 tef genotypes in response to water stress. The authors have shown significant differences among the genotypes. They indicated also that genotypes with high OA survived longer periods under stress than those with low OA. Therefore, they came up with the conclusion that this trait can be used to screen tef germplasm for drought prone areas. This technique is rapid and inexpensive, and hence is suitable for routine screening of breeding populations (Blum, 2005).

2. Water use efficiency (WUE)

Identification of genotypes that have a greater ability to use limited available water is important to enhance productivity of the crop. WUE is equated in a simplistic manner with drought resistance without considering the fact that it is a ratio between two physiological (transpiration and photosynthesis) or agronomic (yield and crop water use) entities (Blum, 2005). It is also defined as total biomass production per unit of water transpired (g kg^{-1}). Plants showing high WUE yield better under water stressed condition (Passioura, 1986). It is virtually impossible to include such a trait in screening large number of genotypes. But, it can be assisted by using morphological traits as markers, like small plant size, small leaf area, or reduced growth duration or use of carbon isotope discrimination for selecting increased WUE (Blum, 2005).

3. Leaf water status and gas exchange

The ability of a plant to survive severe water deficits depends on its ability to restrict water loss through the leaf epidermis after stomata attain minimum aperture. Crop plants avoid dehydration by enhanced capture of soil moisture and by limited crop water loss through stomatal regulation and epicuticular wax layer in the leaf (Blum, 2005). This mechanism has yield penalty, because, stomatal regulation reduces CO_2 fixation, with consequent effects on crop productivity (Mitra, 2001). Belay Shiferaw and Baker (1996b) demonstrated the potential use of this trait for identifying level of drought tolerance among tef cultivars. The thickness of the epicuticular wax layer considered to be an important trait for drought resistance. For example, in sorghum, drought resistance is a trait that is highly correlated with the thickness of the epicuticular wax layer (Nguyen and Babu, 1997).

4. Excised leaf water loss (ELWL)

ELWL, as drought avoidance trait, has been proposed as a selection criterion for drought tolerance in many crops (Dedio, 1975; Clarke and McCaig, 1982). ELWL has been tested in tef to see the level of drought tolerance among tef genotypes (Mulu Ayele, 1993). The author imposed the crop to water stress at vegetative stages and observed the existence of genetic variability of the genotypes in response to water stress. The trait was

positively correlated with grain yield under moisture stress conditions. Low water looser genotypes gave higher yield than high water looser ones. Abuhay Takele (2001) studied tef cultivar differences in ELWL at anthesis stage in response to water stress. The cultivars have shown significant variation of the trait. Tesfaye Teferra *et al*. (2000b) have also tried to see the variability in ELWL of tef genotypes under imposed water stress at different stages (Early stress- at jointing stage and terminal stress-at heading stage). At early stress, ELWL differed significantly among genotypes and it was negatively correlated with grain yield at both heading and grain filling stage. All authors agreed that ELWL is a suitable method for screening drought resistant tef cultivars as there is marked difference of the trait among cultivars.

5. Leaf canopy temperature

Remotely sensed infrared canopy temperatures provide an efficient method for rapid, non-destructive monitoring of whole-plant response to water stress. In plant breeding and selection for drought resistance the interest is in finding genotypes that maintain lower canopy temperature as compared with other genotypes under the same field conditions. Relatively, lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status (Blum *et al*., 1982; Rashid *et al*., 1999). Rashid *et al*. (1999) evaluated the potential use of canopy temperatures to screen drought tolerance in twelve spring wheat genotypes. They observed significant correlations between canopy temperature with yield under moisture-stress conditions and drought susceptibility index values. They recommended that the use of this trait for screening wheat genotypes under stressed condition is valuable. In tef, differential response to drought stress was observed among cultivars with leaf canopy temperature at anthesis (Abuhay Takele, 2001). Twelve tef cultivars of similar maturity group were evaluated under stressed and non-stressed conditions. There was a marked difference in mean excised leaf water loss (ELWL) values between the stressed and non-stressed treatments among genotypes.

6. Root traits

Plant efficiency for water uptaking is detrimental to plants, to resist water stress. It depends on root morphology (size, length, mass, thickness, distribution). Deep and prolific root systems have been positively associated with enhanced avoidance of drought stress. Investigators showed the positive association of deep root system with grain yield in wheat (Hurd, 1974; Ekanayake, *et al*., 1985; Ludlow and Muchow, 1990; Ahmid *et al*., 2007). A deeper root system has been shown to allow upland rice to extract more water from the soil, resulting in a higher yield potential under drought, (Mambani and Lal, 1983). Rooting depth exhibited a moderately positive correlation with grain yield under stress in lowland rice (Venuprasad *et al*., 2002). Root morphology has been employed to screen tef germplasm to develop drought resistant varieties (Mulu Ayele *et al.*, 2001).

7. Yield and yield-related traits

Crop cultivation in drought stress condition causes significant reduction of grain and biological yields. Direct selection for yield under favorable or water-limited conditions is the selection strategy that has been the most commonly used by cereal breeders to improve yield in water-limited environments (Araus *et al*., 2002). Drought indices which provide a measure of drought based on loss of yield under drought-conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988). Yield related traits like days to flowering and maturity, number of tillers, spikelet fertility and low rate of leaf drying under drought condition have been utilized in screening strategy also (Blum, 2005).

8. Drought susceptible index (DSI) and relative growth rate (RGR)

The DSI developed by Fischer and Maurer (1978) and RGR developed by Hunt (1982) gave a robust and extendable opportunity to measure drought resistance level in crop plants. Drought-tolerant genotype shows the lowest mean RGR under moisture stresses and higher RGR under optimum moisture conditions. But in case of DSI, drought-resistant varieties show lowest DSI. DSI and RGR can be calculated as indicated in section 4.1.3. These traits have been utilized as indices to screen drought resistance level

among genotype of tef like that of other cereals (Mulu Ayele *et al.*, 2001; Tesfay Teferra *et al*., 2000a;b).

2.7.2 Genetics of drought-resistance related traits

As discussed above, drought resistance is associated with the action and interaction of many morphological, physiological and biochemical traits. These are controlled by different groups of genes which make drought resistance a very complex trait (Mitra, 2001). Until recently, little achievements have been reported regarding the mechanisms that condition these characters. Some drought related traits were identified as either monogenic or polygenic pattern of inheritance. For example, root inheritance in rice is monogenic. The long root and high root number are controlled by dominant alleles and thick root tip by recessive alleles (Mitra, 2001).

Chozin *et al*. (2006) studied the inheritance of drought-resistance related traits in cowpea using F_1, F_2, F_3, BC_1, BC_2, BC_1S_1 and BC_2S_1 populations. The result indicated that delay in leaf senescence (towards resistance) and leaf temperature (towards susceptibility) were controlled by partial dominance. Heterotic effect towards susceptibility was found on stem diameter. The dominant and epistatic (additive-additive and additive-dominant) gene effects were significant. But, additive-dominant and additive effects were responsible for leaf temperature. In snap beans, resistance to temperature-drought stress was determined by a single dominant gene and by two complementary genes (Bouwkamp and Summers, 1982).

Bhutta *et al.* (2006) have shown the high degree of heritabilities and expected a genetic advance of drought related traits (i.e. Osmotic pressure, leaf water potential, venation, area and thickness of flag leaf) in wheat variety. Root pulling resistance in rice is associated with drought resistance. Dominant and additive genes effects were determined to be effective in controlling the traits (Ekanayake *et al*., 1985). Drought influences the fertility of wheat by inducing apical sterility, a situation in which the terminal spikelets fail to set seed. Mohammady-D *et al.* (2003) studied the mode of inheritance of this trait

in wheat. They developed F_1, BC_1 and F_2 generation from a cross between apical sterile and apical fertile wheat varieties. It was observed that two complementary dominant genes segregating independently were involved in tolerance to water stress induced apical sterility.

2.7.3 Breeding approaches to improve drought resistance in crop plants

Various drought-resistance related traits have been identified and utilized to asses the level of drought resistance in crop plants. When the stress is terminal and predictable, drought escape through the use of shorter duration varieties is often the preferable method of improving yield potential. Drought avoidance and tolerance mechanisms are required in situations where the timing of drought is mostly unpredictable (Blum, 1982; Price *et al*., 2002). Generally, three approaches for drought resistance variety development have been used.

1. Breeding at optimum condition

In this approach, breeding activities are taken place at optimum growth conditions and the best genotypes are selected. Under optimum conditions, the genotypes are expected to express their genetic potentials of yield at maximum level. Here, it is assumed that a genotype superior under optimum level will also yield relatively well under drought. But genotype environment interaction may affect the performance of high yielding variety under stressed environments (Blum, 1988; Hurd, 1974).

2. Breeding at defined drought condition

Breeding of genotypes under actual drought environments is practiced. In this approach, the intensity of drought occurs across year and environments are unmanageable. This will result in selection pressure on breeding materials impose changes drastically from generation to generation (Blum, 1988; Hurd, 1974).

3. Incorporation of drought resistance traits into argonomically superior varieties

An alternative approach to the above two would be to improve drought resistance in high-yielding genotypes through incorporation of morphological, biochemical and physiological mechanisms of drought resistance (Blum, 1988; Hurd, 1974). For example, genetic engineering of a single structural protein (such as *HVA1*) or a single transcription factor (*DREB1A*) has resulted in striking improvements in plant tolerance to drought (Bajaj *et al*., 1999). Tilahun Abebe *et al*. (2003) engineered wheat with *mtlD* gene from *Escherichia coli* and had improved tolerance of wheat to water stress and salinity.

In general, inter-specific variability in *E. pilosa* possesses specific attributes of utility for breeding programs in tef specially for developing drought-resistant tef variety. Therefore, this study was conducted to fill the gap by evaluating the RILs developed from cross between *Eragrostis tef* cv Key Murri and *E. pilosa* (accesition 30-5) under water stressed conditions. Therefore, information on the level of genetic variation among RILs for water stress and examining chromosome stability at meiotic cell division in the hybrids are worthwhile.

3. Objectives

3.1 General objectives

1. To generate information on the level of drought-resistance related traits variation among RILs derived from *E. tef* x *E. pilosa* cross and their parental lines under water stressed and non-stressed conditions and to examine meiotic behavior of F_1- hybrid from *E. tef* x *E. pilosa* cross.

3.2 Specific objectives

1. To see drought-resistance related traits variability among RILs, and compare it with their parental lines and standard check (DZ-Cr-37).
2. To estimate heritability and expected genetic advance of agronomic and drought-resistance related traits among RILs and their parental lines.
3. To estimate the correlation of agronomic and drought-resistance related traits under stressed and non-stressed condition.
4. To see the level of drought resistance index among RILs and grouping the RILs based on the index.
5. To assess meiotic behavior in F_1-hybrid from *E. tef* x *E. pilosa.*
6. To examine pollen fertility of F_1- hybrid, selected RILs and the parental lines.

4. Materials and methods

4.1 Agronomic study

4.1.1 Plant materials

Twenty-five RILs derived from a cross between *E tef* cv. Key Murri and *E. pilosa (*accession *30-5)*, parental lines (*E*. *tef* cv. Key Murri and *E. pilosa,* accession 30-5) and DZ-Cr-37 (standard check) were used as plant material for the study. The descriptions of the plant material are given in Table 1. Key Murri is a tall, thick culmed and late maturing cultivar with a very compact and long panicle, where as, *E. pilosa* accession 30-5 is short, thin culmed and early maturing with a very lax panicle. The RILs have been developed at DZARC by the National Tef Improvement Program since 1995-1999 with the objective of broadening the genetic base of tef germplasm and to assess the potential merit of *E. pilosa* (Hailu Tefera *et al*., 2003a). During the year 1995, a cross was made between *E. tef* cv. Key Murri and *E. pilosa,* accession 30-5. Key Murri was used as female parent and *E. pilosa* accession 30-5 used as pollen source. The progeny lines were developed using single-seed-descent method from F_2 individual plants.

Table 1. List of RILs, the two parental lines (Key Murri, *E. pilosa*) and standard check (DZ-Cr-37) with description of lemma color, panicle form and seed color

No	Plant Materials	Lemma color	Panicle form	Seed color
1	*E. pilosa*	Gray	Very loose	Brown
2	Key Murri	Red	Compact	Yellow white
3	DZ-Cr-37	Yellow	Very loose	white
4	RIL-317	Pink	Loose	White
5	RIL-252	Pink	Very loose	White
6	RIL-118	Variegated (purple & yellow)	Loose	White
7	RIL-172	Gray	Semi-compact	White
8	RIL-124	Variegated (purple & yellow)	Compact	White
9	RIL-12	Red	Compact	White
10	RIL-364	Variegated (purple & yellow)	Very loose	White
11	RIL-36	Pink	Semi-compact	White
12	RIL-290	Variegated (purple & yellow)	Very loose	Light Brown
13	RIL-29	Red	Very loose	Light Brown
14	RIL-337	Red	Loose	Light Brown
15	RIL-173	Red	Loose	Light Brown
16	RIL-66	Pink	Very loose	Light Brown
17	RIL-16	Variegated (purple & yellow)	Very loose	Light Brown
18	RIL-226	Variegated (red & yellow)	Loose	Light Brown
19	RIL-197	Red	Semi-loose	Light Brown
20	RIL-37	Pink	Loose	Deep Brown
21	RIL-9	Variegated (purple & yellow)	Very loose	Deep Brown
22	RIL-233	Pink	Very loose	Deep Brown
23	RIL-139	Variegated (pink& yellow)	Semi-loose	Deep Brown
24	RIL-183	Variegated (pink & yellow)	Semi-compact	Deep Brown
25	RIL-62	Variegated (purple & yellow)	Very loose	Deep Brown
26	RIL-222	Gray	Semi-compact	Deep Brown
27	RIL-275	Pink	Very loose	Deep Brown
28	RIL-313	Pink	Very loose	Deep Brown

4.1.2 Experimental design

The experiment was carried out using 168 plastic pots in the rainout shelter at DZARC. The plant materials were grown in pots with 30cm internal diameter and 30 cm depths under rainout shelter. Homogeneous soil mixture was prepared by mixing the sub-surface soil thoroughly. Each pot was filled with 12 kg of soil. Plant materials were totally blocked from getting rain shower using rainout shelter which has moveable roof. When the rain was expected to shower, the experimental materials were covered with the roof, if not the roof was removed. Ten plants were grown in each pot. Four plants were used for relative growth rate analysis and the rest six plants were allowed to mature. Urea (2.2

g/pot) and DAP (3.8 g/pot) were administered based on 100 kg/ha and 150 kg/ha DAP recommended rate for black soil.

