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LL002 on crops in drought conditions: a multidisciplinary study

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Introduction

Today's agriculture is called to face multiple challenges: to produce more, to cope with the rising food demand, to be more efficient to counter resource-scarcity and to preserve soils, water and air as well as human health.
Plants for Plants (P4P) aims to improve resource efficiency of crops by introducing a new category of biostimulants. In actual practice, biostimulants are often a useful destination for waste materials or by-products, so the discovery of functional active ingredients is rather casual. P4P reverses this approach: first the problem is identified and then tailored compounds, based on specific Standardized Metabolites Phytocomplexes (SMPs) physically extracted from suitable organically grown plants, are produced and tested.
The developed biostimulants enhance crops' ability to use water, nutrients and to get in a stronger physiological status. The LL002 biostimulant is based on a synergistic combination of flavonoids and organic acids: it increases resilience to abiotic stress, and in particular it boosts Water Use Efficiency leading to significant reduction of water deficiency symptoms and increased crop yield and quality. LL002 properties were demonstrated in controlled environments, using cutting-edge phenotyping technologies, biochemical and molecular tools, and in open-field trials on a wide variety of crops across Europe. It supports plants in water deficit by e.g. increasing root biomass in tomato, decreasing wilting in young corn plants and increasing yield in rainfed and poorly irrigated crops. Additionally, it showed positive results in so-called comfort environments proving that even plants grown in optimal conditions are far from exploiting all their genetic potential.

Reversing the approach

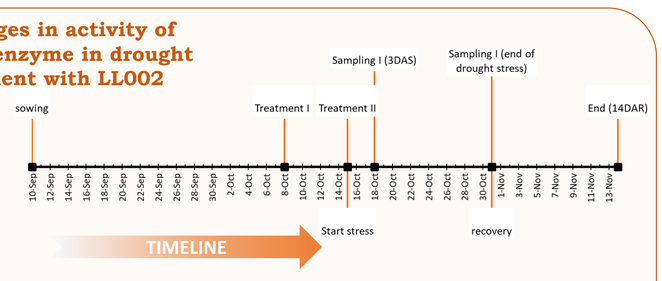
Define the **challenge** → Identify the suitable **SMP*** → Scout for the **source** → Set up the **extraction** method → **Formulation**

With a strong green soul

* Standardized Metabolites Phytocomplex

- Model plant: Sugarbeet
- Drought stress: Stop watering + recovery
- Assessments:
  - Fresh biomass of shoot and root
  - Enzymactic activity: Catalase and Guaiacol Peroxidase
  - H_2O_2 measurement

Study on the changes in activity of some antioxidant enzyme in drought stress upon treatment with LL002



The treatment with LL002 decreased H_2O_2 content (Fig A), thus decreasing oxidative stress. In fact, the enzymes for ROS detoxification where not as active as the UTC in drought conditions (Fig. B). As result, root biomass was not affected by drought stress, at least at the higher dosage (Tab. 1)

Tab. 1 ■ 1st sampling ■ 2nd sampling

	3 DAS				End of drought stress				14 DAR			
Entry	FB shoot		FB root		FB shoot		FB root		FB shoot		FB root	
UTC, drought	5,574	ab	1,961	a	4,151	ab	3,935	a	4,756	a	4,756	a
LL002P, drought, low	5,125	a	1,548	a	3,457	a	3,794	a	4,178	a	4,807	a
LL002P, drought, high	6,832	b	2,110	a	3,475	a	3,317	a	4,172	a	5,898	bc
UTC, well-watered	5,809	ab	2,030	a	5,233	b	3,954	a	6,194	b	6,505	c

Fig. A H_2O_2

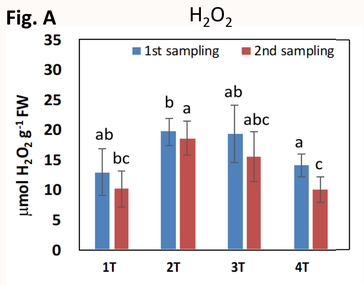
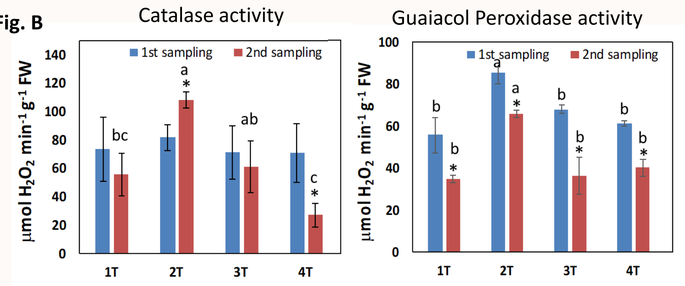


