Analysis of differentially expressed genes induced by drought stress in tef (Eragrostis tef) root

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Abstract

Drought stress is one of the major abiotic stresses which induces root growth in tef. Molecular mechanisms underlying the elongation of roots under drought stress are not known. Therefore, we aimed to study the tef root system to uncover the expression profiles for drought stress using Agilent gene chip of rice. One hundred seventy-five expressed genes were found to be differentially expressed after eight days of drought stress with Eragrostis tef-resistant genotype, Kaye Murri. The drought-responsive genes were isolated and classified into nine categories according to the functional roles in plant metabolic pathways, such as defense, signal transduction, cell wall fortification, oxidative stress, photosynthesis, development, cell maintenance, RNA binding, and unknown functions. The profiles of tef root genes, responsive to drought stress shared common identities with other expression profiles known to be elicited by diverse stresses, including pathogenesis, abiotic stress, and wounding. Well-known drought-related transcription factor-like, WRKY and bHLH were up-regulated. Cell transport-related regulators such as potassium transporter 22-like, auxin transporter-like protein 1, and wall-associated receptor kinase were also involved in the expression profile of tef root under drought stress. Their expression had enhanced the drought-responsive genes, which have a direct role to maintain root growth under drought stress.

Key words: Drought, Genome, Microarray, Root, Tef

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Introduction

Drought stress has been reported as one of the serious threats to staple crops, including Eragrostis tef (Abraha et al., 2016). It causes tremendous economic losses in tef production to the amount of approximately $21.3 million annually in Ethiopia. Drought basically affects the growth of root, which limits nutrient and water absorption. Therefore, researches that help to understand the mechanism of root elongation under drought stress have got increased attentions in order to breed crop plants that cope up drought stresses.

Due to its allotetraploid chromosome structure, tef is one of the least plastic plant species in terms of adaptation. However, the plant is capable of growing at high and low altitudes in tropical and subtropical climates. Eragrostis tef plant has acquired a myriad of developmental and metabolic strategies to optimize water uptake. It also efficiently balances this with water utilization during vegetative growth and reproduction (Hailesillasie et al., 2016).

In the past, a few researches have been done to unravel the molecular processes of drought-induced regulations of tef root (Abraha et al.,
2016; Admas and Belay, 2011; Assefa et al., 2011; Belay et al., 2008, 2009). Studies on shoot physiologỹ have shown that sugars, sugar alcohols, amino acids, and amine function as osmolytes, protecting cellular functions from the effects of dehydration, and are known to accumulate under drought stress (Ayęle, 1999 and Degu et al. 2008). Reduction in vegetative growth, stomatal closure and a decrease in the rate of photosynthesis (Admas and Belay 2011; Degu et al. 2008) are among the earliest responses of tef to drought, protecting the plant from extensive water loss.

Researchers have also identified that there are extensive genetic diversities in the physiological and root length of tef. This genetic variability has been exploited to produce locally adapted drought-tolerant tef cultivars for the dry tropical areas of Ethiopia (Plaza et al., 2013). The morphology of tef primary root and elongation at low water potentials have been studied (Degu et al. 2008) and QTL’s affecting root length mapped (Degu and Fujimura, 2010). However, the expression patterns of root growth to water-deficit have not been sufficiently characterized.

Currently, plant science has entered a new era following the completion of the entire genomic sequence of Arabidopsis and rice (Oryzasativa). Researchers are using model plants to identify the specific functions of plant genes and their expression profiles. The genome, transcriptome, proteome and metabolome tools are used to analyze root system. The result of the transcriptome analysis vary based on the experimental setups, the different germplasm, and accessions used. Thus, there are different transcripts which describe the response of plant roots towards drought stress.

Focusing on tef, one transcriptome characterizations of tef plant in response to 1-week of drought showed 23 and 15 differentially expressed transcriptome (Cannarozzi et al., 2014). The study suggests changes in energy (B-glucanase and ERD), salt-sensitive enzymes (SAL1) and chloroplast regulation (stay-green gene -SGR). This implies that there is a need to study how the root is regulated, and to understand the different transcriptome changes which contributed to the growth and elongation of root length under drought stress.

Rice is a model cereal crop to study the stress response at a molecular level due to the availability of whole-genome information and other molecular tools. Tef is one of the most drought-tolerant cereals, providing a useful platform to understand tolerance mechanisms. Besides, the genome of monocots are characterized by high synteny, and it is feasible to use rice chips to do hybridization with tef RNAs. In the present work, genome-wide transcriptional characterization of tef roots in response to drought deficiency is presented.

Identifying drought-responsive genes in the root, and the understanding of their function can lead to a better breeding of crop plants under drought stress. We applied microarray platforms to identify candidate genes that are associated with a phenotype of drought resistance in tef. The work plan applied the existing rice microarray technology created by the National Agricultural Research Organization of Ethiopia, and tests the feasibility of the orthologous arrays for use in multiple crops. The proposed project will enhance knowledge towards the elucidation of gene function in seminal root elongation under drought stress. Thus, the present study was planned to make a comparative study of drought responsiveness in drought and well-irrigated tef exploring the availability of whole-genome level information and molecular tools in rice.

Materials and Methods

Plant Material and Growth Conditions

The late-maturing improved variety of tef cv. Kaye Murre was used in the study. KayeMurre is capable of elongating its roots under drought stress (Degu and Fujimura, 2010). The seeds were obtained from Debreziet Agricultural Research center of Ethiopia. The seeds were surface sterilized and germinated on filter paper at 25°C in the dark. After 3 days, seedlings with seminal roots about 1 cm long were transplanted to a plastic root box (30 cm in width; 25 cm in diameter and 24 cm in height) containing (1) horticulture nursery soil (Kureha Ltd., Tokyo, Japan); with holes at the bottom. Horticulture nursery soil was porous, consisting of uniformly sized soil particles (0.5–3.0 mm), and containing 0.4 g kg-1 of nitrogen, 1.9 g kg-1
of phosphorus, 0.6 g kg-1 of potassium, and 0.2 g kg-1 of magnesium. The liquid fertilizer was composed of nitrogen: phosphorus: potassium at the rate of 2:1:1. The growth conditions were 12/12 h day/night, one light period supplied 820 μmm-2s- photosynthetically active photon flux density (PPDF), 30/20°C (day/night); and temperature with RH 60 to 70%. For the control experiment, plastic root boxes were placed in tanks for a continuous supply of water through the root system. However, for drought stress treatment, root boxes were kept on a separate tank without the supply of water.

Soil, root and leaf water content measurement

Gravimetric soil water content was determined as described by Singh & Baghini (2014). A soil sample was taken from three points within the plastic basket (both sides and a center) with a borer, and the collected soil was stored in a 1.5-ml Eppendorf tube to equilibrate soil moisture for 2 h. The water content was measured by using electric balance before and after drying the soil in the oven for 48 hrs. The measurement was replicated three times, and the data were averaged. Soil, leaf and root water potential were measured by using a dew point microvoltmeter (model HR33T, WESCOR, Inc. Logan, UT). First, the soil sample was taken using a borer (5 mm in diameter). The collected soil was stored in 1.5 ml Eppendorf tube to equilibrate to the surrounding environment for 2 hrs. Similarly, root sample and leaf sample were taken and kept in 1.5 ml Eppendorf tube.