Randomized complete Block Design (RCBD) with three replications was employed. The treatments were moisture regimes (non-stressed and stressed) and plant materials. Under non-stressed conditions, the plants were regularly watered at field capacity until maturity. While in the stress treatment, water stress was induced by withholding irrigation for 20 days at pre-flowering stage (i.e. 45 days after planting). The weight of the pot at field capacity was determined by the following procedures as described in Klute (1986). Five pots were filled with 12kg of soil each and irrigated upto saturation level. The pots were then covered with plastic to avoid evaporation and allowed for drainage over night. The weight of each pot was measured. Finally, the weight of the pot at field capacity was calculated by taking the average.

The average weight of the pot at field capacity was 15.6 kg (i.e.15 kg the weight of the soil at field capacity plus 0.6 kg the weight of the pot). The moisture level was maintained at 10 to 20% (11.1 kg to 14.1 kg of pot weight) for stressed treatment and 70 to 100% (11.6kg to 15.6kg of pot weight) for non-stressed treatment of the available water. This was done by weighing each pot every other day and the moisture loss were replenished to required level.

4.1.3 Data collected

The following data were collected from both control and stressed conditions as employed by Mulu Ayele *et al*. (2001).

1. **Culm diameter**: The diameter of a whole stem measured on the first (**FCD**) and second (**SCD**) internode of the main tiller in cm. using calipers.
2. **Culm internode length (CIL)**: The average length of internodes of six plants in cm.
3. **Culm strength:** The strength of stem wall was measured on the first (**FCS**) and second internodes (**SCS**) of the main tiller in Ibs using the device Penetrometer.

4. **Days to heading (DTH):** Days from sowing to the stage when 50% of the plants in a pot emerge panicle.
5. **Days to maturity (DTM)**: Days from sowing to the stage when 90% of the panicle per population mature.
6. **Drought susceptibility index (DSI)**: DSI was calculated using the following formula set by Fisher and Maurer (1978). DSI= $[(1-Y_S/Y_C) / (1-Y_{AS}/Y_{AC})]$ where, YS=grain yield from stressed pot of given genotype, Y_C= grain yield from non-stressed pot of the same genotype, Y_{AS}= average grain yield of all genotypes from the stressed pot, Y_{AC}= average grain yield of all genotypes from the non-stressed.
7. **Excised leaf water loss (ELWL)**: ELWL was calculated as (FLW-2DLW)/DLWH, where FLW= Fresh leaf weight, 2DLW=two hours dry leaf weight, DLWH= twenty four hours dry leaf weight. The procedures were as follows, ten fully expanded leaves were randomly sampled from each pot during the middle of the stress period. The fresh weight was measured immediately and the leaves were left to wilt for two hours at 30^{o}C and then weighed. The leaves were then oven-dried at 80^{o}C for twenty for hours and the dried weigh measured (Clark, 1987).
8. **Grain filling period (GFP):** The number of days between heading and maturity.
9. **Grain yield (GY)**: the weight of the seed harvested from six plants in gram.
10. **Grain yield/panicle (PnSW)**: the average weight in gram of the seeds from main tillers of six plants in gram.
11. **Panicle length (PnL)**: The average length of six plants from the base of the panicle of the main tiller to the tip in cm.
12. **Panicle weight (PnW):** the average weight in gram of the panicle from main tillers of six plants.
13. **Peduncle length (PdL)**: The average length of six plants from the last internode of the culm to base of the panicle in cm.
14. **Plant height (PLH)**: The average height of six plants in centimeter from the crown to the panicle tip.

15. **Relative grain yield (RGY):** The grain yield difference between non-stress and stress conditions divided by non-stress conditions expressed in percent.
16. **Relative growth rate (RGR)**: RGR was calculated as (Dm_i/DT) x (1/DM), where, $Dm_{i=}$ Dry matter increment in time DT, DT= Time interval in days, DM=total dry matter (Hunt, 1982)
17. **Root diameter (RtD):** The diameters of a whole root of the main tiller 1 cm below the crown in cm using caliper.
18. **Root length (TRL):** The total root length measured from crown up to root tips in cm. Total root length was measured using bar graph paper.
19. **Root number (RN):** The number of adventitious roots 1cm below the crown.
20. **Root weight (RB**): Oven dry weight (80^0C for 24 h) of roots in gram.
21. **Shoot weight (SB)**: Oven dry weight (80^0C for 24 h) of above ground part in gram at the beginning and end of the stress.
22. **Thousand kernel weight (TSW)**: thousand seeds were taken randomly from the pot seed and weighed on a 0.1g sensitive balance in milligram.
23. **Tiller number (NT)**: the average number of fertile tillers of six plants
24. **Water use efficiency (WUE**): WUE was equated as total dry matter divided by total amount of water used (Blum, 2005).

4.1.4 Data analysis

The collected data for each trait were subjected to statistical analysis of variance using MSTAT-C (Michigan State University, 1991). The analysis helps us to partition the variance into different sources (phenotypic, genotypic and environmental variance) and to see if the difference among genotypes is statistically significant or not for each trait considered (Singh and Chaudhary, 1977).

-Genotypic and phonotypic variances

The variability of each trait was estimated by simple statistical measures such as mean, range, phenotypic and genotypic variances and coefficient of variation.

Variances and coefficient variation were calculated using the following formula suggested by Falconer and Markay (1996).

$\delta^2_P = \delta^2_g + \delta^2_e$ where; δ^2_P= Phenotypic variance, $\delta^2_{g=}$ Genotypic variance, δ^2_e= Environmental variance

$\delta^2_g = (MSg - MSe)/r$ Where; MSg= mean square of genotypes, MSe= mean square of error, r= number of replication

$PCV = [(\delta^2_P)^{1/2}/\mu] \times 100$ Where; PCV= phenotypic coefficient of variation, μ= Population mean

$GCV = [(\delta^2_g)^{1/2}/\mu] \times 100$ Where; GCV= phenotypic coefficient of variation, μ= Population mean

-Heritability and genetic advance

Broad sense heritability (H) expressed as the percentage of the ratio of the genotypic variance to the phenotypic variance. While, genetic advance is the improvement in genotypic value of new population as compared with base population, depends upon the magnitude of differences among genotypic values of individuals in the base population (Allard, 1960; Dabholkar, 1992).

-**Broad sense heritability (H)** was calculated using the following formula given by (Allard, 1960):

$H = Vg/Vp$ Where Vg=genotypic variance; Vp=phenotypic variance

-**Expected genetic advance (GA)** was computed by the following formula.

$GA = K \times H \times (Vp)^{½}$ and GA (% of mean) = (GA/μ) x100%,

where K= selective intensity at 5% (2.06), μ=mean

- Estimating of correlation coefficient

Correlation coefficients (r) among the collected traits were estimated using the formula suggested by Falconer and Markay (1996) and all possible pairing of traits were tested for their significance.

$$r_{pxy\ (phenotypic)} = \frac{\sigma^2 pxy}{\sqrt{(\sigma^2 px)(\sigma^2 py)}} \qquad r_{gxy\ (genotypic)} = \frac{\sigma^2 gxy}{\sqrt{(\sigma^2 gx)(\sigma^2 gy)}}$$

Genotypic covariance between trait x and y (σ^2_{gxy}) = $(MSCPgxy - MSCPexy)/r$

Phenotypic covariance between trait x and y (σ^2_{pxy}) = $\sigma^2_{gxy} + \sigma^2_{exy}$

Environmental covariance between trait x and y (σ^2_{exy}) = $MSCPexy/r$

Where, $\delta^2_P x$ = Variance of trait x; $\delta^2_P y$=Variance of trait y

MSCPgxy= Mean sum of cross product of genotypes

MSCPexy= Mean sum of cross product of error

The significance of phenotypic correlations coefficient of all possible pairs of traits were tested using the following formula (Sharma, 1998).

t (g-2) = $r/\sqrt{1-r2/n-2}$ where, g is the number of genotypes

The significance of genotypic correlation coefficient was tested with the following formula (Robertson, 1959).

$t = r_{gxy}/SE_{rgxy}$ Where, r_{gxy} =genotypic correlation coefficient,

SE_{rgxy} = standard error of genotypic correlation coefficient

$$SE_{rgxy} = \sqrt{(1-r^2gxy)^2/2Hx\ Hy}$$

The calculated 't' value was compared with the 't' tabulated value at g-2 degree of freedom at 5% and 1% level of significance.

4.2 Meiotic study in the inter-specific hybrid

Meiosis analysis was conducted for the F_1-hybrid, which was obtained from the National Tef Research Project at DZARC in Cytogenetics Laboratory of Addis Ababa University in 2008. When the inflorescence just emerged from the flag leaf, it was fixed in freshly prepared ethanol: chloroform: acetic acid (6:3:1 v/v) mixture for about 24 hrs. Then, after 24 hrs of fixation, the fixative was replaced by 70% ethanol and the inflorescence was stored at 4^0C. For meiotic analysis, the fixed inflorescence was hydrolyzed using 1N HCl at 60^0C for 3-5 min and florets was stained in aceto-orcein or propionic-orcein for four

hours at room temperature. Pollen mother cells were released in a drop of 45% acetic acid on a glass slide by macerating florets with a forceps, and squashed under a cover slip (Kifle Dagne, 1994). The preparation was then made semi-permanent by sealing around the edge of the cover slip with a nail varnish and examined under a microscope for any aberrant meiosis such as laggards, bridges, micronuclei. Finally, photomicrographic pictures of appropriate division stages were taken.

4.3 Pollen fertility test

Pollen fertility of the F_1-hybrid, the parental lines and some selected RILs were examined. Pollen grains from freshly dehisced florets were released in a drop of cotton blue lactophenol on a glass slide. The dye was covered with a cover slip and the pollen grains were allowed to stain for three hrs before scoring. Finally, the preparation was examined under the microscope and the number of fully stained pollen grains was scored as normal and the unstained or only partially stained pollen grains as aborted.

5. Results

The present study consisted of two phases; one examining the level of drought-resistance related trait variation among RILs, their parents (Key Murri and *E. pilosa*) and DZ-Cr-37 (standard check) using pots in rainout shelter and the second investigation was to see the meiotic stability and pollen fertility of the F_1-hybrid. The results are presented below separately.

5.1 Agronomic traits

5.1.1 Analysis of variances (ANOVA)

The result indicated that there were significant differences among genotypes for all the characters considered at 1% and 5% probability level (Table 3A and B) at both experimental conditions, except TSW and PdL under non-stressed and PnSW under stressed conditions.

5.1.2 Mean and range values

Mean result indicated that the differences among the means of most genotypes for a given traits were significant (P<0.05%) for both experimental conditions (Table 3 and 4). The range value of the traits for RILs examined is presented in table 5. Range of total root length (110 to 282.6 cm), root biomass (0.32 g to 2.25 g), shoot biomass (22.8 g to 45.3 g) and grain yield (2.48 g to 8 g) under non-stressed conditions and total root length (140.6 cm to 247 cm), root biomass (0.75 g to 1.8 g), shoot biomass (19 g to 49.7 g) and grain yield (0.33 g to 7.9 g) under non-stressed conditions were recorded.

Under the non-stressed condition, nine RILs gave grain yield above the mean value of the parental lines, two RILs less than poor parent (*E. pilosa*) and two RILs greater than better parent (Key Murri). Under stressed condition, eighteen RILs gave above the mean of the parental lines, two below poor parent (Key Murri) and four above better parent (*E. pilosa*). Four and six transgressive lines were obtained for non-stressed and stressed conditions, respectively (Table 6).

Table 2. Mean square for drought-resistance related and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37, 2007 (A: non-stressed, B: stressed)

(A)

		Mean squares													
SV	DF	DTH	DTM	GY	PnW	PnSW	TRL	RN	RTD	FCD	SCD	FCS	SCS	CIL	PLH
Replication	2	68.05**	24.7**	0.676**	0.014**	0.012**	748.54**	0.23**	0.02**	0.029**	0.001**	0.243**	0.039**	51.03**	74.111**
Genotypes	27	54.6**	38.4**	9.487**	0.024**	0.01**	2790.67**	23.7*	0.05*	0.21**	6.51**	0.085**	1.335**	63.3**	145.1**
Error	54	13.097	1.568	0.902	0.01	0.004	187.52	15.29	.0045	0.046	5.546	0.056	1.137	22.016	52.782
CV (%)		8.88	1.29	21.29	28.52	33.08	7.78	22.1	47.5	14.2	20	22.96	20.1	10.57	10.56

(A) Continued

		Mean squares									
SV	DF	NT	SB	RB	TB	TSW	RGR	ELWL	WUE	PnL	PdL
Replication	2	14.40**	0.335**	0.032**	3.518**	9063.5**	3467.726**	0.082**	0.006**	39.723**	5.607**
Genotypes	27	11.7**	0.745**	0.597**	4361.5**	3370.4^{ns}	82.6**	0.268**	0.031**	24.98**	13.67^{ns}
Error	54	5.782	0.308	0.06	1018.32	3051.9	6.232	0.01	0.000	6.121	10.9
CV (%)		29.41	52.17	22.79	10.97	27.16	8.54	5.31	3.97	9.88	16.81

(B)

		Mean squares											
SV	DF	DTH	DTM	GY	PnW	PnSW	TRL	RN	RTD	FCD	SCD	FCS	SCS
Replication	2	8.2	0.7	6.6**	0.03**	10^{-6}	927.9**	14.1**	0.012**	0.052**	0.084**	0.113**	0.053**
Genotypes	27	66.4**	34.1**	0.941**	0.029**	0.01^{ns}	36959**	35.1**	0.02*	0.11**	0.092**	0.113**	0.04*
Error	54	4.5	5.3	22.76	0.01	0.01	254.72	9.2	0.021	0.139	0.095	0.041	0.02
CV (%)		5.3	2.6	2.9	25.7	43.1	8.24	15.7	31.6	24	21.25	20.24	20.23

(B) Continued

		Mean squares												
SV	DF	CIL	PLH	NT	SB	RB	TB	TSW	RGR	DSI	ELWL	WUE	PnL	PdL
Replication	2	25.9**	47.1	20.2**	88.2**	0.2**	127.1**	234.3**	14.33**	0.006	0.015**	0.013*	0.9	68.7*
Genotypes	27	53.4*	111.6**	4.21*	107**	0.68**	145.2**	5301**	87.91 **	1.566**	0.56 **	0.03**	27.9**	14.7**
Error	54	32.4	35	4.01	23.8	0.182	29.851	779.4	0.531	0.769	0.013	0.002	5.7	14.2
CV (%)		16.0	10.2	28.4	16.96	34.2	15.93	14.9	1.80	0.014	7.62	11.21	11.1	22.9

