Deliverable 1.2: Teff drought stress protocol and optimal timepoint for collecting samples

Work Package 1

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UBERN performed a series of experiments in a growth chamber using a Quncho teff variety. The growth conditions of the chamber were set at a relative humidity of 55%, temperature of 24°C day/18 °C night, light intensity of 170 mmol/m2/s photosynthetically active radiation at plant level and a photoperiod of 12 h light and 12 h dark. Plants were 20 days old (V3 stage) when exposed to water withholding. Plants with optimal watering were watered every 2 days until full water capacity (~170 gr/8 cm^2 pot). In each pot, 5 teff plants were grown.

Five treatments were applied:

- 0: the last day of watering
- 1: one day after withholding water
- 2: two days after withholding water
- 3: three days after withholding water
- 4: four days after withholding water

During these periods, the leaf relative water content (RWC) of teff plants was measured using the second leaf from the top [1] (See Image 1), where a value of 60% was considered as an indicator that plants sense as moisture scarcity or withholding.

Image 1. The V3 stage of teff plant when RWC of the leaf was quantified. The second leaf from the top (indicated in the picture) was used for measuring the RWC). At this stage, the plant possesses a total of 3 leaves.

Ours results showed that after 3 days of water withholding, the RWC of the leaves were 65 % (Figure 1A). At this time point, the average soil water RWC was 41 % (Figure 1B) and the average soil moisture content was 30% (Figure 1C). While soil water content is based on the weight basis, the soil moisture content is the recording from TDR/MUX/mpts probe that measures the dielectric water in the soil (making it a more sensitive measurement). Moreover, this instrument was also used by the BOOSTER partners (UDUS) who did similar experiment in maize. Based on these findings, 3 days after water withholding was selected for large-scale study because a relative water content of ~60% is considered optimal for MOA/RNA-Seq analysis based on previous reports.

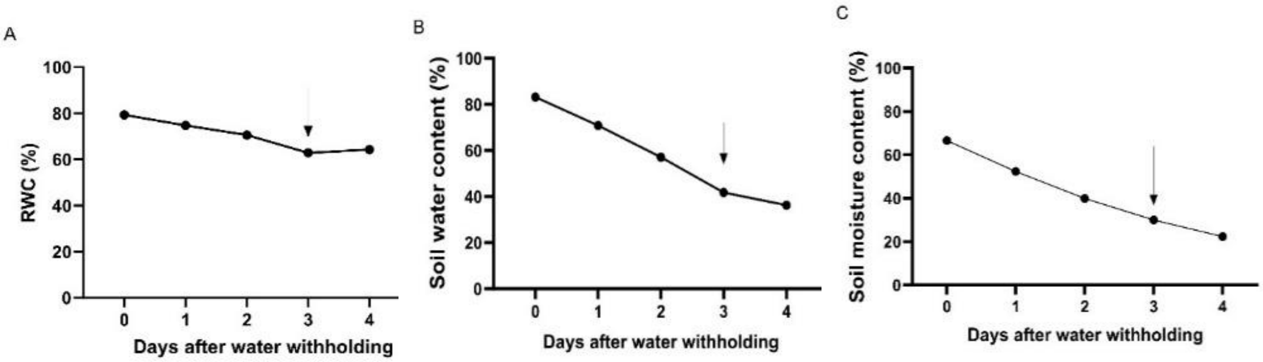

Figure 1. Relative water content of the leaf and soil. Relative water content (%) of the second leaf from the top of Quncho variety. Four biological replicates were used per time point. (B) Soil water content (%) in the pots. The average of four pots were used for each time point. (C) the values of soil moisture content (%) as measured by TDR/MUX/mpts soil moisture probe. Arrow: indicates the treatment chosen for the large-scale experiment.

Criteria to select candidate genes for qPCR analysis of the drought experiment:
Candidate and housekeeping genes were selected based on published work involving drought experiments [2]. For the RT-qPCR analysis, four treatments (0 to 4 days of water withholding) and 3 biological replicates were used in three teff genotypes, namely Quncho, Tsedey and dtt13.

We analyzed a total of six housekeeping and nine other genes. From these, α-tubulin as the most stable housekeeping gene, and two other genes (gene 1 and gene 2) due to their differential expression pattern were selected. The two differentially expressed genes were involved in drought response determined by RNA-Seq in tef (unpublished data from University of Bern). The RT-qPCR results showed that dtt13 had an early response because both gene 1 and gene 2 were upregulated at time point 3, whereas Quncho and Tsedey had a later response at time point 4 (Figure 2).