For relative water content (RWC), two leaves per plant were cut and stored in Eppendorf tube on ice. The fresh weight (FW) was measured following immersing it in double-distilled water (DDW) for 8 hours. This was followed by measuring the turgid weight (TW). The sample was then oven-dried for 24 hrs at 80° centigrade, and dry weight (DW) was measured after cooling it to 50 centigrade. RWC is calculated using the following formula;

\[ \text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \]

For osmotic potential (OP) measurement, leaf and root samples (about 1.5 cm) were collected at 0, 2, 4, 6 and 8 days after drought stress treatment, and were kept in Eppendorf tube at -20 ° centigrade. During measurement, the samples were withdrawn from -20 ° centigrade and left at the room temperature to thaw. The Eppendorf was centrifuged at 14000 g for 15 minutes. The sap was measured by a dew point microvoltmeter (model HR33T, WESCOR, Inc.). Osmotic adjustment (OA) was calculated as the difference between the measured and expected concentration-effect OP of the drought stressed plants. For analysis of the transcription under drought stress, seminal root tips (1 cm) were harvested after 8 days of drought stress from the well-irrigated and drought-stressed sample. All samples were collected at noon. After harvesting, samples were immediately placed in liquid nitrogen and stored at −80°C until RNA extraction.

MicroArray experiments

The Microarray experiment was done following the standard procedure (Figure 1). Total RNAs were extracted from tef root using the RNeasy Maxi kit (Qiagen, Valencia, CA, USA). The RNA samples were treated with DNase I (NipponGene, Chiyoda-ku, Tokyo, Japan) and quantified by spectrophotometer using Nano Drop™ 1000 Thermo Scientific. The RNA quality was checked using the Agilent BioAnalyzer. Next, poly (A) + RNA was isolated from 200 μg of total RNA using an mRNA isolation system (Nippon- Gene, Japan). Linear amplification and labeling were carried out with fluorescent linear amplification kit (Agilent). Transcriptional analysis was carried out using a 22 mer-oligo chip from the Agilent Technologies produced by the Plant Functional Genomics Center, National Institute of Agricultural Science of Japan. The chip (Catalogue array- GA4138A) carries 21,000 genes from the genome of Oryza sativa L. spp. Japonica (Nipponbarre). Redundant probes were randomly distributed in triplicate across the array, each comprising a 22-mer oligonucleotide designed using inkjet-based technology which prints DNA on 1X 3” glass slide. The source of sequence information included a range of genes that can measure the expression of drought, salt and cold stress genes for rice and related plants(Sato et al., 2013). A complete description of the chip is available at the Rice Expression Profile Database (RiceXPro). Three biological replicates per treatment were analyzed. Four (4) microgram of labeled cDNA was hybridized to
the array according to the manufacturer’s recommendations (Maruyama et al., 2014). The array was scanned with the Agilent DNA microarray scanner, and the expression data were extracted with the Agilent Feature Extraction software.

Figure 1. Experimental set up and hybridization of rice microarray on tef cDNA.

**RT-PCR**

Aliquot of total RNAs 10 µg RNA was reverse transcribed and used to synthesize single-stranded cDNA using the First-strand cDNA synthesis kit (TAKARA SHUZO CO. LTD., Otsu, Shiga, Japan) according to the manufacturer’s instructions. RT-PCR reactions were performed with the Access RT-PCR kit (Thermo Fisher Scientific Inc) according to the manufacturer’s protocol. The sequences of the primer were (FW=CGAGCGCTCCAACTCATC and RW=CAGCACCGAGCTGTCCTC with annealing temperature 60°C). The amplicon size was 500 bp; WRKY gene fragments were then amplified using gene-specific primers. Gene expression patterns were normalized to the expression of the 18S ribosomal RNA (FW = AACGGCTACCACATCCAAGG , and RW = TCATTACTCGATCCCGAAG). The PCR consisted of 40 cycles (30 s at 94°C, 1 min at 60°C, and 1 min at 72°C). The PCR-products were sequenced with (Macrogen, Korea).

**Expression patterns of WRKY using real time-Quantitative-PCR**

Quantitative PCR were carried out by designing primer using "Primer 3" software (http://frodo.wi.mit.edu/primer3/) according to the following criteria: melting temperature: 59°C primers' length: 18-24 nucleotides, product size: 110 base pairs (bp) and GC content: 40-55%. Quantitative PCR was performed using cDNA made of 50 ng total RNA, with an Absolute QPCR SYBER Green ROX kit (Thermo Scientific, ABgene UK), using Rotor-Gene 6000 (Corbett life Sciences, Australia). Samples were first heated for 15 min. at 95°C followed by 40 PCR cycles of 10 s at 95°C, 15 s at 59°C and 20 s at 72 °C. Negative controls had no cDNA. Gene expression patterns were normalized to the expression of the QuantumRNA™ Classic 18S Internal Standard (Thermo Scientific, ABgene UK). The fold change is calculated according to the following formula.

\[
\text{Ratio target gene in Drought Stress/Irrigated control} = \frac{\text{Fold change in target gene}}{\text{Fold change in Reference gene}}
\]
Northern Blot Analysis

Root samples were harvested at mid-day in 4, 6 and 8 days of drought-stressed sample, and were frozen in liquid nitrogen and then stored at −80°C until further use. Total RNAs were extracted using the RNeasy Maxi kit (Qiagen, Valencia, CA, USA). Samples were treated with DNase I (NipponGene, Chiyoda-ku, Tokyo, Japan) and quantified by spectrophotometer using Nano Drop™ 1000 Thermo Scientific. A 10μg aliquot of total RNA in a volume of 3.3μl was denatured by incubation with 1.5μl of 6 M glyoxal, 1.2μl of sodium phosphate buffer (0.1 M, pH 7.0) and 6μl of dimethylsulfoxide (DMSO) at 55°C for 1 h. The RNA solution was chilled on ice, and was separated by electrophoresis through a 1.2% agarose gel with 10 mM phosphate buffer. Afterwards, the RNA was transferred onto a Hybond N+ membrane (Amersham Biosciences), and was probed with [α-32P] dCTP-labeled DNA using the BCA Best labeling kit (Takara) according to the manufacturer's instructions in hybridization buffer [5× SSPE (SSPE is 0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA), 1× Denhardt's solution, 0.1% (w/v) SDS and 2 ng ml−1 DNA solutions from salmon sperm (Nippon gene)] for 24 h at 60°C. The blots were washed once in 2× SSC (20× SSC is 3 M NaCl and 300 mM trisodium citrate) for 5 min at room temperature, once with 2× SSC, 0.1% (w/v) SDS for 15 min at 60°C, once with 1× SSC, 0.1% (w/v) SDS for 15 min at 60°C and lastly with 0.1× SSC, 0.1% SDS (w/v) for 15 min at 60°C. Autoradiography was performed at −80°C using BioMax film (Kodak, Rochester, NY, USA) with an intensifying filter. The band intensities were quantified by using 'ImageJ' software (http://rsb.info.nih.gov/ij/)