Total root length=TRL, root number=RN, root diameter=RtD, first culm strength=FCS, second culm strength=SCS, first culm diameter=FCD, second culm diameter=SCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, grain filling period=GFP, relative grain yield=RGY, panicle length=PnL, peduncle length=PdL, culm internode length=CIL, panicle weight=PnW, grain yield/panicle=PnSW, grain yield=GY, 1000 kernel weight=TSW, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE, relative growth rate=RGR

Table 3. Mean values of drought-related and agronomic traits for *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under stressed conditions, 2007

No	RILs	RN	TRL	RtD	FCD	FCS	PLH	NT	PnW	SB	RB	GY
1	***E. pilosa***	21.3^{CDEFG}	206.3^{ABCD}	0.03^{N}	0.2^{ABC}	0.78^{GH}	50.1^{FGHI}	7.0^{ABCD}	0.2^{DE}	30.1^{DE}	1.2^{ABCD}	4.1^{EFG}
2	**Key Murri**	20.7^{EFGHI}	215.0^{ABC}	0.1^{H}	0.2^{ABC}	1.4^{AB}	62.2 ^{ABC}	4.8^{D}	0.3 ^{ABCD}	25.7^{DEFG}	1.7^{AB}	2.6^{IJK}
3	**DZ-cr-37**	22.0^{BCDE}	215.5 ^{ABC}	0.04 ^{M}	0.2^{ABC}	1.1^{ABCDEF}	71.2^{A}	8.0^{ABCD}	0.2^{CDE}	37.4^{B}	0.9^{ABCD}	5.5^{BC}
4	**RIL-183**	16.3^{HIJ}	166.1^{BCD}	0.05^{G}	0.1^{BC}	1.1^{BCDEFG}	58.6^{DEFG}	6.4^{BCD}	0.3^{ABCDE}	18.9^{H}	1.8^{A}	2.5^{JKL}
5	**RIL-62**	13.3^{J}	176.8^{ABCD}	0.05^{I}	0.1^{C}	1.02^{CDEFGH}	57.7^{CDEFG}	9.6^{A}	0.2^{CDE}	30.7^{CD}	1.6^{BC}	5.4 ^{BCD}
6	**RIL-252**	20.7^{EFGHI}	209.1^{ABCD}	0.05^{E}	0.2^{ABC}	1.3^{ABC}	69.4^{AB}	6.7^{BCD}	0.4^{A}	27.6^{DEF}	1.4^{ABCD}	1.9^{KL}
7	**RIL-29**	19.0^{EFGHI}	195.3 ^{ABCD}	0.05^{F}	0.2^{ABC}	0.96^{DEFGH}	56.2^{DEFGH}	5.8^{BCD}	0.3^{ABCD}	24.3^{FGH}	1.4^{BCD}	0.3^{M}
8	**RIL-118**	19.7^{EFGHI}	193.1^{ABCD}	0.1^{D}	0.2^{BC}	0.85^{FGH}	52.5^{EFGHI}	6.3^{BCD}	0.2^{BCDE}	23.2^{FGH}	1.5^{ABCD}	4.3^{EF}
9	**RIL-172**	16.7^{GHIJ}	161.6 ^{CD}	0.1^{F}	0.2^{ABC}	0.7^{H}	47.2^{HI}	5.5^{BCD}	0.2^{CDE}	19.9 ^{GH}	0.9^{ABCD}	3.9^{FGH}
10	**RIL-222**	16.3^{HIJ}	144.2^{CD}	0.1^{F}	0.2^{ABC}	1.5^{A}	61.0^{BCDE}	7.3^{BCD}	0.2^{CDE}	30.3^{DE}	1.3^{ABCD}	1.4^{LM}
11	**RIL-317**	21.7^{BCDEF}	177.8^{ABCD}	0.03^{H}	0.2^{ABC}	0.9^{EFGH}	56.7^{EFGH}	8.1^{ABCD}	0.2^{CDE}	25.2^{EFG}	0.8^{CD}	4.3^{DEF}
12	**RIL-337**	13.3 ^{J}	141.6^{D}	0.04^{L}	0.1^{BC}	0.99^{CDEFGH}	60.4^{BCDE}	6.8^{ABCD}	0.2^{ABCDE}	25.9^{DEF}	1.4^{ABCD}	4.7^{BCDEF}
13	**RIL-173**	22.3 ^{BCD}	163.3 ^{CD}	0.04J	0.1^{BC}	0.9^{EFGH}	59.2^{CDEF}	7.4^{BCD}	0.2^{CDE}	27.7^{DEF}	0.8^{D}	3.9^{EFGH}
14	**RIL-66**	16.3HIJ	174.8 ^{ABCD}	0.1^{G}	0.1 ^{C}	1.0^{CDEFGH}	54.7^{DEFGH}	8.4^{ABC}	0.2^{BCDE}	27.2^{DEF}	1.1^{ABCD}	2.9^{HIJK}
15	**RIL-16**	28.7^{A}	156.1^{CD}	0.03I	0.2^{AB}	1.2^{BCDEF}	60.9^{BCDE}	8.3^{ABC}	0.3^{BC}	49.7^{A}	1.1 ^{BCD}	7.9^{A}
16	**RIL-37**	17.0 ^{FGHIJ}	170.4 ^{BCD}	0.1^{F}	0.2^{BC}	1.2^{ABCDE}	56.7^{DEFGH}	6.3^{ABCD}	0.2^{ABCDE}	25.5^{DEFG}	1.3^{ABCD}	4.7 ^{BCDE}
17	**RIL-226**	16.7^{GHIJ}	194.6^{ABCD}	0.1^{H}	0.2^{ABC}	0.99^{CDEFGH}	59.7^{CDEF}	5.2^{CD}	0.3 ^{AB}	28.6^{CDEF}	0.9^{ABCD}	5.4 ^{BCD}
18	**RIL-9**	17.7^{FGHIJ}	246.9 ^{A}	0.1^{A}	0.1^{BC}	0.78^{GH}	64.0^{ABC}	5.5^{BCD}	0.2^{BCDE}	26.64 ^{DEF}	1.2^{ABCD}	3.8^{EFGH}
19	**RIL233**	16.00 ^{IJ}	241.3 ^{AB}	0.04^{M}	0.2^{ABC}	1.0^{CDEFGH}	62.5^{ABC}	7.0^{ABCD}	0.2^{ABCDE}	25.4^{DEFG}	1.1^{ABCD}	4.1^{EFG}
20	**RIL139**	18.0^{EFGHIJ}	181.6^{ABCD}	0.1^{F}	0.1 ^{C}	0.78^{GH}	52.3^{EFGHI}	8.3^{ABC}	0.2^{CDE}	18.9^{H}	0.8^{CD}	3.7^{EFGHI}
21	**RIL197**	24.0^{AB}	171.6 ^{BCD}	0.04^{M}	0.2^{ABC}	0.7^{H}	54.7^{DEFGH}	8.3^{ABC}	0.3^{ABCDE}	26.5^{DEF}	0.9^{BCD}	3.5^{FGHIJ}
22	**RIL-124**	18. 3^{EFGHI}	204.2^{ABCD}	0.1^{B}	0.2^{A}	1.0^{CDEFGH}	57.5^{CDEFG}	6.4^{ABCD}	0.2^{ABCDE}	24.1^{FGH}	1.7^{A}	3.5^{EFGHIJ}
23	**RIL-364**	24.0^{AB}	166.9^{CD}	0.1 ^{C}	0.2^{ABC}	0.9^{EFGH}	62.0^{ABCD}	7.4^{BCD}	0.2^{CDE}	27.5^{DEF}	1.7^{AB}	3.7^{EFGHI}
24	**RIL-275**	20.3FG^{HI}	182.3^{ABCD}	0.1^{G}	0.2^{ABC}	0.9^{EFGH}	61.6^{ABCDE}	7.7^{BCD}	0.1^{E}	24.9^{EFG}	1.3^{ABCD}	2.8^{HIJK}
25	**RIL-12**	20.0^{EFGHI}	159.0^{CD}	0.03^{N}	0.13^{C}	1.0^{CDEFGH}	57.1^{CDEFG}	8.3^{ABC}	0.2^{CDE}	27.9^{DEF}	1.4^{ABCD}	4.1^{FG}
26	**RIL-313**	17.3^{FGHIJ}	199.5^{ABCD}	0.1^{G}	0.14^{C}	0.87^{EFGH}	43.2 ^{I}	8.5^{AB}	0.1^{E}	23.7^{FGH}	0.9^{ABCD}	2.6^{IJK}
27	**RIL-290**	22.7 ^{BC}	147.5^{CD}	0.04 ^{L}	0.17^{ABC}	1.3^{ABCD}	49.0^{GHI}	6.1^{BCD}	0.2^{CDE}	33.7^{BC}	1.5^{ABCD}	5.8^{A}
28	**RIL-36**	21.0^{DEFGH}	140.6 ^{D}	0.04^{K}	0.15^{ABC}	0.9^{EFGH}	57.9^{CDEFG}	8.0^{ABCD}	0.2^{CDE}	26.5^{DEF}	1.2^{ABCD}	3.5^{FGHIJ}
	Mean	19.3	182.3	0.07	0.017	0.95	57.7	7.1	0.22	26.2	1.24	3.8
	LSD(0.05)	4.9	73.16	0.002	0.052	0.3	9.7	3.3	0.2	5.8	0.8	1.2

-Means followed by a common letter within a column were not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Tests.
Total root length=TRL, root number=RN, root diameter=RtD, first culm strength=FCS, first culm diameter=FCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, panicle seed weight=PnSW, grain yield=GY.

Table 4. Mean values of drought-resistance related and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under non-stressed conditions, 2007

No	RILs	TRL	RN	RtD	FCD	FCS(Ib)	PLH(cm)	NT	PnW(gm)	SB(g)	RB (g)	GY(g)
1	***E. pilosa***	180.6 ^{EFGHIJ}	15.3 ^{CDE}	0.04^{J}	0.1 ^{N}	0.847^{CDE}	68.9^{ABCDEF}	9.4^{ABCD}	0.1 ^{EF}	33.4^{EFGHIJ}	1.1^{CDEF}	4.3^{HIJ}
2	**Key Murri**	169.3 ^{FGHIJK}	16.7 ^{BCDE}	0.04^{J}	0.2 ^{A}	1.270^{AB}	75.8^{AB}	6.3^{CDEF}	0.3^{ABCD}	51.3^{A}	0.6^{HIJ}	6.6^{BCD}
3	**DZ-cr-37**	317.5^{A}	27.0 ^{A}	0.04^{L}	0.1 ^{K}	1.18^{ABC}	78.5^{AB}	7.4^{BCDEF}	0.3^{A}	42.6^{BC}	1.2 ^{BCDE}	5.8^{DEFG}
4	**RIL183**	205.3 ^{CDEFGH}	18.3 ^{BCDE}	0.04^{J}	0.2^{H}	1.09A^{BCDE}	71.4^{ABCD}	8.0^{BCDEF}	0.2^{BCDEF}	25.6^{KLM}	0.7^{FGHI}	2.7^{K}
5	**RIL-62**	110.0 ^{K}	13.33 ^{E}	0.02^{P}	0.1 ^{P}	0.96^{ABCDE}	60.4^{DEF}	9.7^{ABCD}	0.2^{BCDEF}	38.9^{CDE}	0.9^{EFGHI}	7.6^{A}
6	**RIL-252**	199.2^{DEFGH}	18.7 ^{BCDE}	0.1^{F}	0.2 ^{C}	1.04^{ABCDE}	68.1^{BCDEF}	6.1^{DEF}	0.2^{BCDEF}	33.4^{EFGHIJ}	1.3^{BCD}	2.5^{K}
7	**RIL-29**	255.3 ^{ABCD}	18.7 ^{BCDE}	0.1^{C}	0.2 ^{B}	1.13^{ABCD}	71.1^{ABCDE}	4.5^{F}	0.2^{BCDEF}	31.8^{FGHIJ}	2.1^{A}	4.3^{GHIJ}
8	**RIL-118**	175.1 ^{EFGHIJ}	20.0 ^{BCD}	0.1^{B}	0.2 ^{I}	0.787 ^{DE}	68.1^{BCDEF}	9.9^{ABCD}	0.2^{BCDEF}	32.2^{FGHIJ}	0.3 ^{J}	5.1^{FGHIJ}
9	**RIL-172**	231.2^{BCDE}	23.0 ^{AB}	0.04 ^{K}	0.1 ^{O}	0.707 ^{E}	58.8^{F}	12.9 ^{A}	0.1^{CDEF}	28.1^{IJKLM}	0.6^{GHIJ}	4.6^{FGHIJ}
10	**RIL-222**	152.9 ^{HIJK}	14.3 ^{DE}	0.1^{A}	0.1 ^{L}	1.16A^{BCD}	76.6^{AB}	7.3^{BCDEF}	0.2^{BCDEF}	36.2^{DEFG}	1.1^{DEF}	3.7^{JK}
11	**RIL-317**	122.6 ^{JK}	17.7 ^{BCDE}	0.04 ^{H}	0.1 ^{L}	1.03^{ABCDE}	63.8^{CDEF}	7.2^{BCDEF}	0.2^{CDEF}	27.6^{JKLM}	0.8^{FGHI}	4.9^{FGHIJ}
12	**RIL-337**	162.5^{FGHIJK}	16.0 ^{CDE}	0.04^{J}	0.2^{G}	0.85 ^{CDE}	70.1^{ABCDEF}	7.3 ^{BCDEF}	0.2^{CDEF}	28.7^{IJKLM}	1.1^{DEFG}	5.1^{FGHIJ}
13	**RIL-173**	127.9 ^{IJK}	14.7 ^{DE}	0.02 ^{O}	0.2^{F}	1.017^{ABCDE}	70.1^{ABCDEF}	11.1^{AB}	0.2^{CDEF}	30.7^{FGHIJ}	0.8^{EFGHI}	4.6^{FGHIJ}
14	**RIL-66**	145.4 ^{HIJK}	15.0 ^{DE}	0.03 ^{M}	0.2^{D}	1.047^{ABCDE}	69.1^{ABCDEF}	5.3^{EF}	0.2^{BCDE}	23.5^{LM}	1.6^{B}	3.7^{JK}
15	**RIL-16**	158.3 ^{GHIJK}	16.3^{CDE}	0.1^{D}	0.2 ^{F}	0.93^{BCDE}	69.7^{BCDEF}	8.6^{BCDE}	0.3^{AB}	41.6^{BCD}	0.6^{HIJ}	5.2^{FGHIJ}
16	**RIL-37**	224.4 ^{BCDEF}	16.7 ^{CDE}	0.04 ^{I}	0.2 ^{G}	1.343 A	69.7^{ABCDEF}	8.8^{BCDE}	0.2^{ABCD}	32.9^{FGHIJ}	2.3^{A}	5.1^{FGHIJ}
17	**RIL-226**	162.1 ^{FGHIJK}	16.3 ^{CDE}	0.1^{C}	0.2 ^{E}	1.077^{ABCDE}	80.03^{A}	8.1^{BCDEF}	0.2^{BCDEF}	45.3^{AB}	0.9^{DEFGH}	7.1^{ABC}
18	**RIL-9**	182.0 ^{EFGHIJ}	17.3 ^{BCDE}	0.04 ^{I}	0.2 ^{G}	1.05A^{BCDE}	70.3^{ABCDEF}	8.3^{BCDEF}	0.2^{CDEF}	29.4 ^{HIJKL}	0.8 ^{FGHI}	4.5^{FGHIJ}
19	**RIL-233**	177.4 ^{EFGHIJ}	15.7^{CDE}	0.04 ^{J}	0.2 ^{E}	1.35^{A}	69.9^{BCDEF}	6.25^{CDEF}	0.2^{CDE}	41.4^{BCD}	0.9^{DEFGH}	8.0 ^{A}
20	**RIL-139**	162.5 ^{FGHIJK}	18.3 ^{BCDE}	0.1 ^{C}	0.1^{O}	0.70 ^{E}	59.5^{EF}	11.1^{AB}	0.1^{EF}	22.8 ^{M}	0.8 ^{FGHI}	6.4^{BCDE}
21	**RIL-197**	201.6 ^{CDEFGH}	16.7 ^{BCDE}	0.03 ^{N}	0.2 ^{F}	1.02^{ABCDE}	74.6^{ABC}	9.0^{ABCDE}	0.2^{CDEF}	30.1^{GHIJK}	0.94^{FGH}	5.6^{EFGH}
22	**RIL-124**	282.6 ^{AB}	21.7 ^{ABC}	0.1^{D}	0.1^{J}	0.90^{BCDE}	60.7^{DEF}	6.8^{CDEF}	0.2^{CDEF}	29.1 ^{HIJKL}	1.5 ^{BC}	3.9 ^{IJK}
23	**RIL-364**	221.3^{BCDEFG}	19.7 ^{BCDE}	0.1 ^{D}	0.1 ^{M}	1.23^{ABC}	69.8^{ABCDEF}	6.7^{CDEF}	0.2^{ABC}	34.0^{EFGHI}	1.5^{BC}	4.8^{FGHIJ}
24	**RIL-275**	260.4 ^{ABC}	19.0 ^{BCDE}	0.1^{E}	0.1 ^{K}	1.03^{ABCDE}	77.5^{AB}	5.3^{EF}	0.1^{DEF}	29.2^{HIJKL}	1.3^{BCD}	5.9^{DEF}
25	**RIL-12**	189.7 ^{EFGHI}	16.3^{DE}	0.1^{G}	0.1 ^{O}	0.913^{BCDE}	69.3^{ABCDEF}	8.8^{BCDE}	0.2^{CDEF}	34.9^{EFGH}	1.5^{BC}	5.4^{FGHI}
26	**RIL-313**	193.4 ^{DEFGH}	18.3^{BCDE}	0.04 ^{L}	0.1 ^{Q}	0.99^{ABCDE}	46.6^{G}	10.1 ^{ABC}	0.1^{F}	30.1^{HIJK}	0.5^{IJ}	5.6^{FGH}
27	**RIL-290**	262.2^{ABC}	17.0 ^{BCDE}	0.03 ^{N}	0.2 ^{H}	1.173 ^{ABCD}	68.9^{ABCDEF}	10.2 ^{ABC}	0.20^{CDE}	36.8^{CDEF}	1.3^{BCD}	5.7^{CDEF}
28	**RIL-36**	168.4 ^{EFGHIJK}	17.3 ^{BCDE}	0.03 M	0.2 I	0.9 ^{BCDE}	67.9^{CDEF}	8.3^{BCDEF}	0.2^{CDEF}	34.2^{EFGHI}	1.1^{CDEF}	4.3^{FGHIJ}
	Mean	192.9	17.7	0.05	0.14	1.03	68.8	8.2	0.19	33.4	0.88	5.1
	LSD(0.05)	65.0	6.4	0.002	0.002	0.4	11.9	3.9	0.1	6.2	0.4	1.6