Fig. B Catalase activity / Guaiacol Peroxidase activity

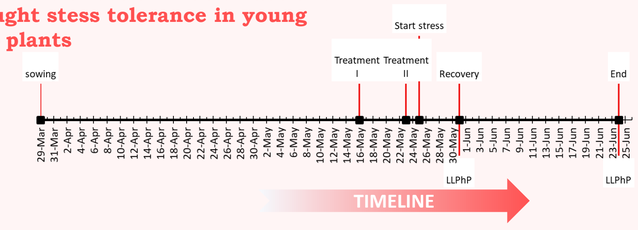


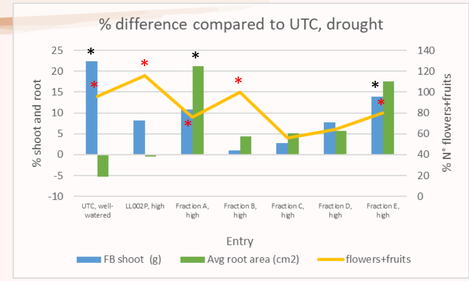
Tab 1. Statistical analysis of fresh weight of roots and shoots.
Fig A. H_2O_2 measurement.
Fig B. Enzymatic activity of Catalase (left) and Guaiacol Peroxidase (right).

1T : UTC, well-watered
2T : UTC, drought
3T : LL002, drought, low
4T : LL002, drought, high

- Model plant: Tomato
- Drought stress: Stop watering + recovery
- Assessments:
  - Fresh biomass of shoot and root
  - Landlab Phenotyping Platform (LLPhP)

Study on the effect of LL002 fractions on drought stess tolerance in young tomato plants



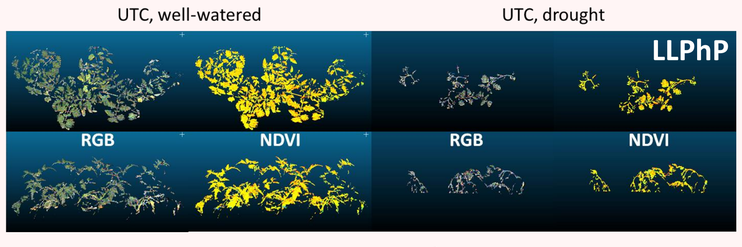


LL002 main fractions were made by SPE fractionation. LL002 and all the fractions had a positive effect on N° of flowers and fruits and on shoot biomass (FB) as a trend, however, only fraction E was statistically significant better than the negative control in term of FB. Instead, for fruits and flowers number, the product *per se* was the best one, followed by fractions B, A and E (Fig. C). The LLPhP parameters show that some of the treatments decreased the senescence index (PSRI) while increasing the NDVI at the peak of drought stress (Tab. 2).

Fig. C

Tab. 2

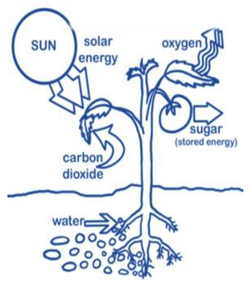
	drought	rec	drought	rec	drought	rec	drought	rec	drought	rec	drought	rec	drought	rec
Entry ↴ LLPhP parameters →	Digital biomass		Leaf angle		3D Leaf area		greeness avg		NDVI avg		NPCI avg		PSRI avg	
UTC, well-watered	1,4E+07	2,3E+07	29,95	31,31	70549	94421	0,08	0,12	0,49	0,51	0,18	0,20	0,12	0,10
UTC, drought	3,0E+06	2,0E+07	21,52	31,28	21550	81376	-0,04	0,13	0,36	0,52	0,11	0,18	0,19	0,09
LL002P, drought, high	4,8E+06	1,9E+07	22,79	31,84	30484	83530	-0,02	0,14	0,40	0,52	0,13	0,19	0,15	0,10
Fraction A, drought, high	3,1E+06	2,0E+07	20,81	32,41	22317	86952	-0,04	0,15	0,38	0,53	0,10	0,19	0,15	0,09
Fraction B, drought, high	1,7E+06	1,9E+07	20,55	31,83	16090	81193	-0,06	0,11	0,34	0,50	0,11	0,19	0,17	0,11
Fraction C, drought, high	1,7E+06	1,9E+07	19,46	32,09	13977	80587	-0,05	0,15	0,32	0,51	0,11	0,19	0,16	0,10
Fraction D, drought, high	1,9E+06	1,8E+07	20,81	31,88	15265	80366	-0,01	0,17	0,37	0,53	0,13	0,19	0,14	0,07
Fraction E, drought, high	2,3E+06	1,9E+07	21,11	32,49	17792	81266	-0,01	0,18	0,39	0,54	0,13	0,19	0,12	0,07