Statistical Analysis

Analysis of variance and mean separation by Fisher's least significant difference methods were performed using Agricolae package with the statistical R programming language. Analysis of microarray raw data was performed using the open-source software of the Bioconductor project (Smyth et al., 2005) with the statistical R programming language (R Core Team 2018). Background adjustment, summarization and quintile normalization were performed using the limma package (Ritchie et al., 2015). Differentially expressed probes were identified by linear models’ analysis (Ritchie et al., 2015) using limma package by applying Bayesian correction, an adjusted p-value of 0.05 and an absolute fold change |FC| ≥ 2. Functional annotation and physical location of the genes represented by the probe sets in the japonica genome were obtained from the Rice XPro website (McCouch, Symbolization, Linkage, & Cooperative, 2008). Genes were grouped into main functional categories according to the "biological" terms of the Gene Ontology (Mi et al., 2016) assigned to each rice EST (Release 12.0) based on the results of BlastP analysis against the UniProt database. Genes without significant BlastP results were classified as "Unknown"; Evalue < 1e-8; identity > 40%.

Results

Morphological change of tef root tip responding to drought stress

Drought stress induced the elongation of the seminal root in tef in accelerated fashion (Figure 2). The root lengths for plants placed under drought stress were 33.3 % longer when compared to the well-irrigated sample. In terms of RWC and leaf water potential, there was no significant difference between drought-stressed and well-irrigated samples (Table 1). And the number of leaves were the same for both drought-stressed and well-irrigated control plants (Figure 2). However, there were significant differences between the two treatments in terms of soil and root water potential. Where the root water potential reduced to -1.2 MPa for drought-stressed sample, it remained -0.4 MPa for well-irrigated tef sample (Table 1). This indicates that when the drought stress increased, the available water content for the root was about 40% at field capacity. The available water content at field capacity was about 80% for well-irrigated control plants. This indicates clear water stress has been created in the drought-stressed sample.
However, OP for the drought-stressed plants was significantly lower than the well-irrigated control sample (Table 1). Specifically, the OA was the highest as the day for drought stress increases, and showed a significant difference between drought-stressed and well-irrigated samples. In the shoot, OA was the highest when compared to the root. The measured value was 0.05 MPa in control and 0.98 MPa in drought-stressed samples, respectively. It is important to note that compared to root under drought stress, the shoot exhibited lower OA, while the decrease in the RWC was significantly different, and it was the lowest for the root (Table 1).

Similarly, after 8 days of drought stress, the soil water potential for the control was about -0.5 MPa while it became -1.5 MPa under drought stress. Even though the soil water potential was significantly different between the control and stressed samples, the leaf water potential measurement and analysis showed no significant difference between the two treatments (Table 1). This was also confirmed by the non-significant difference between the two treatments on the relative water content.

However, the root water potential was significantly different between the two treatments (Table 1). The effect of different water potential between the soil, root, and shoot was revealed on the shoot and root growth (Figure 2). Significant difference (p≤ 0.05) was observed for the shoot and root growth among the control and stressed plants of tef. Although there was a delay in the shoot growth under drought stress, seedlings maintained a healthy green color after 192 hrs of drought stress (Figure 2). On the other hand, there was no significant difference in leaf water potential for both control and drought-stressed plants. (Table 1).
Table 1. Relative water contents (RWC), osmotic potential (OP), and osmotic adjustment (OA) in shoot and root after stopping irrigation.

| Days after water withholding | Control |             | Stress |             |
|-----------------------------|---------|-------------|--------|-------------|
|                             | RWC(%)  | OP (Mpa)    | OA (Mpa) | RWC(%)  | OP (Mpa)    | OA (Mpa) |
| Shoot                       |         |             |        |             |             |          |
| 0                           | 97.6±0.86 | 0.90±0.07  | 0±0.04  | 98.5±0.64  | 0.87±0.1    | 0±0.04   |
| 6                           | 97.8±0.84 | 0.89±0.07  | 0.02±0.03 | 87.3±6.5   | 0.80±0.09   | 0.07±0.08  |
| 7                           | 98.4±0.88 | 0.86±0.05  | -0.02±0.03 | 93.8±6.5   | 0.85±0.08   | 0.03±0.09  |
| 8                           | 97.4±0.83 | 0.80±0.05  | -0.07±0.01 | 91.9±8.7   | 0.83±0.09   | 0.04±0.06  |
| CV                          | 4.9     | 6.7         | -197.8  |             |             |          |
| LSD                         | 6.1     | 0.1         | 0.1     |             |             |          |
| Root                        |         |             |        |             |             |          |
| 0                           | 97.6±0.86 | 0.87±0.04  | 0±0     | 98.48±0.64  | 0.87±0.12   | 0±0      |
| 6                           | 97.8±0.83 | 0.89±0.11  | 0.02±0.04 | 84.02±2.9  | 0.39±0.1    | 0.48±0.12 |
| 7                           | 98.4±0.88 | 0.75±0.15  | -0.12±0.11 | 87.54±4.2  | 0.25±0.14   | -0.61±0.1 |
| 8                           | 97.4±0.83 | 0.79±0.15  | -0.07±0.14 | 86.06±3.4  | 0.31±0.24   | 0.56±0.14 |
| CV                          | 10.7    | 20.2        | -52.8   |             |             |          |
| LSD                         | 12.8    | 0.2         | 0.2     |             |             |          |

An asterisk represents a significantly greater value than the other accession at 5% (*), 1% (**), and 0.1% (***) level. The difference between accessions was statistically analyzed by Tukey pair wise comparison (ANOVA). A hyphen (-) indicates that all pieces of the three tested tissues were withered, and value in parenthesis indicates that 1–2 pieces of the three tested tissues were withered.

Figure 3. Influence of drought stress on leave and root growth of tef for 2, 4, 6 and 8 days. Presented values for leaves and roots are the means of three replications. Vertical bars represent the SD.
Transcriptome analysis of tef root tips in response to drought

The drought-responsive genes were identified by changes in the gene expression patterns of the three replicates; the two-fold difference in the ratio of drought: control transcript abundance and p-value less than 0.05 (Figure 4, Table 2). The scatter plot of data from three replications to compare the control and stressed transcripts showed that there is a linear relationship between most of the genes expressed under control and stressed samples. From the 176 differentially expressed genes, 77 have greater than two-fold changes, but 93 genes were down-regulated with a fold change of less than or equal to minus two. Lesser number of genes were up and down-regulated might be due to the low hybridization signal because of low homology between orthologous genes.

Figure 4. Scatter plot comparisons of microarray gene expression in drought-stressed E. tef . The normalized expression value (signal) for each gene under well-watered (control) vs. drought-stress plotted for E. tef at -2.5 MPa leaf water potential samples (A).

