BOOSTER
BOOSTING DROUGHT TOLERANCE IN KEY CEREALS IN THE ERA OF CLIMATE CHANGE

Deliverable 3.1
Collecting soils with a long history of drought

Project acronym	Booster
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Summary

This deliverable requires collection of soil from áreas with a long history of drought in both Africa and Europe for growing and testing of drought tolerance of teff and maize respectively. This has been fulfilled in the case of the European soils (collected by Alphea. Bio) and Ethiopian soils (collected by EIAR). Due to the project starting date, budget availability, the necessity to collect in the growing and flowering season of Eragrostis nindensis and Eragrostis curvula, as well as the bureaucratic logistics of collecting soil from these species, this has been also fullfilled in the best posible by UCT in South Africa. However, to further improve the quality and the amount of the collected simples to be used for developing novel microbial-based biostimulants, UCT will also collect soil from these species in an additional seven distantly located área of South Africa. This will be completed before the end of the year.

Document History

Date	Author	Action	Status
08/10/2023	Jill Farrant	Wrote document and requested input from EIAR and Alphe Bio	draft
18/10/23	Jill Farrant	Circulated to all partners	draft
27/10/23	Jill Farrant	Sent to the coordinator	final

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Acronyms and abbreviations

APBIO	Aphea.Bio
AT-TR & RS:	Alem Tena teff rhizosphere soil & root sample
AT-PRS & RS:	Alem Tena pilosa rhizosphere soil & root sample
AT-BSS:	Alem Tena bulk soil sample
CRO's	Contract Research Organizations
EIAR	Ethiopian Institute of Agricultural Research
UCT	University of Cape Town
VIB	Vlaams Instituut voor Biotechnologie

1. Introduction

The aim of this deliverable in WP3, reported here, is to collect rhizosphere associated soil from drought prone areas of Africa where Eragrostis species occur naturally for work on teff, and in such areas in Europe for work on maize. UCT are responsible for collection of soil samples from *Eragrostis nindensis* and *Eragrostis curvula* (4 different areas each) and EIAR are responsible for collecting soils associated with *Eragrostis pilosa* and *Eragrostis tef* (4 different areas each). UCT will send soils to EIAR for growing of teff and testing for drought tolerance. Aphea.Bio are responsible for collection soils (20 different areas) in Europe. These will be sent to VIB for testing of maize drought tolerance.
The African researchers have initiated a project within this deliverable, not described in the original WP3. They are isolating bacteria from the collected soils and EIAR will use these to test drought tolerance of teff along side soil trials.

2. Work completed in Africa

2.1 UCT.

Soil samples from 2 sites at Aggeneys (S 33(deg) 55.968; E 18 (deg) 29.554; Elevation 891) Northern Cape South Africa, were collected in early May, flowering season for *E.nindensis* in that area. Note that this is a Winter rainfall área, hence flowing in May. Due to the need to collect in the growing season and because the bureaucratic logistics of collecting soil from these species, which includes application for permits and the extremely remote sites and long distances that need to be travelled, an additional collecting trip will take place in Summer rainfall áreas in November and December 2023

2.1.1 Bacteria have been isolated from the soil collected from Aggenys using 3 different media, M9, Jensen's and King's B likely to select for drought tolerant and nitrogen fixing strains. These will be sent to EIAR with all the soils and further bacteria isolated from newly collected soils.

2.2 EIAR.

Soil samples were collected from four locations with a long history of drought namely; Alme Tena (Oromia Region), Dhera (Oromia Region), Humbo (South Ethiopia Region) and Abala Abaya (South Ethiopia Region). Geographic location (latitude, longitude, and

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altitude), region, district, specific local names of the four sample collection areas are given in the tables below.