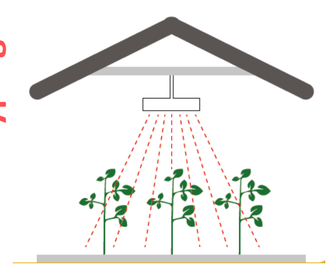
Tab 2. Statistical analysis of the LLPhP parameters. NPCI (chlorophyll index), PSRI (senescence index).
Green / Orange:
Positive / negative statistical difference with the UTC, drought; Light green / orange: positive / negative trend compared to the UTC, drought
Fig C. FB of the shoot (g), roots area (sqcm) and N° of flowers and fruits at the end of the trial. The asterisks indicate significant differences with the negative control in shoot and root (black) and in flowers and fruits (red).

Biochemistry

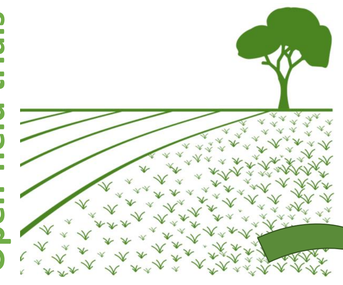
Physiology



High-throughput Phenotyping

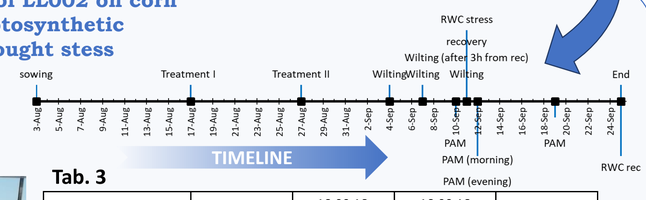


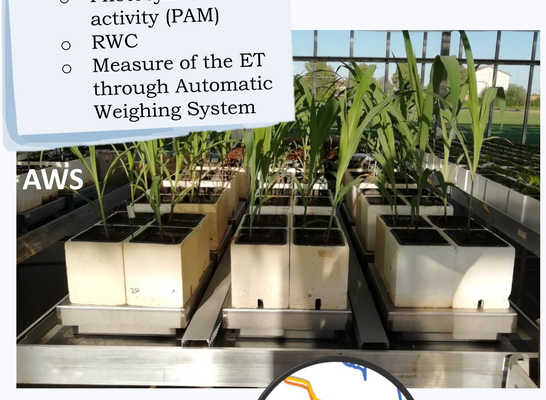
Open-field trials



- Model plant: Corn
- Drought stress: Stop watering + recovery
Assessments:
  - Wilting
  - Photosynthetic activity (PAM)
  - RWC
  - Measure of the ET through Automatic Weighing System

Study on the effect of LL002 on corn traspiration and photosynthetic efficiency during drought stess





Tab. 3

Entry PAM LA	10.09.18		12.09.18 (morning)		12.09.18 (evening)		19.09.18	
UTC, drought	0,19	a	0,64	a	0,67	a	0,55	a
LL002P, low, drought	0,22	a	0,67	ab	0,71	a	0,60	b
LL002P, high, drought	0,24	a	0,68	b	0,67	a	0,58	ab
UTC, well-watered	0,68	b	0,72	c	0,67	a	0,57	ab

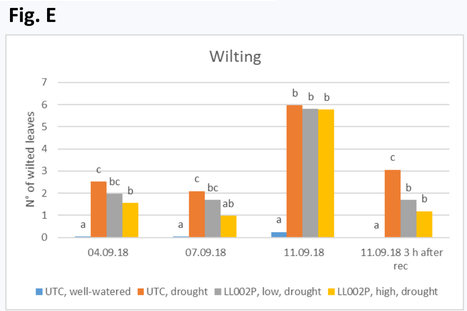
Entry PAM DA	10.09.18		12.09.18 (morning)		12.09.18 (evening)		19.09.18	
UTC, drought	0,54	a	0,72	a	0,76	a	0,76	a
LL002P, low, drought	0,56	a	0,74	ab	0,78	a	0,77	a
LL002P, high, drought	0,54	a	0,75	b	0,75	a	0,77	a
UTC, well-watered	0,75	b	0,80	c	0,75	a	0,76	a

Tab. 4

Entry	RWC stress		RWC rec	
UTC, drought	56,51	a	87,67	a
LL002P, low, drought	57,47	a	88,41	ab
LL002P, high, drought	58,46	a	88,48	ab
UTC, well-watered	89,53	b	89,75	b