Table 2. Selected drought responsive genes in O sativa highly hybridized resulted in up or down regulation of E. tef transcript (significant at 5% level)

| ProbeName  | GeneName | Gene symbol | Locus tag        | Fold Change | Log p-value | Gene description                                |
|------------|----------|-------------|-----------------|-------------|------------|------------------------------------------------|
| A_71_P120462 | AK109080 | LOC4344714  | OSNPB_08015970 0 | 4.2         | 4.8        | AT-hook motif nuclear-localized protein 23     |
| A_71_P113410 | AK067302 | LOC4333169  | OSNPB_03042880 0 | 4.4         | 4.9        | Uncharacterized                                |
| Entry          | Accession     | Description                                                                 |
|---------------|---------------|-----------------------------------------------------------------------------|
| A_71_P103875  | AK058902      | OSNPB_02056240, Uncharacterized                                            |
| A_71_P103875  | LOC4329688    | OSNPB_02056240, Uncharacterized                                            |
| A_71_P126315  | AK069006      | OSNPB_12057400, Uncharacterized                                            |
| A_71_P126315  | LOC4352600    | OSNPB_12057400, Uncharacterized                                            |
| A_71_P126315  | AK069943      | OSNPB_08010950, -                                                          |
| A_71_P126315  | LOC4344472    | OSNPB_08010950, -                                                          |
| A_71_P110996  | AK100257      | OSNPB_04064120, -                                                          |
| A_71_P110996  | LOC4337170    | OSNPB_04064120, -                                                          |
| A_71_P102759  | AK102031      | OSNPB_01019460, 2.9                                                       |
| A_71_P102759  | LOC4327316    | OSNPB_01019460, 2.9                                                       |
| A_71_P104162  | AK102271      | OSNPB_02081680, 2.9                                                       |
| A_71_P104162  | LOC4331143    | OSNPB_02081680, 2.9                                                       |
| A_71_P125325  | AK102853      | OSNPB_11055560, -                                                          |
| A_71_P125325  | LOC4350717    | OSNPB_11055560, -                                                          |
| A_71_P102968  | AK109457      | OSNPB_10051050, -                                                          |
| A_71_P102968  | LOC4324110    | OSNPB_10051050, -                                                          |
| A_71_P123735  | AK109491      | OSNPB_12040380, 2.9                                                       |
| A_71_P123735  | LOC4349090    | OSNPB_12040380, 2.9                                                       |
| A_71_P126097  | AK059940      | OSNPB_02046120, 2.9                                                       |
| A_71_P126097  | LOC4352037    | OSNPB_02046120, 2.9                                                       |
| A_71_P106066  | AK063459      | OSNPB_12024290, 2.9                                                       |
| A_71_P106066  | LOC4329297    | OSNPB_12024290, 2.9                                                       |
| A_71_P126249  | AK064505      | OSNPB_02060550, 2.9                                                       |
| A_71_P126249  | LOC4351872    | OSNPB_02060550, 2.9                                                       |
| A_71_P104593  | AK067496      | OSNPB_02067950, 2.9                                                       |
| A_71_P104593  | LOC4329918    | OSNPB_02067950, 2.9                                                       |
| A_71_P106979  | AK067772      | OSNPB_12073220, 2.9                                                       |
| A_71_P106979  | LOC4330316    | OSNPB_12073220, 2.9                                                       |
| A_71_P106439  | AK102091      | OSNPB_08012780, 2.9                                                       |
| A_71_P106439  | LOC4330628    | OSNPB_08012780, 2.9                                                       |
| A_71_P119754  | AK103321      | OSNPB_09052750, 2.9                                                       |
| A_71_P119754  | LOC4344573    | OSNPB_09052750, 2.9                                                       |
| A_71_P122240  | AK110546      | OSNPB_02052720, 2.8                                                       |
| A_71_P122240  | LOC4347641    | OSNPB_02052720, 2.8                                                       |
| A_71_P105146  | AK058851      | OSNPB_07051690, 2.8                                                       |
| A_71_P105146  | LOC4329525    | OSNPB_07051690, 2.8                                                       |
| A_71_P118869  | AK061900      | OSNPB_02012310, 2.8                                                       |
| A_71_P118869  | LOC9270800    | OSNPB_02012310, 2.8                                                       |
| A_71_P124449  | AK063047      | OSNPB_05047000, 2.8                                                       |
| A_71_P124449  | LOC4328135    | OSNPB_05047000, 2.8                                                       |
| A_71_P114932  | AK065359      | OSNPB_08056160, 2.8                                                       |
| A_71_P114932  | LOC4339070    | OSNPB_08056160, 2.8                                                       |
| A_71_P121233  | AK066884      | OSNPB_03014290, 2.8                                                       |
| A_71_P121233  | LOC4346328    | OSNPB_03014290, 2.8                                                       |
| A_71_P109377  | AK067103      | OSNPB_11019960, 2.8                                                       |
| A_71_P109377  | LOC4331586    | OSNPB_11019960, 2.8                                                       |
| A_71_P125413  | AK101774      | OSNPB_01089920, 2.8                                                       |
| A_71_P125413  | LOC4350003    | OSNPB_01089920, 2.8                                                       |
| A_71_P102782  | AK108736      | OSNPB_01076640, 2.7                                                       |
| A_71_P102782  | LOC4325031    | OSNPB_01076640, 2.7                                                       |
| A_71_P102538  | AK073493      | OSNPB_09055740, 2.7                                                       |
| A_71_P102538  | LOC4325494    | OSNPB_09055740, 2.7                                                       |
| A_71_P121607  | AK099503      | OSNPB_09055740, 2.7                                                       |
| A_71_P121607  | LOC4347825    | OSNPB_09055740, 2.7                                                       |