Soil sample collection was made following the standard protocol given by Barillot et al. (2013). Accordingly, five individual samples of teff rhizosphere soil and roots were collected at five-points (four from each corner and one from center) from the 1,250 m^2 plot of each sampling location. For rhizosphere soil sample collection, individual teff and pilosa plants with their tillers were uprooted and vigorously shacken to remove bulk soils. Moreover, the soil close to the root surface was gently scraped off using sterile cotton swabs and placed into a labeled polyethylene bags for the rhizosphere soil colonizing microbe isolation. A total of 300 samples of cultivated tef rhizosphere soil, *Eragrostis pilosa* rhizosphere soil and root with adhering soils were collected and kept in a ice-box and transported to the microbiology laboratory. The other remaining soil samples will be used to grow the four selected teff genotypes (two susceptible and two tolerant genotypes) in the green house.

2.2.1 Isolation of microbes is simultaneously being conducted in the Microbiology laboratory. To this end, a laboratory protocol for microbes isolation has been developed and the necessary chemicals and consumables procured and currently, the microbe isolation work is in progress.

Table 2.1 Sample collection sheet (Dhera)

FIELD SAMPLE COLLECTION SHEET - Dhera District									
Date of Sample Collection: 19 September 2023									
	Sample code	**Location of sampling area**			**GPS data**				
No	**Code**	**Region**	**District**	**Specific area**	**Latitude**	**Longitude**	**Altitude**	**Teff Variety/wild**	**Crop Stage**
1	DR-TRS&RS	Oromia	Dhera	Dilfegar	8º17'37"	39º19'29"	1690	Local	Flowering
2	DR-TRS&RS	Oromia	Dhera	Dodota	8º17'0.8"	39º18'19"	1721	Boset	Flowering
3	DR-TRS&RS	Oromia	Dhera	Fechiso	8º21'24"	39º19'43"	1593	Local	Flowering
4	DR-TRS&RS	Oromia	Dhera	Dilfegar	8º17'34"	39º19'31"	1692	Wild	Flowering
5	DR-TRS&RS	Oromia	Dhera	Dodota Alem	8º16'59"	39º18'17"	1717	Wild	Flowering
6	DR-TRS&RS	Oromia	Dhera	Dhera (02)	8º19'10"	39º19'11"	1680	Wild	Flowering
7	DR-TRS&RS	Oromia	Dhera	Dodota Alem	8º17'19"	39º18'38"	1713	Non-Agr	-
8	DR-TRS&RS	Oromia	Dhera	Dhera (02)	8º19'6"	39º19'14"	1686	Non-Agr	-
9	DR-TRS&RS	Oromia	Dhera	Bodecha	8º22'20"	39º19'46"	1577	Non-Agr	-

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Table 2.2 Sample collection sheet (Alem Tena)

FIELD SAMPLE COLLECTION SHEET - Dhera District									
Date of Sample Collection: 15 September 2023									
	Sample code	Location of sampling area			GPS data				
No	Code	Region	District	Specific area	Latitude	Longitude	Altitude	Teff Variety/wild	Crop Stage
1	AT-TRS&RS	Oromia	Alemtena	Elen	08º19'28"	38º58'19"	1609	Boset	Flowering
2	AT-TRS&RS	Oromia	Alemtena	Tuka Langand	08º15'52"	38º54'39"	1686	Boset	Flowering
3	AT-TRS&RS	Oromia	Alemtena	Station	08º18'26"	38º57'09"	1646	Boni	Flowering
4	AT-PRS&RS	Oromia	Alemtena	Elen	08º18'26"	38º58'19"	1609	Pilosa	Flowering
5	AT-PRS&RS	Oromia	Alemtena	Tuka Langand	08º19'28"	38º58'43"	1668	Pilosa	Flowering
6	AT-PRS&RS	Oromia	Alemtena	Elen	08º19'28"	38º57'09"	1645	Pilosa	Flowering
7	AT-BSS	Oromia	Alemtena	Elen	08º19'28"	38º58'21"	1608	Non agr.	NA
8	AT-BSS	Oromia	Alemtena	Tuka Langand	09º17'15"	38º56'16"	1660	Non agr.	NA
9	AT-BSS	Oromia	Alemtena	Station	08º18'30"	38º57'09"	1640	Non agr.	NA

Table 2.3 Sample collection sheet (Humbo)