-Means followed by a common letter within a column were not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Tests.
-Total root length=TRL, root number=RN, root diameter=RtD, first culm strength=FCS, second culm diameter=SCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, grain yield=GY.

Table 5. Range value of drought-resistance related and agronomic traits of *E. tef, E. pilosa,* and their RILs grown under stressed and non-stressed condition, 2007

Traits	**RILLs**					
	Minimum		Mean of parental lines (Key Murri *and E. pilosa*)		maximum	
	non-stressed	stressed	non-stressed	Stressed	non-stressed	stressed
TRL(cm)	110.0(RIL62)	140.6(RIL36)	174.95	210.65	282.6(RIL124)	247.0 (RIL9)
RN	13.3(RIL62)	13.3 (RIL62)	16	21	23 (RIL172)	28.7 (RIL16)
RtD (cm)	0.02 (RIL62)	0.03 (RIL12)	0.04	0.04	0.08 (RIL222)	0.06 (RIL9)
FCD(cm)	0.1 (RIL313)	0.13 (RIL66)	0.165	0.165	0.08 (RIL222)	0.2 (RIL124)
SCD(cm)	0.11 (RIL62)	0.12 (RIL66)	0.2	0.15	0.22(RIL290)	0.18 (RIL16)
FCS	0.7 (RIL139)	0.72(RIL172)	1.06	1.09	1.4 (RIL233)	1.5 (RIL222)
SCS	0.44(RIL139)	0.53(RIL172)	0.735	0.705	0.93 (RIL16)	0.92(RIL252)
RB (g)	0.32(RIL118)	0.75(RIL173)	0.855	1.405	2.25 (RIL37)	1.8 RIL(183)
RGR	0.008(RIL197)	0.01(RIL275)	0.017	0.017	0.025(RIL66)	0.03(RIL313)
ELWL	1.35 (RIL36)	0.85 (RIL62)	1.745	1.615	2.29 (RIL124)	2.07(RIL252)
DSI	-	0.27 (RIL37)		1.22	-	3.25 (RIL29)
WUE	0.33 (RIL252)	0.3 RIL172)	0.605	0.39	0.67 (RIL226)	0.74 (RIL16)
DTH	34 (RIL317)	35.7(RIL173)	43.7	42.45	47.7 (RIL252)	49.3(RIL222)
DTM	91.7 (RIL139)	82.3(RIL290)	87.65	90.8	103 (RIL16)	95 (RIL252)
CIL(cm)	29.8(RIL313)	25 (RIL222)	44.85	33.95	51.1 (RIL226)	41.4 (RIL9)
PLH(cm)	46.6(RIL313)	43.2(RIL313)	72.35	56.15	80.0 (RIL226)	69.4(RIL252)
NT	4.5(RIL29)	5.2(RIL226)	7.85	5.9	4.5 (RIL29)	9.6 (RIL62)
PnL(cm)	17.4 (RIL313)	16.0 RIL313)	27.15	22.6	30.0 (RIL16)	27.7 (RIL252)
PnW(g)	0.18 (RIL313)	0.23(RIL275)	0.37	0.3415	0.49 (RIL364)	0.61 (RIL29)
TSW (mg)	0.18(RIL118)	0.13 RIL124)	0.275	0.195	0.23 (RIL9)	0.27 (RIL62)
GY(g)	2.48 (RIL252)	0.33 (RIL29)	5.45	3.34	8.0 (RIL233)	7.9 (RIL16)
SB (g)	22.8(RIL139)	19.0 RIL183)	42.35	27.91	45.3 (RIL226)	49.7 (RIL16)
TB (g)	28.2(RIL66)	22.6(RIL183)	48.6	32.35	53.2 (RIL226)	59.5 (RIL16)

Total root length=TRL, root number=RN, root diameter=RtD, First culm strength=CS, , second culm strength=SCS second culm diameter=SCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, panicle length=PnL, peduncle length=PdL, panicle weight=PnW, grain yield=GY, 1000 kernel weight=TSW, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE.

Table 6. Mean grain yield of RILs, better parental line, poor parental line and parental lines mean

No	RILs	Non-stressed				RILs	Under stressed			
		GY (g)	**>Key Murri (6.6g)**	***<E. pilosa* (4.3g)**	>Parental mean (5.45g)		GY (g)	**<Key Murri (2.6g)**	***>E. pilosa* (4.1g)**	>Parental mean (3.35g)
1	**RIL-252**	2.5^{K}		√		**RIL-29**	0.3^{M}	√		
2	**RIL183**	2.7^{K}		√		**RIL-222**	1.4^{LM}	√		
3	**RIL-222**	3.7^{JK}				**RIL-252**	1.9^{KL}			
4	**RIL-66**	3.7^{JK}				**RIL-183**	2.5^{JKL}			
5	**RIL-124**	3.9^{IJK}				**Key Murri**	2.6^{IJK}			
6	***E. pilosa***	4.3^{HIJ}				**RIL-313**	2.6^{IJK}			
7	**RIL-36**	4.3^{FGHIJ}				**RIL-275**	2.8^{HIJK}			
8	**RIL-29**	4.3^{GHIJ}				**RIL-66**	2.9^{HIJK}			
9	**RIL-9**	4.5^{FGHIJ}				**RIL-124**	3.5^{EFGHIJ}			√√
10	**RIL-172**	4.6^{FGHIJ}				**RIL197**	3.5^{FGHIJ}			√√
11	**RIL-173**	4.6^{FGHIJ}				**RIL-36**	3.5^{FGHIJ}			√√
12	**RIL-364**	4.8^{FGHIJ}				**RIL139**	3.7^{EFGHI}			√√
13	**RIL-317**	4.9^{FGHIJ}				**RIL-364**	3.7^{EFGHI}			√√
14	**RIL-118**	5.1^{FGHIJ}				**RIL-9**	3.8^{EFGH}			√√
15	**RIL-337**	5.1^{FGHIJ}				**RIL-173**	3.9^{EFGH}			√√
16	**RIL-37**	5.1^{FGHIJ}				**RIL-172**	3.9^{FGH}			√√
17	**RIL-16**	5.2^{FGHIJ}				**RIL233**	4.1^{EFG}			√√
18	**RIL-12**	5.4^{FGHI}				**RIL-12**	4.1^{EFG}			√√
19	**RIL-197**	5.6^{EFGH}			√√	***E. pilosa***	4.1^{FG}			
20	**RIL-313**	5.6^{FGH}			√√	**RIL-317**	4.3^{DEF}			√√
21	**RIL-290**	5.7^{CDEF}			√√	**RIL-118**	4.3^{EF}			√√
22	**RIL-275**	5.9^{DEF}			√√	**RIL-37**	4.7^{BCDE}			√√
23	**RIL-139**	6.4^{BCDE}			√√	**RIL-337**	4.7^{BCDEF}			√√
24	**Key Murri**	6.6^{BCD}			√√	**RIL-62**	5.4^{BCD}		√√	√√
25	**RIL-226**	7.1^{ABC}			√√	**RIL-226**	5.4^{BCD}		√√	√√
26	**RIL-62**	7.6^{A}	√√		√√	**RIL-290**	5.8^{A}		√√	√√
27	**RIL-233**	8.0^{A}	√√		√√	**RIL-16**	7.9^{A}		√√	√√
Mean										

√√=RILs that gave grain yield greater than better parental line and parental lines mean
√=RILs that gave grain yield less than poor parental line
Key Murri= better parental line for non-stressed condition, while poor for stressed conditions.
E. pilosa= better parental line for stressed condition, while poor for non-stressed conditions.

5.1.3 Phenotypic and genotypic coefficients of variation

The result in the present study indicated that there were high PCV and GCV values for most traits (Table 7 and 8). Under non-stressed condition, GCV and PCV ranges of 1.4 (SB) to 58.9 (RB) and 1.8 (SB) to 52.6 (PnW) were estimated, respectively. But under stressed condition, the ranges of GCV were 3.5 (DTM) to 71.9 (DSI), while the PCV ranges were 3.8 (DTM) to 72.3 (DSI).

5.1.4 Heritability and genetic advances

The result is shown in Table 7 and 8. Under non-stressed condition, agrononomic and drought-resistance related traits considered showed high level of heritability estimate ranged from 45.5% to 100%. Regarding GA (as % of mean) low to high genetic advance were obtained. 2.5% (SB) and 8.9% (DTM) were the lowest value. Traits like, RB (117.1%), GY (80.9%), PnW (75.9%) and WUE (53.2%) gave highest value and the rest traits had medium value. The heritability ranged from 4.5% to 99.7%. DTM (6.7%), PLH (15.1%) and NT (1.64%), had lowest GA (as % of mean). Maximum value was obtained for DSI (147.5%), RtD (102.6%), GY (68.6%) and RB (57.9%).

Table 7. Mean, genotypic (GVC) and phenotypic (PCV) coefficients of variation, genotypic (V_g), phenotypic (V_P) and environmental variances (V_e), heritability and genetic advance of drought-resistance related traits and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under non-stressed conditions, 2007.

Traits	Mean	V_P	V_g	V_e	PCV	GCV	H (%)	GA	GA % mean
DTH	40.8	25.14	20.77	4.37	12.3	11.2	82.6	8.53	20.9
DTM	97.3	18.91	18.391	0.523	4.5	4.4	97.3	8.7	8.9
GY	5.1	4.59	4.289	0.301	42	40.6	93.4	4.1	80.9
PnW	0.19	0.010	0.007	0.003	52.6	44	70	0.14	75.9
TRL	192.9	1364.1	1301.6	62.507	19.2	18.7	95.4	72.6	37.6
RN	17.7	9.302	4.205	5.097	17.2	11.6	45.2	2.84	16.1
FCD	0.14	0.097	0.082	0.015	20.7	19.0	84.5	0.54	38.7
FCS	1.03	0.033	0.015	0.0187	17.6	11.9	45.5	0.17	16.5
PLH	68.8	63.75	46.159	17.59	11.6	9.9	72.4	11.9	17.3
NT	8.2	4.898	2.971	1.927	27.1	21.0	60.7	2.77	33.7
SB	32.4	0.321	0.219	0.103	1.8	1.4	68.2	0.8	2.5
RB	0.88	0.289	0.269	0.02	61.1	58.9	93.1	1	117.1
RGR	0.024	$3.3x10^{-7}$	$3.3x10^{-7}$	10^{-5}	4.3	4.2	97.1	0.1	43.6
ELWL	1.9	0.132	0.129	0.003	19.1	18.9	97.7	0.73	38.5
WUE	0.49	0.016	0.016	0	25.8	25.8	100	0.26	53.2

Root length=TRL, root number=RN, root diameter=RtD, culm strength=CS, culm diameter=SCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, panicle length=PnL, peduncle length=PdL, culm internode length=CIL, panicle weight=PnW, grain yield/panicle=PnSW, grain yield=GY, 1000 kernel weight=TSW, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE, relative growth rate=RGR

Table 8. Mean, genotypic (GVC) and phenotypic (PCV) coefficients of variation, genotypic (V_g), phenotypic (V_P) and environmental variances (V_e), heritability and genetic advance of drought-resistance related traits and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under stressed conditions, 2007.