**Comments:**
- Ankyrin repeat protein SKIP35
- Beta-galactosidase 11-like
- (+)-neomethyl dehydrogenase
- Uncharacterized
- U-box domain-containing protein 11
- Helicase-like transcription factor CHR28
- Probable E3 ubiquitin-protein ligase BAH1-like 1
- Probable calcium-binding protein CML27
- Uncharacterized
- Probable receptor-like serine/threonine-protein kinase
- Uncharacterized
- RINT1-like protein MAG2L
- Uncharacterized
- Anamorsin homolog 1-like
- Protein CDI
- Unknown expressed protein
- 50S ribosomal protein L31
- Unknown expressed protein
- Glycine-rich RNA-binding protein 2, mitochondrial
- Uncharacterized
- 50S ribosomal protein L28, chloroplastic
- Indole-3-glycerol phosphate synthase, chloroplastic
- Anaphase-promoting complex subunit 15
- Fasciclin-like arabinogalactan protein 13
- Mitogen-activated protein kinase 14
| Accession | Gene ID     | Length | Expression | Description                                                                 |
|-----------|-------------|--------|------------|-----------------------------------------------------------------------------|
| A_71_P100151 | AK100105    | LOC4327103 | 0          | OSNPB_01025100 - 2.7 like Protein trigalactosyldiacylglycerol 4, chloroplastic |
| A_71_P106899 | AK100401    | LOC4328582  | 0          | OSNPB_02019210 - 2.7 Uncharacterized                                         |
| A_71_P115743 | AK101156    | LOC4340300  | 0          | OSNPB_06017920 - 2.7 Uncharacterized                                         |
| A_71_P110642 | AK103022    | LOC4333590  | 0          | OSNPB_03065090 - 2.7 origin of replication complex subunit 1-like            |
| A_71_P102476 | AK112105    | LOC9270556   | 0          | OSNPB_01035420 - 2.7 Unknown expressed protein                               |
| A_71_P103041 | AK065358    | LOC4324159  | 0          | OSNPB_01073350 - 2.7 Uncharacterized                                         |
| A_71_P115357 | AK069970    | LOC4332513  | 0          | OSNPB_04059270 - 2.7 Uncharacterized                                         |
| A_71_P112428 | AK101611    | LOC4336833  | 0          | OSNPB_01085450 - 2.7 Uncharacterized                                         |
| A_71_P114265 | AK103819    | LOC4339464  | 0          | OSNPB_05054010 - 2.7 Uncharacterized                                         |
| A_71_P112572 | AK068391    | LOC4346642  | 0          | OSNPB_09029400 - 2.7 Non-specific lipid-transfer protein 2-like              |
| A_71_P115357 | AK069632    | LOC4337885  | 0          | OSNPB_05016060 - 2.7 Uncharacterized                                         |
| A_71_P110380 | AK072940    | LOC4328666  | 0          | OSNPB_02020370 - 2.7 Uncharacterized                                         |
| A_71_P1102854 | AK102203    | LOC4324824  | 0          | OSNPB_01085450 - 2.7 Uncharacterized                                         |
| A_71_P127945 | AK101068    | LOC4326544  | 0          | OSNPB_01074220 - 2.7 Uncharacterized                                         |
| A_71_P128119 | AK100386    | LOC4329433  | 0          | OSNPB_02050490 - 2.7 Uncharacterized                                         |
| A_71_P125176 | AK064740    | LOC4350576  | 0          | OSNPB_11051280 - 2.7 Uncharacterized                                         |
| A_71_P117169 | AK073271    | LOC4340699  | 0          | OSNPB_06026430 - 2.7 Uncharacterized                                         |
| A_71_P111395 | AK101488    | LOC4334912  | 0          | OSNPB_04010630 - 2.7 Uncharacterized                                         |