FIELD SAMPLE COLLECTION SHEET – Humbo District									
Date of Sample Collection: 11 October 2023									
	Sample code	Location of sampling area			GPS data				
No	Code	Region	District	Specific area	Latitude	Longitude	Altitude	Teff Variety/wild	Crop Stage
1	HU-TRS&RS	South Eth	Humbo	Ampokoishe	6º43'31"	37º47'27"	1599	Gomadie	Flowering
2	HU-TRS&RS	South Eth	Humbo	Ampokoishe	6º43'43"	37º48'24"	1590	Gomadie	Flowering
3	HU-TRS&RS	South Eth	Humbo	Ampokoishe	6º43'39"	37º47'55"	1593	Gomadie	Flowering
4	HU-PRS&RS	South Eth	Humbo	Ampokoishe	6º43'47"	37º48'29"	1590	Pilosa	Flowering
5	HU-PRS&RS	South Eth	Humbo	Ampokoishe	6º43'46"	37º48'23"	1589	Pilosa	Flowering
6	HU-PRS&RS	South Eth	Humbo	Ampokoishe	6º43'54"	37º48'22"	1595	Pilosa	Flowering
7	HU-BSS	South Eth	Humbo	Ampokoishe	6º43'48"	37º48'23"	1590	Grass	-
8	HU-BSS	South Eth	Humbo	Ampokoishe	6º43'30"	37º47'23"	1597	Grass	-

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9	HU-BSS	South Eth	Humbo	Ampokoi she	6º43'34"	37º47'24"	160 8	Grass	-

Table 2.4 Sample collection sheet (Abela Abaya)

FIELD SAMPLE COLLECTION SHEET – Abela Abaya									
Date of Sample Collection: 12 October 2023									
	Sample code	**Location of sampling area**			**GPS data**				
No	**Code**	**Region**	**District**	**Specific area**	**Latitude**	**Longitude**	**Altitude**	**Teff Variety/wild**	**Crop Stage**
1	AB-TRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'23"	37º47'26"	1430	Gomadie	Flowering
2	AB-TRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'24"	37º47'34"	1420	Tsedey	Flowering
3	AB-TRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'29"	37º47'40"	1417	Tsedey	Flowering
4	AB-PRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'19"	37º47'28"	1430	Pilosa	Flowering
5	AB-PRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'18"	37º47'28"	1433	Pilosa	Flowering
6	AB-PRS&RS	South Ethiopia	Abela Abaya	Sipa	6º40'17"	37º47'22"	1440	Pilosa	Flowering
7	AB-BSS	South Ethiopia	Abela Abaya	Sipa	6º40'23"	37º47'26"	1430	Grass	NA
8	AB-BSS	South Ethiopia	Abela Abaya	Sipa	6º40'23"	37º47'31"	1422	Grass	NA
9	AB-BSS	South Ethiopia	Abela Abaya	Sipa	6º40'29"	37º47'40"	1417	Grass	NA

References

Barillot, C.D.C., Sarde, CO., Bert, V. et al. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. Ann Microbiol 63, 471–476 (2013). https://doi.org/10.1007/s13213-012-0491

3. Work completed in Europe

Representative soil samples enriched in plant rhizosphere were collected from regions with a long history of drought. Locations were chosen based on the knowledge and experience of the EU farmers “Contract Research Organizations”. Soil history cannot be proven by hard data. Locations were chosen where severe drought is experienced every year and where field drought is frequently recurring. Samples from 20 different sites in Italy and Spain (Table 3.1) were taken based on APBIO CRO’s network. The table below gives an overview of the soils that were chosen based geographic spread and soil types. Rainfall in the South West of Spain decreases annually, according to data from the Guadalquivir Hydrographic Confederation, it is the seventh driest year in the historical series and shows that the average rainfall is 22% lower than that recorded in the last 25 years. During last summer, high temperatures reached maximum temperatures ever recorded in the peninsula, in which Andalusia has once again been in the lead, according to the Spanish Meteorological Agency (Aemet). The latest annual Environment report published by the Ministry of Sustainability, Environment and Blue Economy reports that the average temperature in Andalusia in 2022 was 0.4 degrees higher than the historical average of the territory. The lack of rain and high temperatures form the perfect storm for the emergence of a situation of scarcity that is extreme due to the overexploitation of water resources due to economic activities such as intensive agriculture and tourism,