Traits	**mean**	V_P	V_g	V_e	**PCV**	**GCV**	**H (%)**	**GA**	**GA % mean**
DTH	40.3	22.13	20.6	1.5	11.7	11.3	93.1	9	22.4
DTM	88.2	11.37	9.6	1.77	3.8	3.5	84.5	5.87	6.7
GY	3.8	2.18	1.87	0.31	38.9	36	85.7	2.6	68.6
PnW	0.22	0.01	0.006	0.003	44.8	35.2	61.9	0.13	57
TRL	140.6	1231.6	1146.7	84.91	25	24.1	93.1	67.3	47.9
RN	19.3	11.7	8.63	3.07	17.7	15.2	73.8	5.2	27.1
RtD	0.07	0.01	0.003	0.004	110.7	74.2	45	0.07	102.6
FCD	0.02	0.03	0.03	0.003	11.5	11.0	95.2	0.35	22.6
FCS	0.95	0.038	0.024	0.014	20.5	16.3	63.2	0.25	26.7
PLH	57.7	37.2	25.53	11.67	10.6	8.8	68.6	8.6	15.1
NT	7.1	1.40	0.07	1.34	16.7	3.7	4.8	0.12	1.64
SB	26.2	35.67	27.73	7.93	22.8	20.1	77.8	9.6	36.5
RB	1.24	0.23	0.17	0.06	38.4	32.9	73.1	0.72	57.9
RGR	0.02	10^{-5}	10^{-5}	10^{-5}	24.8	17.6	50	0.46	27.5
ELWL	1.47	0.19	0.18	0.004	29.3	29	97.9	0.87	59.1
WUE	0.43	0.009	0.01	0.001	21.7	20.8	92.1	0.18	41.1
DSI	1.0	0.522	0.517	0.005	72.3	71.9	99.1	1.48	147.5

Root length=TRL, root number=RN, root diameter=RtD, culm strength=CS, culm diameter=SCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, grain filling period=GFP, relative grain yield=RGY, panicle length=PnL, peduncle length=PdL, culm internode length=CIL, panicle weight=PnW, grain yield/panicle=PnSW, grain yield=GY, 1000 kernel weight=TSW, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE, relative growth rate=RGR

5.1.5 Estimation of correlation coefficient

1. Phenotypic correlation coefficient

The phenotypic association of agronomic and drought resistance related traits were analyzed for each genotype and the following result were obtained (Table 9 and 10). Most of the drought-resistance related traits have shown significant relationship with agronomic traits. Under non-stressed conditions, grain yield was positively and significantly correlated with DTH (0.18*), DTM (0.24**), FCS (0.24**), NT (0.15*), SB (0.33**), ELWL (0.21**) and WUE (0.71**) but a negative correlation was seen for TRL (-0.3**), RTD (-0.11*), FCD (-0.17*), RB (-0.22**) and RGR (-0.7**). WUE and RGR had shown strong correlation with GY. The relationship obtained for grain yield and drought-resistance related traits under stressed condition were different from the relation seen for non-stressed condition. For example, RN (0.25**), TRL (0.38**), RB (0.21*), SB (0.5**) and RGR (0.55**) were positively correlated. DTH (-0.3**), DTM (-0.3**), DSI (-0.21**) and ELWL (-0.4**) had negative strong correlation. WUE (0.83**) were positively correlated like non-stressed condition.

2. Genotypic correlation coefficient

The genotypic correlations between all the pairs of characters studied are presented in Table 9 and 10. The pattern of relationships among agronomic and drought-resistance related traits observed for phenotypic correlation were more or less similar with genotypic results for both experimental conditions for each traits. Under non-stressed condition, the correlation coefficients for most of the pairs of characters revealed the presence of strong genotypic association between GY with NT (0.24**), ELWL (0.31**), WUE (0.76**), TRL (-0.5**), RB (-0.3**), and RGR (-0.81**). The correlation coefficient between GY and these traits under stressed condition were, NT (0.34**), ELWL (-0.4**), WUE (0.87**), TRL (0.58**), RB (0.31**), and RGR (0.68**). The coefficient values of both types of correlations are comparable but for some traits the genotypic correlations are of higher in magnitude than their corresponding phenotypic correlations.

Table 9. Genotypic (upper triangle) and phenotypic (lower triangle) correlation coefficients among drought-resistance related traits and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under non-stressed conditions, 2007.

Traits	DTH	DTM	RN	TRL	RtD	FCD	FCS	PnL	PLH	NT	SB	RB	GY	WUE	RGR	ELWL
DTH	**1.0**	0.56**	0.06	0.16**	-0.5**	0.66**	0.77**	0.71**	0.5**	-0.46**	0.71**	0.14*	0.11**	0.21*	-0.12*	0.25**
DTM	0.42**	**1.00**	-0.08	0.07	-0.4**	0.42**	0.43**	0.12*	0.06	-0.35**	0.07	0.02	0.07	0.11*	-0.07	0.17*
RN	-0.09	-0.06	**1.00**	0.81**	0.75**	-0.19*	-0.37**	0.01	-0.2**	-0.58**	-0.4**	0.09	-0.2**	0.16*	0.26*	-0.21**
TRL	0.12*	0.09	0.35**	**1.00**	0.70**	0.06	-0.15*	-0.13*	-0.2**	0.06	-0.3**	0.46*	-0.5**	-0.28**	0.38**	-0.09
RtD	0.11*	-0.08	0.08	0.08*	**1.00**	-0.44**	-0.33**	0.93**	1.1**	-1.59**	-0.2**	0.68**	-1.08*	-0.09*	0.82**	1.11**
FCD	0.45**	0.35**	-0.15*	0.01	0.11*	**1.00**	0.79**	0.72**	0.7**	-0.83**	0.58**	0.20**	-0.2**	0.28**	0.03	0.21**
FCS	0.27**	0.13*	-0.06	-0.04	-0.04	0.27**	**1.00**	0.89**	0.74**	-1.22**	1.18**	1.15**	0.2**	0.37**	-0.3**	0.46**
PnL	0.44**	0.07	0.08	-0.08*	0.08	0.54**	0.27**	**1.00**	0.88**	-0.92**	0.87**	0.04	0.17*	0.45**	-0.03	0.05
PLH	0.32**	0.04	0.02	-0.11*	0.07	0.44**	0.34**	0.73**	**1.00**	-0.96**	0.54**	0.27**	0.07	0.28**	-0.14*	0.11*
NT	-0.13*	-0.16*	0.25**	0.06	-0.09	-0.29**	-0.29**	-0.2**	-0.17*	**1.00**	-0.6**	-0.7**	0.24**	-0.11*	-0.06	-0.57**
SB	0.27**	0.04	0.11*	-0.15	0.07	0.22**	0.20**	0.41**	0.28**	-0.12*	**1.00**	0.17*	0.52**	0.14*	-0.8**	0.59**
RB	0.15*	0.09	0.04	0.30**	-0.04	0.14*	0.21**	0.16*	0.19**	-0.12*	0.12**	**1.00**	-0.3**	0.15*	-0.14*	0.12*
GY	0.18*	0.24**	-0.07	-0.3**	-0.11	-0.17*	0.24**	0.09	0.08	0.15*	0.33**	-0.2**	**1.00**	0.76**	-0.81*	0.31**
WUE	0.18**	0.07	0.44**	0.6**	-0.3**	0.33**	0.33**	0.35**	0.29**	0.27**	0.34**	0.31**	0.7**	**1.00**	-0.78*	0.78**
RGR	-0.07	-0.05	0.11*	0.32**	0.11*	0.05	-0.11*	-0.04	-0.11*	-0.04	-0.2**	-0.03	-0.7**	-0.6**	**1.00**	-0.20**
ELWL	0.18**	0.17**	-0.08*	-0.08	0.17*	0.15*	0.18*	0.03	0.06	-0.28**	0.33**	0.06	0.21**	0.66**	-0.18*	**1.00**

Total root length=TRL, root number=RN, root diameter=RtD, first culm strength=FCS, first culm diameter=FCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, relative grain yield=RGY, panicle length=PnL, grain yield=GY, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE, relative growth rate=RGR

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 10. Genotypic (upper triangle) and phenotypic (lower triangle) correlation coefficients among drought-resistance related traits and agronomic traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 under stressed conditions, 2007.

Traits	DTH	DTM	RN	TRL	RtD	FCD	FCS	PnL	PLH	NT	SB	RB	GY	WUE	RGR	ELWL	DSI
DTH	**1.00**	0.53**	0.16*	-0.17**	0.33**	1.9 **	0.99**	0.6**	0.52**	-1.2**	0.4**	0.27**	-0.4**	0.08*	-0.11*	0.35**	0.2**
DTM	0.41**	**1.00**	0.24**	-0.01	0.31**	0.69 *	0.4**	0.5 **	0.6 **	1.5 **	0.3**	-0.09*	-0.31*	0.12*	-0.06	0.20*	0.12*
RN	0.07	0.06	**1.00**	-0.02	-0.8**	3.12**	-0.03	0.12 *	0.1	0.01	0.6**	0.11*	0.3**	0.46**	0.14**	-0.12*	-0.13*
TRL	-0.12*	-0.01	-0.01	**1.00**	-0.16*	0.96 *	0.22**	0.24**	0.07	-0.5**	0.4**	0.4 **	0.58**	0.63**	0.49**	-0.3**	-0.3**
RtD	0.03	-0.01	0.06	-0.02	**1.00**	-4.9**	-0.5**	-0.10*	0.20*	-4.9**	0.9**	1.60*	-0.5**	-0.3**	-0.9 **	0.19**	0.15*
FCD	0.21**	0.06	0.24**	0.08	0.21*	**1.00**	3.89**	2.7 **	2.95**	-1.3**	2.5**	4.08**	0.30**	0.3**	0.30**	0.29**	0.12*
FCS	0.49**	0.2**	0.06	0.06	0.1	0.06*	**1.00**	0.58**	0.49**	-1.1**	0.5**	0.51**	0.3**	0.32**	0.16*	0.17*	0.23**
PnL	0.47**	0.26 **	0.13*	0.16**	0.12*	0.20 *	0.45**	**1.00**	1.0	-2.1**	0.5**	0.44**	-0.2**	0.4**	-0.17*	0.04	0.10*
PLH	0.34**	0.27**	0.14*	-0.01	0.03	0.05	0.37**	0.73**	**1.00**	-0.8**	0.4**	0.35**	-0.3**	0.31**	-0.27**	0.003	0.10*
NT	-0.2**	0.04	0.18**	-0.04	-0.08	-0.2**	-0.11*	-0.09*	0.01	**1.00**	1.2**	-1.75*	0.34**	0.3**	1.82**	-0.27**	-0.01
SB	0.17*	0.1	0.44**	0.17**	-0.06	0.15**	0.36**	0.36**	0.4**	0.3 **	**1.00**	0.46**	0.47**	0.31**	0.44**	-0.02	0.23**
RB	0.18*	-0.03	0.16*	0.27**	0.07	0.22**	0.29**	0.27**	0.25**	-0.08*	0.4**	**1.00**	0.31**	0.21**	0.11*	0.13*	0.08*
GY	-0.3**	-0.30**	0.25**	0.38**	-0.09	-0.07	0.09*	-0.02	0.03	0.17**	0.5**	0.21*	**1.00**	0.87**	0.68**	-0.4**	-0.25**
WUE	0.04	0.07	0.5**	0.6**	-0.3*	0.4**	0.31**	0.4**	0.3*	0.3**	0.3**	0.19*	0.83**	**1.00**	0.59**	-0.71**	-0.4**
RGR	-0.10	-0.06	0.1	0.45**	-0.3**	0.02	0.07 *	-0.13*	-0.18*	0.19*	0.3**	0.09	0.55**	0.57**	**1.00**	-0.26	-0.3**
ELWL	0.31**	0.13**	-0.08*	-0.2 **	0.08*	0.03	0.14*	0.05	-0.01	-0.02	0.01	0.08	-0.4**	-0.7**	-0.25**	**1.00**	0.6**
DSI	0.2*	0.21**	-0.1	-0.3**	0.1*	0.1	0.2**	0.06	0.01	-0.04	0.3**	0.06	-0.2**	-0.3**	-0.3**	0.59**	**1.00**

Total root length=TRL, root number=RN, root diameter=RtD, first culm strength=FCS, first culm diameter=FCD, shoot weight=SB, root weight=RB, plant height=PLH, tiller number=NT, days to heading=DTH, days to maturity=DTM, relative grain yield=RGY, panicle length=PnL, grain yield=GY, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE, relative growth rate=RGR

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

5.1.6 Response of recombinant inbred lines (RILs) to water stress

5.1.6.1 Phenological development and plant growth rate (RGR)

In the present study, moisture stress slowed down the time to anthesis in Key Murri, RIL-252, RIL-18 and RIL-222 (Figure 1A) but not for *E. pilosa*, DZ-Cr-37 and in most other RILs which accelerated the time to anthesis. Moisture stress hastened maturity in all the experimental materials at different degree. Nine RILs headed before the standard check (DZ-Cr-37). The number of days taken to head ranged from 33.7 (*E. pilosa*) to 53.7(Key Murri) under non-stressed and 28.0 (*E. pilosa*) to 56.7 (Key Murri) under stressed conditions (Table 11). Significant differences were observed among genotypes for grain filling periods. Under non-stressed conditions, 49 to 65.3 days were taken for grain filling periods but 36.7 to 56 days for stressed. Considerable variability was observed among lines for RGR which was ranged from 0.009 to 0.033 for non-stressed and 0.011 to 0.034 for stressed.

5.1.6.2 Root growth

The performance of root growth of the RILs and parental lines is indicated in figure 1 (B) for both experimental conditions. The over all mean of root length, number of root and root biomass had shown a significant difference ($P<0.05$) between stressed and non-stressed conditions (Table 12). The result indicated that there were considerable variations of RILs for root growth in response to stress. Drought resistant RILs and the standard check produced long roots under stressed than non-stressed condition. Root growth retardation was highest in RIL-364, RIL-275, RIL-290, RIL-36 and Key Murri.