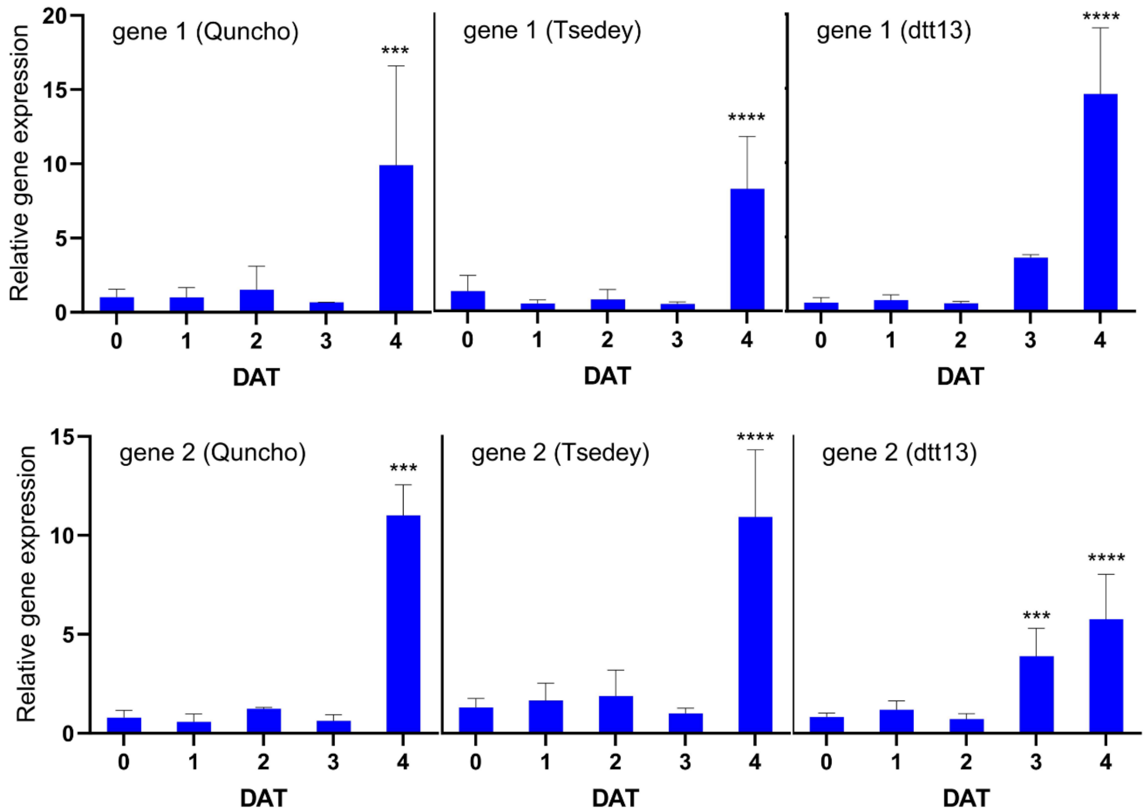

Figure 2. Relative expression of two candidate genes (gene 1 and gene 2) involved in drought response in dtt13, Quncho and Tsedey teff genotypes. Three biological replicates were used for each treatment where each biological replicate contained a pool of three plants. DAT: days after drought treatment where DAT 0 is the last day of watering. The error bars represent the mean ± SD. Comparisons were performed using one-way ANOVA with Dunnett's post hoc testing. Statistical significance was accepted at P < 0.05. Significant differences shown are compared to the control (DAT=0).

The measurement of RWC % in the leaf during the five time points indicated that the three teff genotypes had similar values (Figure 3). The mean RWC after three days of water withholding were 62% for Quncho, 66% for Tsedey and 64% for dtt13 genotypes. This shows that an early maturing genotypes of dtt13 and Tsedey and a late maturing genotype of Quncho retain a similar level of moisture in their leaves after 4 days of no watering.