| Accession | Gene ID     | Length | Expression | Description                                                                 |
|-----------|-------------|--------|------------|-----------------------------------------------------------------------------|
| A_71_P102743 | AK100090    | LOC4324749 | 0          | OSNPB_01053950 - 2.7 Uncharacterized                                         |
| A_71_P114270 | AK103672    | LOC4339458  | 0          | OSNPB_01053950 - 2.7 Uncharacterized                                         |
| A_71_P105946 | AK072940    | LOC4328666  | 0          | OSNPB_02020370 - 2.7 Uncharacterized                                         |
| A_71_P127945 | AK110168    | LOC4326544  | 0          | OSNPB_01074220 - 2.7 Uncharacterized                                         |
| A_71_P128119 | AK110386    | LOC4329433  | 0          | OSNPB_02050490 - 2.7 Uncharacterized                                         |
| A_71_P125176 | AK064740    | LOC4350576  | 0          | OSNPB_11051280 - 2.7 Uncharacterized                                         |
| A_71_P108336 | AK066383    | LOC9266989   | 0          | OSNPB_03010005 - 2.7 Uncharacterized                                         |
| A_71_P119552 | AK066544    | LOC4342173  | 0          | OSNPB_07010210 - 2.7 Uncharacterized                                         |
| A_71_P117169 | AK073271    | LOC4340699  | 0          | OSNPB_06026430 - 2.7 Uncharacterized                                         |
| A_71_P111395 | AK101488    | LOC4334912  | 0          | OSNPB_04010630 - 2.7 Uncharacterized                                         |
| Accession       | Gene ID      | Description                                                                 |
|-----------------|--------------|------------------------------------------------------------------------------|
| A_71_P110635    | AK105583     | LOC4333158 eukaryotic translation initiation factor 2 subunit alpha homolog |
| A_71_P112010    | AK109273     | LOC10727872 ubiquitin-like modifier-activating enzyme 5                      |
| A_71_P109593    | AK109470     | LOC4332731 auxin-responsive protein SAUR32                                   |
| A_71_P102642    | AK059715     | LOC4324964 Uncharacterized                                                    |
| A_71_P105802    | AK063438     | LOC4328135 Uncharacterized                                                    |
| A_71_P126815    | AK068049     | LOC4351356 glucose-6-phosphate isomerase 1, chloroplastic                    |
| A_71_P123898    | AK070579     | LOC4348282 Uncharacterized                                                    |
| A_71_P111111    | AK101835     | LOC4336102 G-type lectin S-receptor-like serine/threonine-protein kinase     |
| A_71_P116173    | AK102365     | LOC4342018 wall-associated receptor kinase 1                                 |
| A_71_P112259    | AK062775     | LOC4333169 Uncharacterized                                                    |
| A_71_P106457    | AK064402     | LOC4329874 chloroplastic import inner membrane translocase subunit HP30-2   |
| A_71_P117853    | AK067620     | LOC4344339 Uncharacterized                                                    |
| A_71_P111034    | AK102124     | LOC4337380 WUSCHEL-related homeobox 9-like                                   |
| A_71_P119970    | AK103306     | LOC4346187 Uncharacterized                                                    |
| A_71_P117941    | AK106266     | LOC4342464 Uncharacterized                                                    |
| A_71_P117316    | AK109313     | LOC4329438 ubiquitin-like modifier-activating enzyme 5                       |
| A_71_P110278    | AK061178     | LOC4332206 elongation factor 1-alpha-like                                    |
| A_71_P117528    | AK064179     | LOC4340325 B3 domain-containing protein Os02g0598200-like                    |
| A_71_P119501    | AK065696     | LOC4343831 Uncharacterized                                                    |
| A_71_P118242    | AK069889     | LOC4342267 Uncharacterized                                                    |
| A_71_P124967    | AK072582     | LOC4349984 protein MAIN-LIKE 2                                               |
| A_71_P124115    | AK106467     | LOC4348087 Uncharacterized                                                    |
| A_71_P124066    | AK107022     | LOC4348241 Uncharacterized                                                    |
| A_71_P110063    | AK058803     | LOC4332352 17kDa alpha-amylase/trypsin inhibitor 2-like                       |
| A_71_P107096    | AK066613     | LOC4330727 serine/arginine repetitive matrix protein 1                        |
| A_71_P105265    | AK069956     | LOC4330113 ran-binding protein 1 homolog a                                    |
| Accession  | Gene ID     | OSNPB    | Log2 Fold Change | Description                                                                 |
|------------|-------------|----------|------------------|-----------------------------------------------------------------------------|
| A_71_P121291| AK101010    | LOC9266867 | 4.4              | NAC transcription factor 29                                                  |
| A_71_P126741| AK106509    | LOC4351270 | 4.4              | Uncharacterized                                                             |
| A_71_P121378| AK108589    | LOC4344743 | 4.4              | probable methyltransferase PMT19                                             |
| A_71_P104421| AK109015    | LOC10727524| 4.4              | Uncharacterized                                                             |
| A_71_P109351| AK062506    | LOC4331362 | 4.4              | probable phospholipid hydroperoxide glutathione peroxidase                  |
| A_71_P108529| AK062977    | LOC4331483 | 4.4              | cyclin-dependent kinase A-2-like                                             |
| A_71_P105792| AK068761    | LOC4330225 | 4.4              | Bifunctional aspartokinase/homoserine dehydrogenase 2, chloroplastic        |
| A_71_P103675| AK073433    | LOC4325382 | 4.4              | mitochondrial proton/calcium exchanger protein                              |
| A_71_P109785| AK099818    | LOC4332656 | 4.4              | Uncharacterized                                                             |
| A_71_P1117690| AK068356   | LOC4331210 | 4.4              | auxin transporter-like protein 1                                            |
| A_71_P105792| AK068761    | LOC4330225 | 4.4              | dihydroceramide fatty acyl 2-hydroxylyase FAH2                              |
| A_71_P128158| AK110434    | LOC4339717 | 4.4              | auxilin-related protein 1                                                   |
| A_71_P110945| AK058848    | LOC4335799 | 4.4              | photosystem I subunit O                                                     |
| A_71_P100324| AK065125    | LOC4325653 | 4.4              | beta-1,4-mannosyl-glycoprotein 4-beta-Nacetylglucosaminyltransferase         |
| A_71_P105147| AK067722    | LOC9267259 | 4.4              | rho GTPase-activating protein 5                                              |
| A_71_P104323| AK068122    | LOC4331210 | 4.4              | Uncharacterized                                                             |
| A_71_P1110156| AK073459   | LOC4333085 | 2.5              | mitochondrial substrate carrier family protein B                            |
| A_71_P112354| AK101902    | LOC4335831 | 2.5              | mitochondrial substrate carrier family protein B                            |
| A_71_P110583| AK105818    | LOC4332538 | 2.5              | probable carboxylesterase 15                                                |
| A_71_P106058| AK109318    | LOC4329438 | 2.5              | RNA-binding protein CP29B, chloroplastic mitogen-activated protein kinase    |
| A_71_P123431| AK069758    | LOC4348063 | 2.6              | kinase 5                                                                    |
| A_71_P126492| AK072652    | LOC4351897 | 2.6              | Uncharacterized                                                             |
| A_71_P123717| AK100196    | LOC4325457 | 2.6              | mitochondrial substrate carrier family protein B                            |
| A_71_P118094| AK101955    | LOC4343623 | 2.6              | monothiol glutaredoxin-S2-like                                              |
| A_71_P107503| AK103085    | LOC4334311 | 2.6              | calcium-dependent protein kinase 21-like                                     |
| A_71_P119592| AK109447    | LOC4343868 | 2.6              | gallate 1-beta-glucosyltransferase                                          |
| A_71_P114630| AK111471    | LOC4338289 | 2.6              | soluble inorganic pyrophosphatase                                           |
| Accession | GenBank | LOC | OSNPB | Score | Evalue | Description |
|-----------|---------|-----|-------|-------|---------|-------------|
| A_71_P110290 | AK060019 | LOC4332213 | OSNPB_03024160 | 0.0 | 2.6 | Uncharacterized |
| A_71_P107682 | AK061464 | LOC4331811 | OSNPB_03017750 | 0.0 | 2.6 | Uncharacterized |
| A_71_P124888 | AK063652 | LOC9270608 | OSNPB_11053340 | 0.0 | 2.6 | exosome complex component RRP41-like |
| A_71_P104586 | AK064473 | LOC4328332 | OSNPB_02015300 | 0.0 | 2.6 | DNA polymerase alpha subunit B |
| A_71_P119866 | AK069066 | LOC4346248 | OSNPB_08054920 | 0.0 | 2.6 | dehydrodolichyl diphosphate synthase 6 |
| A_71_P123084 | AK069924 | LOC4348749 | OSNPB_10044610 | 0.0 | 2.6 | bromodomain-containing factor 2 |
| A_71_P118954 | AK070767 | LOC4342293 | OSNPB_01094620 | 0.0 | 2.6 | probable alkaline/neutral invertase F |
| A_71_P104150 | AK065136 | LOC4329854 | OSNPB_02059510 | 0.0 | 2.6 | methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial-like |
| A_71_P111210 | AK100967 | LOC4335200 | OSNPB_11015330 | 0.0 | 2.6 | Uncharacterized |
| A_71_P125379 | AK060118 | LOC4349805 | OSNPB_02059510 | 0.0 | 2.6 | Uncharacterized |
| A_71_P104150 | AK065136 | LOC4329854 | OSNPB_04022160 | 0.0 | 2.6 | Uncharacterized |
| A_71_P111210 | AK100967 | LOC4335200 | OSNPB_04049150 | 0.0 | 2.6 | Uncharacterized |
| A_71_P113166 | AK101116 | LOC4336249 | OSNPB_10051640 | 0.0 | 2.6 | Uncharacterized |
| A_71_P123021 | AK103655 | LOC4349117 | OSNPB_07021670 | 0.0 | 2.6 | Uncharacterized |
| A_71_P118139 | AK107633 | LOC4342730 | OSNPB_07068320 | 0.0 | 2.6 | Uncharacterized |
| A_71_P128030 | AK110267 | LOC9266867 | OSNPB_08041690 | 0.0 | 2.6 | protein NRT1/ PTR FAMILY 4.5 |
| A_71_P120556 | AK062863 | LOC4345581 | OSNPB_01096320 | 0.0 | 2.6 | Uncharacterized |
| A_71_P117434 | AK063377 | LOC4329688 | OSNPB_03027670 | 0.0 | 2.6 | Uncharacterized |
| A_71_P107424 | AK063885 | LOC4329485 | OSNPB_12065080 | 0.0 | 2.6 | Uncharacterized |
| A_71_P126067 | AK065165 | LOC4352741 | OSNPB_05056360 | 0.0 | 2.6 | Uncharacterized |
| A_71_P114418 | AK099313 | LOC4339617 | OSNPB_02079760 | 0.0 | 2.6 | Uncharacterized |
| A_71_P104020 | AK104474 | LOC4331018 | OSNPB_03037660 | 0.0 | 2.6 | Uncharacterized |
| A_71_P107567 | AK109412 | LOC4332957 | OSNPB_12010530 | 0.0 | 2.6 | PHD finger protein ALFIN-LIKE 9-like |
| A_71_P128033 | AK110273 | LOC9269069 | OSNPB_01080650 | 0.0 | 2.6 | protein CHUP1, chloroplastic |
| A_71_P101793 | AK111757 | LOC4325072 | OSNPB_04055630 | 0.0 | 2.7 | delta(14)-sterol reductase |
| A_71_P111748 | AK062772 | LOC4336627 | OSNPB_04045050 | 0.0 | 2.7 | Oryza sativa glutathione peroxidase 1 (GPX1) |
| Gene ID       | Accession   | LOC  | OSNPB      | Description                                           |
|--------------|-------------|------|------------|-------------------------------------------------------|
| A_71_P107943| AK068484    | LOC4333501 | OSNPB_03062670 0 | Uncharacterized                                      |
| A_71_P107609| AK068746    | LOC4333878 | OSNPB_03071060 0 | probable lipid phosphate phosphatase beta            |
| A_71_P124577| AK072924    | LOC4350916 | OSNPB_11061490 0 | transcription initiation factor TFIID subunit 15b    |
| A_71_P113689| AK099719    | LOC9267958 | OSNPB_05014350 0 | YTH domain-containing family protein 2               |
| A_71_P119849| AK101404    | LOC4344441 | OSNPB_08010480 0 | arginase 1, mitochondrial-like                        |
| A_71_P111324| AK109889    | LOC4336181 | OSNPB_08043430 0 | Unknown expressed protein                             |
| A_71_P121341| AK058477    | LOC4345657 | OSNPB_09046560 0 | probable lipid phosphate phosphatase beta            |
| A_71_P122196| AK068061    | LOC4347311 | OSNPB_02055850 0 | Uncharacterized                                      |
| A_71_P106957| AK072724    | LOC4329681 | OSNPB_04041290 0 | Uncharacterized                                      |
| A_71_P112363| AK073418    | LOC4335786 | OSNPB_07059830 0 | Uncharacterized                                      |
| A_71_P118271| AK101648    | LOC4343803 | OSNPB_02081890 0 | zinc finger CCHC domain-containing protein 10        |
| A_71_P117346| AK108060    | LOC4331157 | OSNPB_01069890 0 | F-box protein SKIP23                                  |
| A_71_P128236| AK110559    | LOC9270256 | OSNPB_07068200 0 | nascent polypeptide-associated complex subunit alpha-like protein 2 |
| A_71_P128274| AK110618    | LOC4344309 | OSNPB_05010140 0 | protein PYRICULARIA ORYZAE RESISTANCE 21-like        |
| A_71_P111288| AK067481    | LOC4337526 | OSNPB_11017540 0 | TNF receptor-associated factor 6                      |
| A_71_P124567| AK070884    | LOC4349916 | OSNPB_04044950 0 | NAC domain-containing protein 48-like                 |
| A_71_P110906| AK100347    | LOC4335990 | OSNPB_04022160 0 | protein FLX-like 2                                   |
| A_71_P113574| AK100817    | LOC4335199 | OSNPB_12046730 0 | Uncharacterized                                      |
| A_71_P125669| AK101496    | LOC9272227 | OSNPB_04062930 0 | transducin beta-like protein 3                       |
| A_71_P112718| AK102284    | LOC4337089 | OSNPB_07061170 0 | probable isoprenylcysteine alpha-carbonyl methyltransferase ICMEI1 |
| A_71_P117818| AK109158    | LOC4343876 | OSNPB_01054270 0 | rust resistance kinase Lr10                           |
| A_71_P103769| AK110526    | LOC4326871 | OSNPB_01012110 0 | Uncharacterized                                      |
| A_71_P100337| AK071475    | LOC4326040 | OSNPB_03042880 0 | ankyrin repeat protein SKIP35                        |
| A_71_P109704| AK060233    | LOC4333169 | OSNPB_08047020 0 | Uncharacterized                                      |
| A_71_P120608| AK059773    | LOC9268297 | OSNPB_05053570 0 | alpha carbonic anhydrase 7                            |
| A_71_P113554| AK061590    | LOC4339442 | OSNPB_08011680 0 | polyadenylate-binding protein RPB45                  |
| A_71_P120282| AK063695    | LOC4344519 | OSNPB_08011680 0 | exosome complex component RRP41-                      |
| Accession | Description                                                                 | Log2 Fold Change | Log2 Fold Change |
|-----------|------------------------------------------------------------------------------|-----------------|-----------------|
| A_71_P109622 | A_71_P109622 | 0               | OSNPB_03081690   | 3.3 5.2 potassium transporter 22-like |
| A_71_P111475 | A_71_P111475 | 0               | OSNPB_04049680   | 3.3 5.2 Uncharacterized |
| A_71_P119110 | A_71_P119110 | 0               | OSNPB_07060770   | 3.3 5.2 Uncharacterized |
| A_71_P101086 | A_71_P101086 | 0               | OSNPB_01019890   | 3.3 5.2heavy metal-associated isoprenylated plant protein 7 |
| A_71_P125732 | A_71_P125732 | 0               | OSNPB_12054810   | 3.3 5.2 condensin-2 complex subunit D3 |
| A_71_P104256 | A_71_P104256 | 0               | OSNPB_02081890   | 3.3 5.2 Uncharacterized |
| A_71_P120258 | A_71_P120258 | 0               | OSNPB_08011680   | 4.5 4.0 Uncharacterized |
| A_71_P120991 | A_71_P120991 | 0               | OSNPB_08015270   | 4.8 9.8 sphingoid long-chain bases kinase 1 |
| A_71_P111306 | A_71_P111306 | 0               | OSNPB_03042880   | 5.8 7.4 Uncharacterized |
| A_71_P105174 | A_71_P105174 | 0               | OSNPB_02069880   | 8.5 7.9 probable WRKY transcription factor 14 |
| A_71_P118665 | A_71_P118665 | 0               | OSNPB_07065980   | 8.5 7.9 E3 ubiquitin-protein ligase DIS1-like |
| A_71_P110167 | A_71_P110167 | 0               | OSNPB_06050730   | 5 4.0 E3 ubiquitin-protein ligase DIS1-like |
| A_71_P116544 | A_71_P116544 | 0               | OSNPB_03033270   | 6.3 4.8 BTB/POZ domain-containing protein POB1 |
| A_71_P110549 | A_71_P110549 | 0               | OSNPB_01070750   | 12.5 5.8 ABC transporter I family member 6, chloroplastic |
| A_71_P102603 | A_71_P102603 | 0               | OSNPB_0327679    | 9.4 6.7 bHLH transcription factor |