5.1.6.3 Excised leaf water loss (ELWL) and water use efficiency (WUE)

Mean values of ELWL and WUE for RILs are presented in Table 12. All RILs showed high ELWL value when grown under non-stressed experimental conditions than under stressed except RIL-29, RIL-222, RIL-66, RIL-364 and RIL275. Considerable variations

of ELWL were observed among RILs under stressed (0.7 to 2.3) and non-stressed condition (1.5 to 2.32). The values of WUE for most RILs were higher in non-stressed condition (0.324 to 0.731) than stressed conditions (0.295 to 0.74) except RIL-16 and RIL-66.

5.1.6.4 Drought susceptible index (DSI)

DSI was calculated for each genotype (Table 12). Wide DSI values were observed among lines ranged from 0.27 to 3.25. Five RILs had DSI value of less than 0.5, 12 RILs between 0.5 to 1 and eight RILs grater than 1.

5.1.6.5 Relative yield performances

The data from relative yield performance under stressed condition of the genotypes showed that most of RILs gave relatively similar grain and biomass yield under both experimental conditions (Table 13) except, Key Murri, RIL-29, RIL-275, RIL-197 and RIL-233 which had incurred huge percentage of grain yield loss when grown under stress. Under stressed, DZ-Cr-37, *E. pilosa*, RIL-183, RIL-337, RIL-37 and RIL-290 attained comparable yield with non-stressed condition. RIL-16 gave highest yield under stressed condition than non-stressed i.e. 52.7% yield advantage. In general, the relative yield ranged from -52.7% to 92.3% grain yield and -25%.6 to 49.7% for biomass yield.

Table 11. Summary of phenological development of *E, tef, E. pilosa*, their RILs and DZ-Cr-37, 2007.

		Non-stressed			Stressed			HDD	MDD	GFP	
No	**Lines**	**DTH**	**DTM**	**RGR**	**DTH**	**DTM**	**RGR**			**Non-stressed**	**Stressed**
1	***E. pilosa***	33.7 ^{I}	88.0 ^{K}	0.014 ^{EF}	28.0I	82.0I	0.014 ^{O}	5.7	6	54.3	54
2	**Key Murri**	53.7 ^{A}	104.0 ^{A}	0.011^{H}	56.7 ^{A}	93.3^{ABC}	0.017^{KL}	-3	10.7	50.3	36.7
3	**DZ-Cr-37**	42.3 ^{BCDE}	96.3 ^{FGH}	0.012^{GH}	41.0^{CDEF}	90.0^{BCDE}	0.021^{GH}	1.3	6.3	54	49
4	**RIL-183**	42.7 ^{BCDE}	94.3 ^{HI}	0.033 ^{A}	40.3^{CDEF}	86.3^{EFGH}	0.018^{JK}	2.4	8	51.7	46
5	**RIL-62**	42.0 ^{BCDE}	97.3 ^{DEFG}	0.013 ^{FG}	41.3^{CDE}	87.7^{EFG}	0.027 ^{C}	0.7	9.6	55.3	46.3
6	**RIL-252**	47.67 ^{B}	100.3 ^{BC}	0.024 ^{B}	49.0^{B}	95.0^{A}	0.014^{NO}	-1.33	5.3	52.7	46
7	**RIL-29**	42.0^{BCDE}	97.7 ^{DEF}	0.020 ^{C}	41.0^{CDEF}	87.0^{EFGH}	0.015 ^{MN}	1	10.7	55.7	46
8	**RIL-118**	37.0^{EFGHI}	98.7 ^{CDE}	0.024 ^{B}	38.3^{EFGH}	87.0^{EFGH}	0.014 ^{NO}	-1.3	11.7	61.7	48.7
9	**RIL-172**	39.0^{EFGHI}	97.3 ^{DEFG}	0.020 ^{C}	39.3^{DEFG}	87.3^{EFG}	0.016 ^{LM}	-0.3	10	58.3	48
10	**RIL-222**	41.7 ^{CDE}	95.3 ^{GH}	0.019 ^{C}	49.3B	87.7^{EFG}	0.015^{MN}	-7.6	7.6	53.7	38.3
11	**RIL-317**	34.7 ^{HI}	97.0 ^{DEFG}	0.015^{DE}	37.7^{GH}	88.0^{DEFG}	0.024^{DE}	-3	9	62.3	50.3
12	**RIL-337**	41.0 ^{DEFG}	96.7 ^{EFG}	0.012^{GH}	38.7^{EFGH}	89.7^{CDE}	0.023 ^{EF}	2.3	7	55.7	51
13	**RIL-173**	35.7 ^{FGHI}	97.7 ^{DEF}	0.013^{FG}	35.7^{HI}	87.0^{EFGH}	0.020^{HI}	0	10.7	62	51.3
14	**RIL-66**	37.0^{EFGHI}	102.3 ^{AB}	0.025 ^{B}	40.3^{CDEF}	94.3^{A}	0.022^{FG}	-3.3	8	65.3	54
15	**RIL-16**	46.3 ^{BC}	103.0 ^{A}	0.012^{GH}	43.3^{C}	93.7^{AB}	0.031^{B}	3	9.3	56.7	50.3
16	**RIL-37**	42.7 ^{BCDE}	100.3 ^{BC}	0.014^{EF}	39.3^{DEFG}	89.0^{DEF}	0.020^{HI}	3.4	11.3	57.7	49.7
17	**RIL-226**	40.7^{CDEFG}	92.33 ^{IJ}	0.012^{GH}	41.0^{DEF}	84.3^{GHI}	0.025^{D}	-0.3	8.03	51.7	43.3
18	**RIL-9**	41.0^{CDEFG}	97.0 ^{DEFG}	0.0127^{FG}	38.3^{EFGH}	87.0^{FGH}	0.018 ^{JK}	2.7	10	56	48.7
19	**RIL-233**	40.3^{DEFGH}	96.00 ^{FGH}	0.009 ^{I}	37.7^{FGH}	88.0^{EFG}	0.024 ^{DE}	2.6	8	55.7	50.3
20	**RIL-139**	37.3 ^{EFGHI}	91.67 ^{J}	0.016 ^{D}	36.0^{GHI}	83.3^{HI}	0.022 ^{FG}	1.3	8.37	54.3	47.3
21	**RIL-197**	44.3 ^{BCD}	102.3 ^{AB}	0.009^{I}	41.7^{CDE}	91.7^{ABCD}	0.024^{DE}	2.6	10.6	58	50
22	**RIL-124**	37.$^{7\ EFGHI}$	94.33 ^{HI}	0.020^{C}	37.7^{FGH}	85.7^{FGHI}	0.019 ^{IJ}	0	8.63	56.7	48
23	**RIL-364**	41.7^{CDE}	97.7 ^{DEF}	0.016^{D}	42.0^{CD}	86.7^{EFGH}	0.017^{KL}	-0.3	11	56	44.7
24	**RIL-275**	37.3 ^{EFGHI}	97.67 ^{DEF}	0.013^{FG}	35.7^{HI}	91.7^{ABCD}	0.011 $^{P\ N}$	1.6	5.97	60.3	56
25	**RIL-12**	41.7 ^{CDE}	100.0 ^{C}	0.013 ^{FG}	40.3^{CDEF}	86.3^{EFGH}	0.021^{GH}	1.4	13.7	58.3	46
26	**RIL-313**	35.3^{GHI}	99.00 ^{CD}	0.012 ^{GH}	35.7^{HI}	89.7^{CDE}	0.027^{C}	-0.4	9.3	63.7	54
27	**RIL-290**	44.0^{BCD}	93.00 ^{IJ}	0.015 ^{DE}	40.3^{CDEF}	82.3^{I}	0.034^{A}	3.7	10.7	49	42
28	**RIL-36**	41.3 ^{CDEF}	97.0 ^{DEFG}	0.015 ^{DE}	41.3^{CDE}	87.7^{EFG}	0.020^{HI}	0	9.3	55.7	46.3
	Mean	40.8	97.3	0.24	40.3	88.4	0.02				
	LSD(0.05)	5.924	2.05	0.0016	3.477	3.78	0.0017				
	EMS	13.1	1.568	0.001	4.512	5.343	0.001				

-DTH= days to heading, DTM= day to mature, RGR= relative growth rate, GFP= Grain filling periods, HDD=Heading day differences between non-stressed and stressed treatments, MDD= Maturity day differences between non-stressed and stressed treatments

-Means followed by a common letter within a column were not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests.

Table 12. Mean value for drought-resistance related traits of *E. tef, E. pilosa,* their RILs and DZ-Cr-37, 2007.

		ELWL		WUE		DSI	Classification based on DSI
No	Variety	Non-stressed	Stressed	Non-stressed	Stressed	Stressed	
1	***E. pilosa***	1.82^{HIJK}	1.033^{MN}	0.483^{LM}	0.409^{EFGHI}	0.34^{KLM}	√√
2	**Key Murri**	2.22^{BC}	2.21^{AB}	0.731^{A}	0.36^{HIJKL}	2.11^{B}	√
3	**DZ-Cr-37**	1.85^{HIJ}	1.06^{LMN}	0.620^{E}	0.534^{B}	0.49^{HIJKL}	√√
4	**RIL-183**	2.13^{DEF}	2.30^{A}	0.345^{W}	0.326^{JKL}	0.51^{HIJK}	√√
5	**RIL-62**	2.32^{AB}	1.246^{JKL}	0.589^{F}	0.532^{BC}	1.03^{E}	√
6	**RIL-252**	1.5^{NO}	2.073^{BC}	0.324^{X}	0.310^{KL}	0.92^{EF}	√
7	**RIL-29**	2.08^{CDEF}	2.171^{AB}	0.648^{C}	0.343^{IJKL}	3.25^{A}	√
8	**RIL-118**	2.11^{CDE}	1.348^{IJK}	0.491^{J}	0.481^{BCDE}	0.50^{HIJKL}	√√
9	**RIL-172**	1.93^{GHI}	0.9987^{N}	0.485^{K}	0.295^{L}	0.54^{HIJK}	√√
10	**RIL-222**	1.54^{MN}	1.882^{DEF}	0.388^{V}	0.42^{DEFGH}	2.22^{B}	√
11	**RIL-317**	2.15^{CD}	1.22^{KLM}	0.402^{U}	0.43^{DEFGH}	0.39^{JKLM}	√√
12	**RIL-337**	2.22^{BC}	1.06^{LMN}	0.423^{S}	0.429^{DEFGH}	0.31^{LM}	√√
13	**RIL-173**	1.67^{KLM}	1.43IJ	0.413^{T}	0.42^{DEFGH}	0.54^{HIJ}	√√
14	**RIL-66**	1.37^{O}	1.93^{CDE}	0.345^{W}	0.38^{HIJK}	0.67^{GH}	√√
15	**RIL-16**	1.95^{EFGH}	0.70^{O}	0.590^{F}	0.74^{A}	0.51^{HIJK}	√√
16	**RIL-37**	1.88^{GHI}	1.22^{KL}	0.510^{H}	0.491^{BCD}	0.27^{M}	√√
17	**RIL-226**	2.11^{CDE}	1.2^{KLM}	0.665^{B}	0.54^{B}	0.81^{FG}	√√
18	**RIL-9**	1.69^{JKLM}	1.09^{LMN}	0.445^{P}	0.45^{DEFG}	0.59^{HI}	√√
19	**RIL-233**	2.4^{A}	1.15^{LMN}	0.629^{D}	$0.49B^{CD}$	1.36^{D}	√
20	**RIL-139**	1.95^{FGH}	1.65^{GH}	0.439^{Q}	0.37^{HIJK}	1.45^{D}	√
21	**RIL-197**	1.78^{IJKL}	1.41^{IJ}	0.497^{I}	0.39^{FGHIJ}	1.27^{D}	√
22	**RIL-124**	1.62^{LMN}	1.47^{HI}	0.427^{R}	0.38^{GHIJK}	0.36^{JKLM}	√√
23	**RIL-364**	1.55^{MNO}	1.73^{FG}	0.453^{O}	0.46^{CDEF}	0.80^{FG}	√√
24	**RIL-275**	$1.79I^{JKL}$	2.02^{BCD}	0.454^{O}	0.36^{HIJKL}	1.88^{C}	√
25	**RIL-12**	2.03^{DEFG}	1.17^{KLMN}	0.482^{M}	0.485^{BCD}	0.82^{FG}	√√
26	**RIL-313**	2.05^{DEF}	1.75^{EFG}	0.484^{KL}	0.35^{IJKL}	1.82^{C}	√
27	**RIL-290**	1.96^{EFGH}	1.11^{LMN}	0.514^{G}	0.52^{BC}	0.42^{JKLM}	√√
28	**RIL-36**	1.65^{KLMN}	1.47^{HI}	0.477^{N}	0.43^{DEFGH}	0.66^{GH}	√√
	Mean	1.9	1.47	0.49	0.43	0.959	
	LSD (5%)	0.164	0.187	0.0016	0.073	0.194	

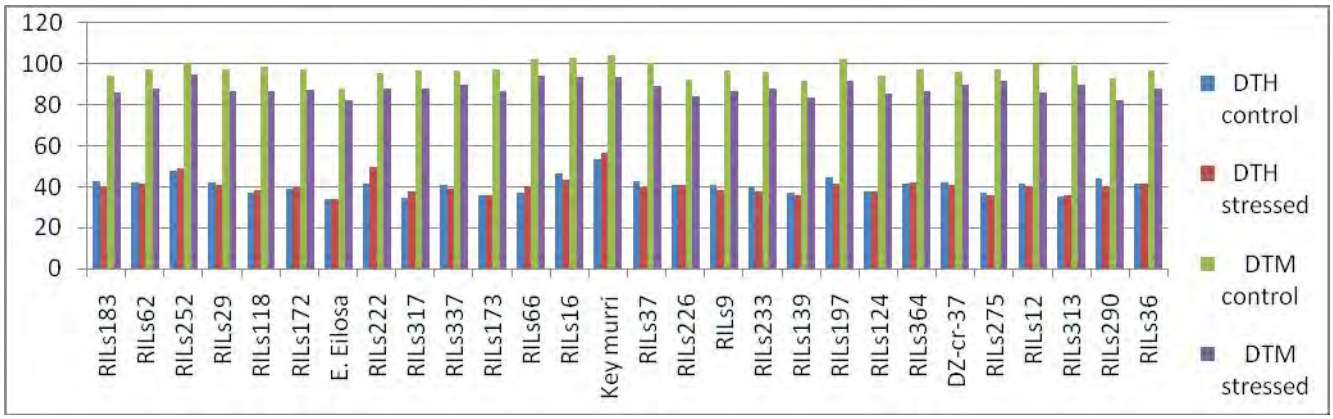
-Means followed by a common letter within a column were not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Tests.