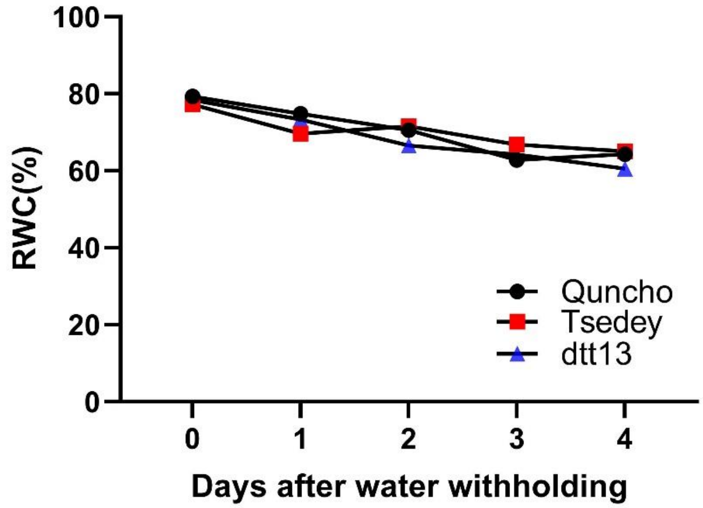

Figure 3. The relative water content (RWC) of Quncho, Tsedey, and dtt13 teff genotypes under different periods of withholding water.

Planning for the large-scale drought experiment

Based on the above controlled experiments in growth chambers, design and conditions for the large-scale experimentation was optimized to quantify the leaf relative water content (%), soil water relative water content (%), soil moisture content as measure by dielectric water (%) and the relative expression of selected drought responsive genes. It is expected that the time point selected above (i.e, 3 days after water withholding) will also reveal differential expression of genes in the RNA-Seq analysis of the 10 F1 teff genotypes to be used in the large-scale drought experiment. Similarly, the same time point will also be used for MOA-Seq analysis in the large-scale drought experiment.

RWC and soil water content of F1 hybrid teff genotypes

The 10 Male parental lines used for crossing to the Quncho female parental line were obtained from the large-scale selection involving phenotypic and genotypic diversity on over three thousand teff accessions collected from different eco-geographic areas in Ethiopia [3]. Descriptions for the 10 crosses and parental lines are presented in the Appendix 1. Quncho was included as a female parent in all of the 10 crosses due to its wide-spread use in many crossing programs involving teff. Twenty-day old plants were exposed to either optimal water condition or to water withholding for 3 days. The RWC from the second leaf from the top was quantified, where four biological replicates were included in the experiment. Interestingly, the leaf RWCs in dtt13, Quncho, and Tesdey genotypes were about 60% after three days of water withholding. Surprisingly, several hybrids particularly cross No 4, 7 and 9 did not show significant differences in RWC of the leaf when exposed to three days of water withholding (Figure 4). In contrast, in crosses No 2, 11 and 12, the RWC was significantly lower after three days of water withholding treatment compared to the well-watered plants. This shows the existence of considerable difference in genotypes in their response to different moisture regimes.

Unlike RWC values, where the differences between the two moisture regimes were more similar across all genotypes, the values of soil water content were significantly different ($p<0.001$) between the two water regimes in 12 crosses (Figure 5). Despite huge differences in the soil water content between well-watered (WW) and drought (WD) treatment, the differences in the soil water content among different teff crosses were negligible. It is, however, important to note that the soil used in this experiment lost its moisture from about 75 % to below 40 % in just three days of water withholding. The minor differences observed among the genotypes might require further investigation where it is related to the rooting pattern or water absorption capacity of different tef genotypes.

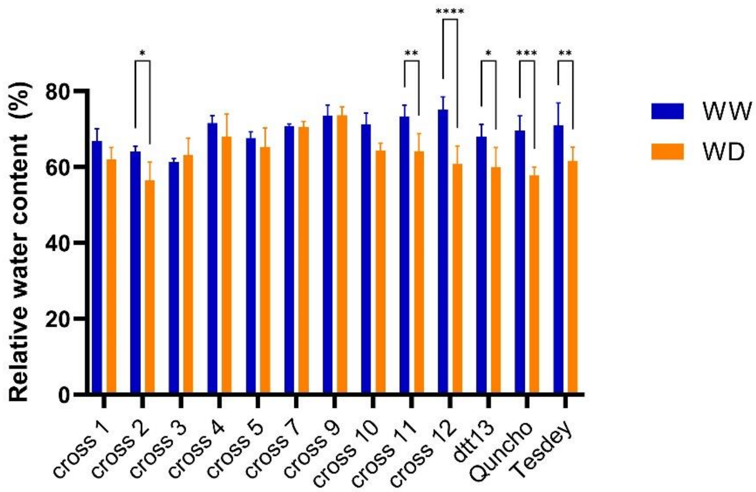

Figure 4. RWC of 10 hybrids crosses, and dtt13, Quncho and Tesdey genotypes. WW: well-watered plants and WD: drought treated plants. The error bars represent the mean ± SD. Comparisons were performed using one-way ANOVA with Dunnett's post hoc testing. Statistical significance was accepted at P < 0.05. Significant differences shown are compared to the control (DAT=0).