**Functional Characterization of drought-responsive genes**

The differentially expressed genes were classified into functional categories. The functional annotation of these genes is based on sequences which match to other sequences in the GenBank using BLAST analysis. The result is set with the threshold of expectation value less than 10-10. Among the 171 differentially expressed transcripts, 57 belong to genes with unknown functions. The dissection of the expressed gene profile of tef under drought stress showed that most of the transcripts (93) were down-regulated (Figure 4). On the other hand, 77 transcripts were up-regulated under drought stress. Those differentially expressed genes between drought-stressed and well-irrigated tef root plant (77 up-regulated and 93 down-regulated) were identified by Linear Models for Microarray (LIMMA) (adjusted p-value ≤ 0.05; with fold change (FC) of |2|).
Figure 5. Distribution of drought responsive genes in E. tef for their functional classes. Percentage of drought responsive genes in the various functional categories; Up-regulated (top) and Down-regulated (bottom).

The resulting unregulated and down-regulated genes were further analyzed using the Gene Ontology (GO) Enrichment analysis to identify their molecular function. The majority of the transcripts belong to the unknown function 44% and 36% for up-regulated and down-regulated genes, respectively. The rest were categorized into cell wall (10%), Mitochondrion (9% and 12%), cytoplasmic membrane-bound vesicles (11% and 13%), secretory pathway (6% and 8%), membrane (5% and 6%), ATP binding (3% and 4%), electron transport (3% and 4%), cellular process (21%), localization (7%), DNA binding (2%), Nucleus (2%), metabolism(2%), and Oxidoreductase activity (2%), were for up-regulated and down-regulated genes, respectively (Figure 5).