-Total root length=TRL, drought susceptibility index=DSI, excised leaf water loss=ELWL, water use efficiency=WUE,

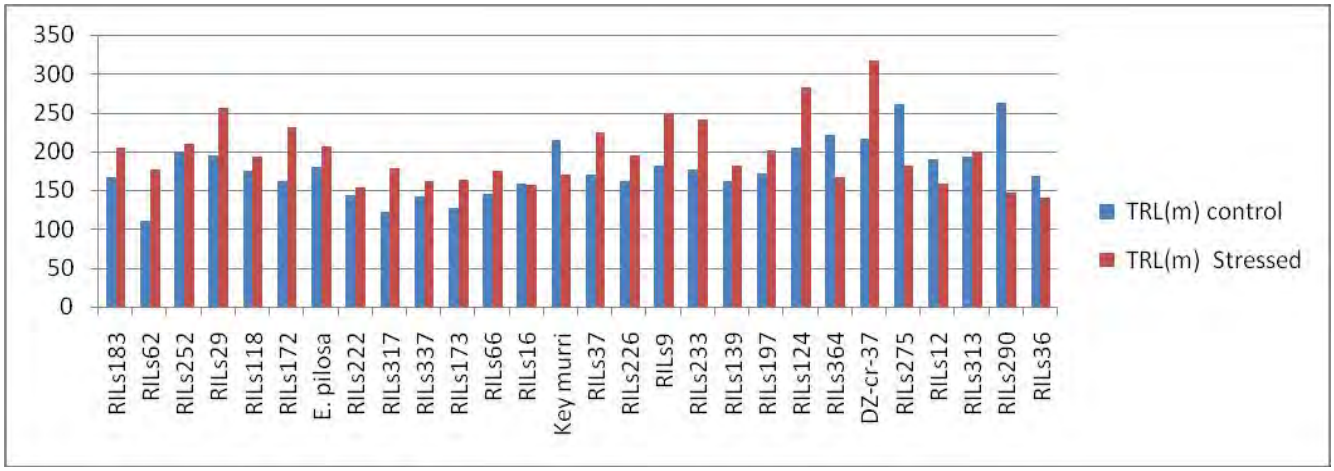
- √= drought susceptible and √√=drought resistant based on Clarke *et al*. (1982)

Table 13. Relative mean grain and biomass yield reduction of *E. tef, E. pilosa,* their RILs and DZ-Cr-37 due to water stress, 2007.

No	Varieties	Grain yield		Relative loss (%)	Total Biomass		Relative loss (%)
		Non-stressed	Stressed		Non-stressed	Stressed	
1	***E. pilosa***	4.3	4.1	4.0	38.7	35.3	8.6
2	**Key Murri**	6.6	2.6	61.4	58.5	29.4	49.7
3	**DZ-Cr-37**	5.8	5.5	5	49.6	44.1	11.2
4	**RIL-183**	2.7	2.5	8.3	29.1	22.6	22.4
5	**RIL-62**	7.6	5.4	28. 9	47.4	37.6	20.8
6	**RIL-252**	2.5	1.9	20. 2	37.2	31.2	16.2
7	**RIL-29**	4.3	0.3	92.3	38.2	26.3	31.2
8	**RIL-118**	5.1	4.3	16. 5	37.5	28.5	24.2
9	**RIL-172**	4.6	3.9	15.7	33.3	24.3	27.3
10	**RIL-222**	3.7	1.4	63.5	40.9	33.4	18.3
11	**RIL-317**	4.9	4.3	11.6	33.2	30.7	7.6
12	**RIL-337**	5.1	4.7	8.6	34.9	31.3	10.2
13	**RIL-173**	4.6	3.9	15.7	36.1	32.4	10.3
14	**RIL-66**	3.7	2.9	20.3	28.2	30.6	-8.5
15	**RIL-16**	5.2	7.9	-52. 7	47.4	59.5	-25.6
16	**RIL-37**	5.1	4.7	7.8	40.8	31.5	22.8
17	**RIL-226**	7.1	5.4	24.2	53.2	35.5	33.4
18	**RIL-9**	4.5	3.8	15.1	34.6	32.5	6.1
19	**RIL-233**	8.0	4.1	48.4	50.3	30.8	38.9
20	**RIL-139**	6.4	3.7	42.2	29.8	23.2	22.0
21	**RIL-197**	5.6	3.5	37. 7	36.6	31.0	15.4
22	**RIL-124**	3.9	3.5	10.4	34.5	30.1	12.8
23	**RIL-364**	4.8	3.7	22.9	40.4	32.6	19.4
24	**RIL-275**	5.9	2.8	53.0	36.3	28.7	21.1
25	**RIL-12**	5.4	4.1	25	41.8	32.8	21.6
26	**RIL-313**	5.6	2.6	53.8	36.3	27.6	23.9
27	**RIL-290**	5.7	5.8	-0.52	43.9	41.1	6.4
28	**RIL-36**	4.3	3.5	19.4	39.6	31.0	21.7
	Mean	5.1	3.8	22.4	39.6	32.3	17.5



(A)



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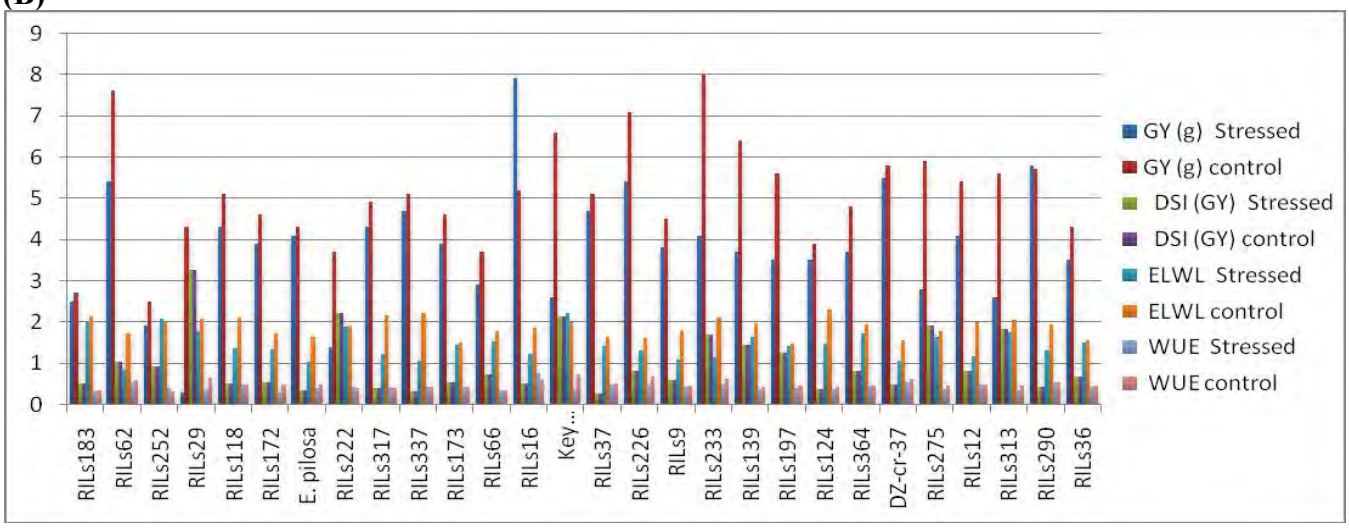

(C)

Figure 1. Performance of *E. tef, E. pilosa,* DZ-Cr-37 and their RILs for drought-resistance related traits grown under non-stressed and stressed conditions

5.2 Meiotic study

5.2.1 Meiotic analysis

In the present study, no meiotic abnormalities were detected during Metaphase I (Fig. 2B), Metaphase II (Fig. 2G & H), anaphase I (Fig. 2C), anaphase II (Fig. 2I), and telophase I (Fig. 2D), except two laggards in Telophase II observed in a single PMC (Fig. 2J). These indicated all the bivalents moved and aligned on the equator of the spindle at equal pace and also segregation of the bivalents in either pole was normal. Of total of 154 PMCs examined included 41 prophase I, 27 metaphase I, 26 metaphase II, 19 anaphase I, 8 anaphase II, 24 telophase I and 9 telophase II PMCs. The formations of tetrads were also found to be normal. A total of 160 PMCs at tetrad stage were examined and all produced normal tetrads with four microspores each (Fig. 2L). No micronuclei formation was detected during tetrad formations. Each PMC produced four equal sized microspores.

5.2.2 Pollen fertility test

Pollen fertility was examined for the parental lines, F_1-hybrid and some selected RILs. All showed high percentage of pollen fertility which ranged from 90 to 95.1%. Small and large sized pollen having circular or oval shapes were appeared in mixture in all RILs and parental lines (Table 14).

5.2.3 Morphology of the F_1-hybrid

In the present study, comparisons were done between F_1-hybrid and its parental lines (Figure 3). The F_1-hybrid had very loose panicle and brown seed color like that of *E. pilosa* but purple lemma color. The F_1-hybrid plant looked similar stand establishment with Key Murri except a little bit vigor of the former. Plant height (98cm) and number of tiller (15) were observed which is higher in F_1-hybrid plant than its parental lines (*E. pilosa*: 56cm, 7; Key Murri: 87cm, 6). The F_1-hybrid gave better yield (1.2g/pl)

accompanied by excellent stand establishment than the parental (*E. pilosa*; 0.46g/pl; key murri:.0.61g/pl).

Table 14. Summary table for pollen fertility percentage for *E. tef, E. pilosa*, F_1-hybrid and some selected RILs, 2008.

Variety	NLCP		NSCP		NLOP		NSOP		Fertility (%)
	Normal	Aborted	Normal	Aborted	Normal	Aborted	Normal	Aborted	
Key Murri	112	4	53	4	77	3	47	4	95.1
E. pilosa	99	5	42	3	76	3	42	5	94.2
F_1-Hybrid	100	3	29	3	54	3	16	3	94.3
RIL317	89	7	21	2	37	4	23	5	90.4
RIL183	99	5	34	7	78	8	19	3	90.9
RIL118	105	6	31	3	42	3	21	4	92.6
RIL222	134	3	33	7	37	8	25	6	90.5
RIL317	74	3	23	6	27	5	29	3	90
RIL172	83	2	22	9	57	5	55	5	91.2
RIL16	65	4	35	5	46	7	39	3	90.7
RIL252	77	8	32	5	56	4	67	7	90.6
RIL275	97	6	25	3	52	7	37	4	91.3
RIL62	87	7	26	3	61	5	61	8	91.1
RIL290	79	6	39	3	30	4	35	2	92.4
RIL36	69	4	37	2	38	3	49	4	93.7

NLCP=№ of large circular pollen, NSCP=№ of small circular pollen, NLOP=No of large oval pollen, NSOP=No of small oval pollen

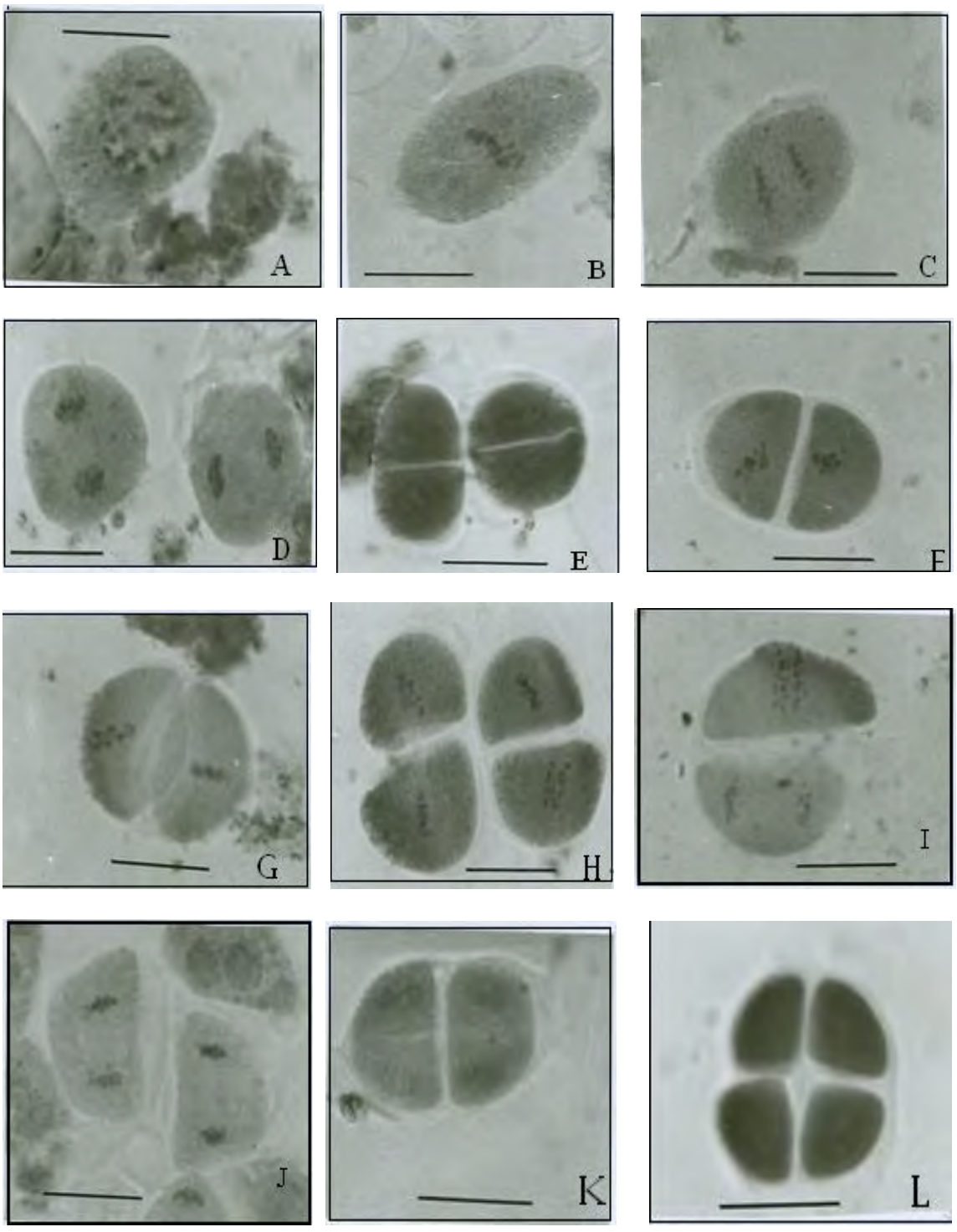

Figure 2. Meiosis in F_1-hybrid of *E. tef* and *E. pilosa*. A) Diakinesis, B) Metaphase I showing normal configuration, C) Anaphase I, D) Telophase I aggregated at the pole, E) Cytokinesis, F) Prophase I, G & H) Metaphae II showing normal configuration, I) Anaphase II, J) Telophase II with two chromosomes lagging behind, K) Anaphase II, L) tetrad formation. One bar represents 20 µm for all phases.