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which makes the demand for the use of water rise exponentially, most acutely during the summer months. The Guadalquivir and Guadiana basins are currently at 18.10% and 23.70% of their respective capacities, which greatly limits the use of water not only in agriculture but also for the consumption of the population.

Mazarrón: Soil was sampled from Mazarrón (Murcia), in the South-East of Spain, where climate is characterized by hot, arid summers and cool, dry and windy winters. Annual average temperatures range between 5ºC and 30ºC, and precipitation is around 280 mm per year.

Canals: Soil was sampled from Canals (Valencia), in the East of Spain, where climate is characterized by hot, short summers and long, cool and windy winters. Annual average temperatures range between 4ºC and 33ºC, and precipitation is around 500 mm per year, most of them in winter.

Garrapinillos: Soil was sampled from Garrapinillos (Zaragoza), in the North-East of Spain, where climate is characterized by hot, dry, sunny summers and cold winters. Wind can be present at every season, it is especially strong in winter and spring. Annual average temperatures range between 2ºC and 33ºC, and precipitation is around 315 mm per year, most of them in spring and autumn.

Bea: Soil was sampled from Bea (Teruel), in the North-East of Spain, where climate is characterized by short, hot, dry summers and long and very cold winters. Annual average temperatures range between -2ºC and 27ºC, and precipitation is around 250 mm per year, most of them in spring and autumn.

Daroca: Soil was sampled from Daroca (Zaragoza), in the North-East of Spain, where climate is characterized by short, hot, dry summers and long and very cold winters. Annual average temperatures range between 0ºC and 29ºC, and precipitation is around 250 mm per year, most of them in spring and autumn.

Table 3.1 Description of sites of soil collection

Research Number	ID	Country	Region	Other
780635	**ES01**	Spain	Badajoz	Montijo
780636	**ES02**	Spain	Badajoz	Bienvenida
780630	**ES03**	Spain	Cadiz	San Lucar de Barrameda
780626	**ES04**	Spain	Cadiz	Rota
780620	**ES05**	Spain	Sevilla	Marchena
780492	**ES06**	Spain	Sevilla	Los Palacios
780632	**ES07**	Spain	Sevilla	El Viso del Alcor
780631	**ES08**	Spain	Huelva	Rociana del Condado
780623	**ES09**	Spain	Huelva	Chucena
780619	**ES10**	Spain	Huelva	Almonte

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780622	**ES11**	Spain	Zaragoza	Bea
780624	**ES12**	Spain	Murcia	Mazarron 1
780625	**ES13**	Spain	Murcia	Mazarron 2
780628	**ES14**	Spain	Valencia	Canals
780627	**ES15**	Spain	Zaragoza	Garrapinillos
780629	**ES16**	Spain	Zaragoza	Daroca
780618	**FR01**	France	Bourdic	
780633	**FR02**	France	Tolouse	Le Fousseret
780621	**IT01**	Italy	Martini	EU-23-1873-04
780634	**IT02**	Italy	Zanellati	EU-23-1873-03

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Risk Description	Likelihood	Impact	Proposed risk-mitigation measures	WP
General and Management Risks				
Promblem in sending soils and microbes from South Africa to Ethiopia	Low	Low	Both countries are party to the Nagoya Protocol. We will ensure that we follow exact legislation to safely get the material into Ethiopia.	
Soils from Italy	Low	Low	With the exception of Italy, all countries from which soils were collected have signed the Nagoya Protocol. However, there are no ABS rules in Italy regarding genetic resources in and from Italy and thus soil samples from Italy used in the project are free of ABS obligations.	3
Technical risks				
NA				
Dissemination and exploitation risks				
Too early				

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