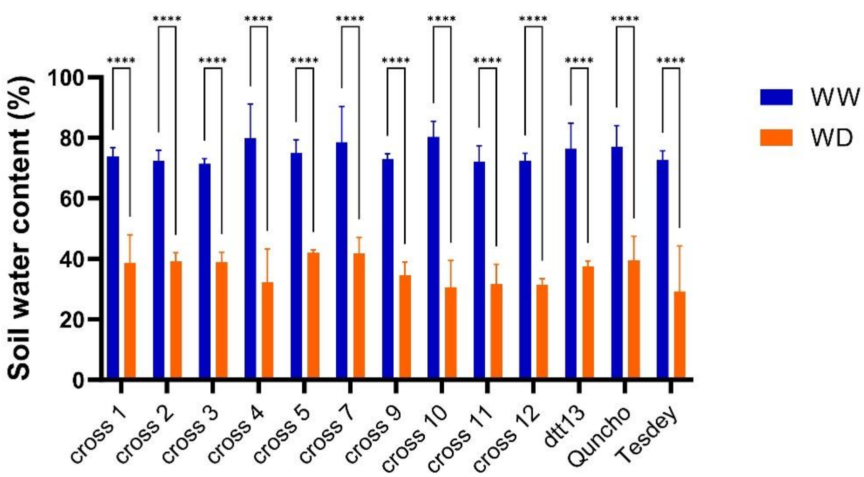

*Figure 5. Soil water content of 10 hybrids crosses, and dtt13, Qunch
o and Tesdey genotypes. WW: well-watered plants and WD: drought treated plants. The error bars represent the mean ± SD. Comparisons were performed using one-way ANOVA with Dunnett's post hoc testing. Statistical significance was accepted at P < 0.05. Significant differences shown are compared to the control (DAT=0).*

References

1. Pardo, J. et al. (2020) Intertwined signatures of desiccation and drought tolerance in grasses. Proceedings of the National Academy of Sciences 117 (18), 10079-10088.
2. Kumar, K. et al. (2013) Reference genes for quantitative real-time PCR analysis in the model plant foxtail millet (Setaria italica L.) subjected to abiotic stress conditions. Plant Cell, Tissue and Organ Culture (PCTOC) 115, 13-22.
3. Woldeyohannes, A.B. et al. (2020) Current and projected eco-geographic adaptation and phenotypic diversity of Ethiopian teff (Eragrostis teff) across its cultivation range. Agriculture, ecosystems & environment 300, 107020.

csv files representing data in D1.2

- Data 1. Relative water content (RWC) of the leaf and soil water content (SWC) obtained during 5 days of water withholding (linked to Figure 1)
- Data 2. Relative gene expression of two candidate genes involved in drought response (linked to Figure 2)
- Data 3. The relative water content (RWC) of Quncho, Tsedey, and Dtt13 genotypes under drought stress(linked to Figure 3)
- Data 4. The relative water content (RWC) of the F1 plants and dtt13, Quncho and Tsedey for Large-scale experiment(linked to Figure 4)
- Data 5. The soil water content (SWC) of the F1 plants and dtt13, Quncho and Tsedey for Large-scale experiment(linked to Figure 5)

Appendix 1. Description of teff lines from Ethiopia used for crossing to generate F1 lines.

Name of the cross	Parent		Description of the male parent			
	Female	Male	Region of collection	Altitude (masl)	Main features	Number of crosses made
Cross 1	Quncho	T_33	Amhara	2352	Very white seed color Very loose panicle type	30
Cross 2	Quncho	T_366	Tigray	2509	Loose panicle type Very white seed color	50
Cross 3	Quncho	T_379	Tigray	1396	Early mature type (<90 days) Very loose panicle type Very white seed color	23
Cross 4	Quncho	T_304	Oromia	2551	Loose panicle type Brown seed color	31
Cross 5	Quncho	T_116	Amhara	1795	Early mature (<90 days) Compact panicle type Dark brown seed color (very strong brown)	26
Cross 7	Quncho	T_87	Amhara	2319	Medium maturity date (90-110 days) Loose panicle and very white seed color	27
Cross 9	Quncho	T_345	SNNP	1372	Loose panicle type Pale white seed color (medium to yellowish)	27
Cross 10	Quncho	T_224	Oromia	2014	Very compact panicle type White seed color	28
Cross 11	Quncho	Boni	Improved variety from dtt2 x dtt13	1400-1600	Loose panicle type Early maturing	25
Cross 12	Quncho	Tesedy	Improved variety	1400-1700	Loose panicle type Early maturing	26