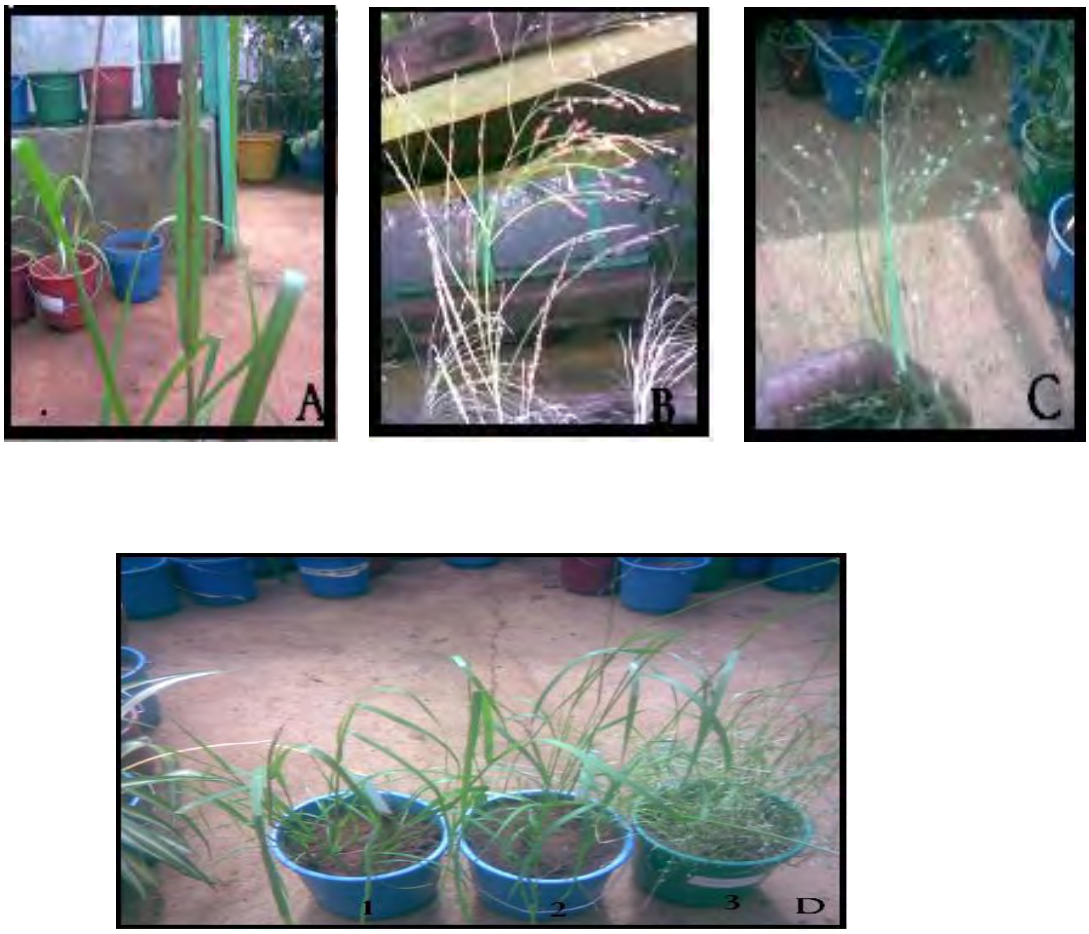

Figure 3. Panicle form and Stand establishment of Key Murri, F_1-hybrid and *E. pilosa.* (A, D-1= Key Murri, B, D-2=F_1-hybrid and C, D-3= *E. pilosa*)

6. Discussion

Results from ANOVA and mean separation test ($p<0.05$ %) revealed that the presence of significant differences among genotypes except TSW and PdL under non-stressed and PnSW under stressed conditions. This result has shown the existence of sufficient variability of agronomic and drought-resistance related traits among RILs.

The mean and range values of the RILs indicated that there are RILs performing better than the parental lines for some traits. For grain yield, there are RILs performing better not only than either of the parental lines but also the standard check (DZ-Cr-37). There are also RILs that performed lower than the poor parent. This phenomenon is termed as transgressive variation (Allard, 1960). This is due to the recombination of desirable alleles in the better RILs and non-desirable alleles in the poor RILs from *E. pilosa* and Key Murri. Transgression of traits observed in some RILs can give an opportunity for breeders to develop superior varieties from crosses of Key Murri with *E. pilosa* for water stressed areas. RIL-37, RIL-337, RIL-290 and RIL-16 are found to be superior under water stressed conditions, while RIL-139 and RIL-233 for non-stressed conditions. RIL-62 and RIL-226 perform well at both experimental conditions. Transgressive lines were also reported for intra- and inter-specific crosses in tef (Hailu Tefera, 2003a;b). RIL-16 has given a surprising grain (52.7%) and biomass (25.6%) yield advantage under stressed condition. The moisture level of non-stressed conditions may be beyond the optimum requirement of RIL-16.

The variability in a population arises as a result of the differences in the genetic make up of individual in the population, the environment and the interaction between genes with the environments. These three sources of variation can be measured and expressed in terms of variance. The variation in the population that is due to the genotypic composition of individuals is called genotypic variation (Allard, 1960; Mather and Jinks, 1971, Agrawal, 1998). In the present study, results from the estimation of GCV and PCV of most drought resistance-related and agronomic traits have shown the possibility of creating new genetic pool in tef germplasm through interspecific crosses. The genetic

variation observed in RILs is explained by the segregation, crossing over and recombination of alleles of the two parental lines in their progeny lines (Allard, 1960). There is no wide variation between GCV and PCV for most traits which indicates that the variation observed in the lines have strong genetic bases. The variations observed among RILs were wide for most traits as compared to created in intra-specific crosses of tef (Hailu Tefera *et al.,* 2003b). The result agreed with the report from Hailu Tefera *et al.* (2003a) conducted under field conditions.

Heritability provides information about correspondence between the genotypic and phenotypic variance. Its estimation is an important aspect of inheritance of quantitative traits as it indicates the genetic gains that may be achieved through selection (Pondery and Tiwari, 1983). The heritability estimates of characters for all genotypes are very high for both experimental conditions, although the estimated values under stressed condition are lower than under non-stressed conditions. High H alone is not enough to make sufficient improvement through selection in advanced generations. It must be accompanied by substantial amount of GA. So, GA should be considered along with H in coherent breeding program (Johanson *et al*., 1995; Bhargave *et al*., 2003). High percentage of H and GA (% mean) are required for genetic advance through selection. These were observed in most traits of RILs except DTM and SB. Generally, the data indicates that it is possible to improve the level of drought-resistance of tef through wide hybridization followed by selection. High degree of heritability and expected genetic advance of drought related traits were demonstrated in wheat also (Bhutta *et al*., 2006; Ahmid *et al*., 2007).

The result from correlation coefficient attested that most traits were correlated strongly with each other. This may result from pleiotropic effects of genes or linkage of genes governing inheritance of two or more characters (Dabholkor, 1992). A strong positive correlation between two traits means that increasing one trait would be accompanied by an increasing in the other trait also. But, if the correlation is negative, increasing one trait would result in the reduction of the other. Simultaneous selection to improve two negatively correlated traits together is impossible. Similar results were reported for most

traits by different authors (Mulu Ayele *et al*., 1993; Tesfay Teferra *et al*. 2000a,b; Abuhay Takele, 2001; Mulu Ayele *et al*., 2001; Hailu Tefera *et al*., 2003b; Yifru Teklu and Hailu Tefera, 2005) for phenotypic correlation. Strong genotypic correlations were reported for other cereals, for example Ezeaku and Mohammed (2006) for sorghum and Izge *et al*. (2006) for pearl millet hybrids.

The effect of drought varies depending on the intensity of drought stress, genotypes and stages of growth at which the stress commences. In tef, the effect is more sever when the stress occurs during the vegetative growth stages (early stress) than at grain feeling periods (terminal stress) (Tesfaye Teferra *et al*., 2000b). Morphological and phenological traits such as plant type, root systems and early flowering play a major role in adaptation of plants to specific drought conditions (Blum, 2005). The variation observed among the RILs for these traits in response to drought can give an opportunity for plant breeder to develop drought resistant RILs.

The data of RGR revealed that this trait is also essential to screen for the level of drought resistance among RILs. Most RILs hastened the growth rate during the stress period, while the susceptible one delayed their growth. Rapid development of a deep-root system is considered a drought avoidance strategy for plants as it enables absorption of water in deep soil layers (Kamoshita *et al*., 2002; Serraj, 2004). The drought resistant lines have produced higher TRL and RB in the stressed than in non-stressed condition. Drought resistant lines have hastened root development, while Key Murri and susceptible RILs have shown a retarded root development under stressed conditions. Similar results were reported for tef homozygous lines from Abuhay Takele (2001); Tesfaye Teferra *et al*. (2000a;b); Mulu Ayele *et al*., (2001).

The activity of water in plants can be regulated genetically. Those resistant RILs lose less water under water stressed condition and maintain optimum moisture level inside the cell. The mechanism is through stomatal regulation (Blum, 2005). Considerable variations of ELWL were seen among RILs under stressed and non-stressed condition. Lines showing lower ELWL give better yield than that has less value grown under water

stress. The traits has shown good indices for measuring the level of drought resistance among tef homozygous line (Mulu Ayele, 1993; Abuhay Takele, 2001; Mulu Ayele *et al*., 2001; Tesfaye Teferra, *et al*., 2001b).

The levels of drought resistance among genotypes were evaluated using DSI which can only be estimated by comparing the performance of lines under stress and non-stress conditions. Resistant lines gave low DSI index while the susceptible ones had high index value. According to Clarke *et al*. (1987), RILs can be classified into susceptible or resistant using DSI. If the index is less than unity, it is considered as medium to highly resistant. But if it is greater, it is highly susceptible. So, 17 of the RILs are resistant and eight are drought susceptible (Table 12).

The use of many wild species is limited by their poor crossability and high degree of F_1 sterility in interspecific hybrids. These limitations were not seen in the parental materials. It is possible to make crosses and produce viable seed of the two parental species. This will give chance to introgress genes from *E. pilosa* to tef (Hailu Tefera *et al*., 2003a). Confirmation of the exact F_1-hybrid and examining the meiotic stability of PMCs of the F_1-hybrid are required critically in conventional sexual hybridization. Morphological markers like panicle form, lemma and seed color are employed to identify F_1-hybrid in tef (Hailu Tefera *et al*., 2003a).

Grain yield is not only affected by biotic and abiotic factors but also the inherent characteristics of the species like, meiotic abnormality. Studies in different plant species have revealed the role of meiotic irregularities for declining seed setting and production capabilities (Kifle Dagne, 1994; Majumdar *et al*., 2004; Adamowski, 2008). Pollen fertility is the most important criterion for genetic superiority of a variety, because the ability of a variety to reproduce and to survive depends on it. Pollen fertility directly or indirectly is dependent upon the efficiency of the meiotic process. Meiotic stability is manifested in pollen fertility insuring high fertility of the F1-hybrid (Majumdar *et al*., 2004; Adamowski, 2008).

Meiosis was studied for pollen mother cells (PMCs) collected from florets of the main tiller of F_1-hybrid. Normally, meiotic division proceeds from pairing of chromosome, to tetrad formation in a coordinated and sequential manner. These different stages of meiosis were examined in the F_1-hybrid except chromosome pairing and chiasmata formation. Chromosome pairing and chiasmata formation in tef is not clearly detectable due to the very minute size of tef chromosome (i.e., 0.8 µm-2.9 µm; Mulu Ayele *et al*., 1996).

The F_1-hybrid has shown regular meiotic cell division and high level of pollen fertility which confirmed the chromosome homology between the genomes of the two species. High levels of pollen fertility and seed setting in the F1-hybrid and RILs were detected, which are additional evidences for meiotic stability in the hybrid exist. The meiotic stability observed in F_1-hybrid can be extrapolated to the RILs as well so that any difference in the yielding capacity among RILs would not be attributed to meiotic instability among RILs.

The present findings about the meiotic regularity and seed fertility of the F_1-hybrid between *E. tef* and *E. pilosa* cross shows not only that gene transfer is possible from the latter to the former, but it also further strengthen the view that the two species are very closely related. According to Tavassoli (1986), meiosis is most frequent in tef at late-booting stage and proceeds through emergence. But, this was not the case in the present study. Meiosis was found to be most frequent when the panicle just emerged from the flag leaf. This might be due to environmental effect.

7. Conclusion and recommendation

In the present study the possibility of transferring drought resistance related traits and the level of variations among RILs derived from cross between *E. tef* and *E. pilosa* was assessed. Based on the result, wide genetic variability in response to water stress was observed among RILs. Highly drought resistant (even better than *E. pilosa* and standard check, DZ-Cr-37) to highly drought susceptible (even more susceptible than Key Murri) RILs were obtained from the interspecific cross. Meiotic analysis result also indicated that interspecific hybridization could be used in tef breeding programs, since tef and *E. pilosa* have shown crossbility and regular meiosis resulting in normal seed fertility of the F_1 and subsequent segregant generations. So, *E. pilosa* can have a role in tef genetic improvement to diversify tef germplasm for water stress through conventional crossing.

The above conclusions were made based on one year result conducted using pot. So, the following are recommended for further studies. The variability among RILs should be tested across locations and seasons with more number of RILs involved, to confirm the present study and give additional information about the stability and degree of variability of drought-resistance related traits and evaluate the best performing lines. Drought-resistance variability among RILs should also be assessed using molecular markers. To improve the capacity of tef to withstand water stress, indirect selection of drought-resistance related traits are valuable. Selection for low level of DSI and ELWL, high RGR, WUE and root production are important indices.

Identification of drought resistant wild relatives of tef and also studies like crossability with tef and behavior of the hybrids are recommended to diversify tef germplasm more. The mode and extent of inheritance of drought related traits in tef should also be investigated like that of most cereals. Identification of molecular marker linked genes associated with drought-resistance related traits is required to use marker assisted selection (MAS) in tef breeding to supplement conventional breeding.

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DECLARATION

I, the undersigned, declared that this thesis is my original work, has not been presented for

a degree in any University and that all sources of materials used for the thesis have been duly acknowledged.

Name: Sintayehu Admas

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