RT-PCR, Real-Time Quantitative PCR and Northern blot analysis of highly differentially expressed transcript.
Preliminary assessment of gene expression patterns under the normal control (well-irrigated) and drought stress in tef root (for 2, 4, 6, and 8) was performed for WRKY gene (Table 2) by quantitative RT-PCR, Real-time Quantitative PCR and Northern blot (Figure 6). The expression patterns under well irrigated and drought stress conditions differed significantly (LSD0.05 = 0.49). This gene was not expressed at all under the well-irrigated condition as evidenced in expression analysis. This implies the gene was significantly induced by drought in the roots.

Figure 6. (a) The elongation of tef root (scale bar 35 cm) under drought stress compared to control from 0 to 8 DAS (days after stress). (b) RT-PCR analysis of tef’s root WRKY gene after treatment for 2, 4, 6 and 8 days under drought stress. (c) Effect of drought stress on gene expression of WRKY in roots of tef. (d) Effect of drought stress on WRKY gene in tef root cells. Total RNAs were isolated from tef root which grow under drought stress for 4, 6 and 8 days. Total RNAs (10 μg each) were electrophores on an agarose gel, transferred onto a membrane and were probed with 32P-labeled isoform specific DNA fragments. The lower panel shows the quantification of ethidium bromide-stained total RNA after 4, 6 and 8 days after drought stress. Symbols, C and D indicate the well irrigated and drought stressed sample, respectively. To show equal amount of RNA for all used for all experiment, 18S ribosome cDNA is control.

Discussion
Well irrigated and drought-stressed Kaye Murri showed significant variation in terms of shoot and root RWC, OP and OA suggest that drought stress tolerance mechanism by OA is operating on tef.

The drought-stressed plants had higher OA value and elongated root length, which indicated that some drought tolerance mechanism is operating by OA and deep root system under drought stress. This implies that the two traits can be studied at the molecular level to elucidate the tolerant mechanism of tef under drought stress. The increased seminal root length in tef under the drought stress is due to
the shift in elongation of the shoot as compared to the well-irrigated leaf sample (Figure 3).

To unravel the transcripts related to the elongation of the root in tef, rice microarray chips with the known gene was used in this experiment. Rice is a model plant where extensive studies related to drought has been carried out (Moon and Jung, 2014; Moon et al., 2014a, 2018; Ramamoorthy et al., 2008). Multiple whole-genome sequences of rice and transcription studies and other molecular tools are available for this crop. Tef is one of the most drought-tolerant cereals, providing a useful platform to understand the mystery of root elongation under drought stress. Since many of the monocot genomes had high synteny (Choe et al., 2018), it is feasible to use rice chips to do hybridization with tef RNAs. However, from the hybridization of tef and rice transcripts, only 171 genes are differentially regulated. This might be the low homology of the rice orthologs to tef RNA.

Several ABC transporter family members are up-regulated in tef root under drought stress. This transcript is essential in transporting compounds which are important in drought adaptation (Lane et al., 2016). The larger number of ABC transporter genes are important in their ability to sequester and transport foreign chemicals and compound to protect the plant under drought stress (Hwang et al., 2016; Moon et al., 2014b). Basic helix–loop–helix (BHLH) transcription factor is also up-regulated in drought-stressed tef root. This transcription factor is responsible for the initiation of root development in plants (Schlereth et al., 2010). This involves the embryonic root signal for initiation of root elongation in tef root under drought stress.

However, WRKY transcription factor 14 is highly up regulated under drought stress. The role of WRKY transcription factors has been studied by many researchers on many crop plants under abiotic stress (Dong et al., 2003; Eulgem and Somssich, 2007; Eulgem et al., 2000; Mangelsen et al., 2008; Pandey and Somssich, 2009; Ren et al., 2010; Ross et al., 2007; Rushton et al., 2010; Shen et al., 2012). The expression of this transcription factor contributes to the various signaling pathways in plants. The translated protein regulates different functions as a negative or positive regulator. The up-regulation of this gene under drought stress and its confirmation by real-time and the Northern blot analysis indicate that it is involved in various regulatory functions.

We also observed that there is the down regulation of important transcription factors like CCHC-type zinc finger protein, GTPase-activating protein, BTB/POZ, and carbonic anhydrase protein under the drought-stressed root of tef. Gene regulation involves regulation of cis-acting transcription factors like CCHC both in DNA and RNA (Yang et al., 2017). The GAF BTB/POZ domain has also contributed to interactions with non-BTB/POZ proteins which reduced programmed cell death, and is an indication of the negative regulation of plant immune pathway (Orosa et al., 2017). Mitochondrial genes were also down-regulated (Table 1). The biosynthesis and morphological regulation of mitochondria were highly affected by the gene carnitine (Piemontese et al., 2017). This protein was involved in the metabolic pathways to regulate plants under drought stress (Rao et al., 2017). GTP binding proteins are up-regulated under drought stress and are involved in the root hair formation (Wang et al., 2016). In tef root, these genes were up-regulated and the morphology of tef roots under drought stress had less root hair development (Figure 1, Figure 6). Carbonic anhydrase plays a major role in all photosynthetic organism (DiMario et al., 2017) and is involved in the synthesis of lipid molecules in the plant (Ludwig, 2016). It seems that multiple genes of the Carbonic anhydrase are involved in the conversion of lipid in the root cell to facilitate the energy biosynthesis under drought stress; thus, facilitating the root elongation process by fueling for an energy source.

The molecular function of down-regulated genes includes transcription factor, hydrolyzing activity, RNA binding, transmembrane transporter, enzyme inhibiting, oxidoreductase and DNA binding activity (Table 2). TCP encodes plant-specific transcription factors like Transcription factor PCF8. Researches indicated that the down-regulation of PCF8 results in an increased tolerance towards abiotic stress (Yang et al., 2013). Membrane-anchored ubiquitin-fold
protein 3 is involved in all biological process of eukaryotic plants. Mostly, E3 ligase binds to this protein and is involved in the regulation, production, and signaling of plant hormones (Nagels Durand et al., 2016). BTB/POZ PROTEIN 1 is involved in the regulation of crown rootless gene (CRL3) (Yu et al., 2016). The fact that this gene is down regulated in tef indicates its major involvements in delaying the crown root formation while facilitating seminal root elongation. Thus, the morphology of tef root is elongated seminal root with a few crown root formations.

**Conclusion**

Tef responds to drought by elongating the seminal root. One hundred seventy-six (176) differentially expressed transcripts were identified in the roots of the tef plants, under drought and well-irrigated conditions. The differentially regulated transcripts confirm the presence of key regulatory elements controlling the elongation of root related to the drought response. Extensive responsive transcripts are up-regulated under drought stress to sense the amount of water in the soil for proper growth and development. Transcripts linked to biosynthesis are expressed in response to drought stress. Thus, this transcriptome analysis allowed us to find putative targets for further functional investigation of tef root under drought stress. Further studies are essential to characterize the molecular functions of root and leaf transcripts under drought stress. Thus, the information would give us a better understanding to unravel the adaptation of tef under drought specific environmental conditions.

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