Fig. D

Fig. E

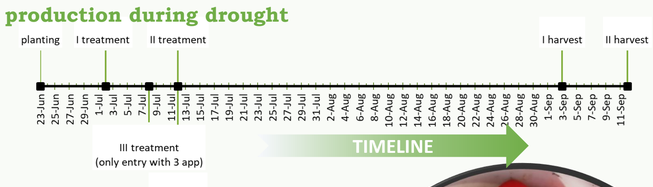


Treatment decreased wilting compared to the negative control during drought stress (Fig. E), and accelerate recovery (in terms of wilting, Fig. E, photosynthetic activity, Tab. 3, and RWC, Tab. 4). The decreased water loss during drought stress is confirmed by the reduced transpiration observed through the Automatic Weighting System (AWS) (Fig. D).

Tab 3. Photosynthetic activity measured with a portable PAM of light adapted (LA) or dark adapted (DA) plants.
Tab 4. RWC at the peak of drought stress (RWC stress) and after recovery (RWC rec)
Fig D. Evapotranspiration trend during the drought stress (1=UTC, well-watered; 2=UTC, drought; 3=LL002, low, drought; 4=LL002, high, drought)
Fig E. N° of wilted leaves at different timepoints.

- Model plant: Processing tomato
- Drought stress: -30% irrigation
- Assessments:
  - Weight and N° of fruits in two harvests
  - Brix and Firmness

Study on the effect of LL002 on tomato production during drought stress



Tab. 5

Entry	Weight/plot (g) I		N°/plot I		Weight/plot (g) II		N°/plot II		Green/plot (g) II		N green/plot II	
UTC, 70%	4755,8	a	91,7	a	2787,6	a	64,1	a	2747,8	a	66,4	ab
LL002 dosage 1, 70%	5268,5	ab	104,9	ab	2971,6	ab	69,0	a	1620,6	a	45,9	a
LL002 dosage 2, 70%	6261,0	abc	119,3	abc	3457,2	ab	68,3	a	2901,2	a	68,8	ab
UTC, 100%	7103,0	bc	135,0	abc	4337,7	b	88,5	a	3681,1	a	90,5	b
LL002 dosage 2, 70%, 3 app	7589,2	c	155,4	c	3516,0	ab	77,9	a	2218,4	a	57,6	ab

Tab. 6

Entry	°Brix I		Firmness I		°Brix II		Firmness II	
UTC, 70%	4,7	a	4,1	a	4,0	a	4,8	a
LL002 dosage 2, 70%, 3 app	4,8	a	4,4	a	4,1	ab	5,1	a
UTC, 100%	4,6	a	4,1	a	4,4	bc	4,7	a
LL002 dosage 2, 70%	4,5	a	4,0	a	4,6	c	4,8	a
LL002 dosage 1, 70%	4,7	a	4,3	a	4,7	c	5,0	a

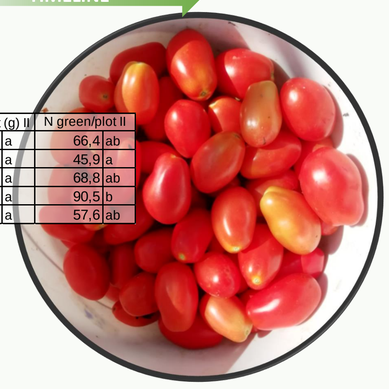
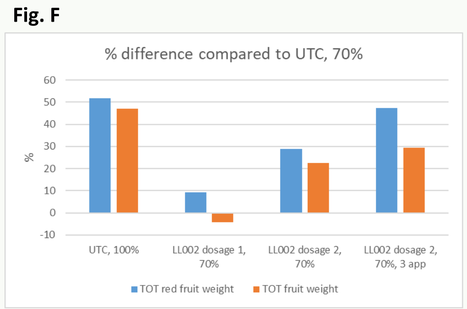


Fig. F



Treatment with LL002 decreased drought stress effect on fruits production in processing tomato (Tab.5) and slightly increased the Brix at the second harvest (Tab. 6). At the end the production of the treated plants (at high dosage) was comparable with the one of the untreated with 100% irrigation (Fig. F).

Tab 5. Harvests parameters: weight and N° of fruits per plot.
Tab 6. Analysis of the fruits: °Brix and Firmness
Fig F. % difference compared to UTC 70% of TOT red fruits weight and TOT fruits weight.

Conclusions:

With the water becoming a scarse resource and a precious commodity and the climate change, the need of products that increase the efficiency of water usage by crops is rising. The plantsforplants project has created a new biostimulant made to overcome drought stress related issues on a wide variety of crops. In this work, we show that treatment with LL002 was able to oppose the negative effect of drought stress (both as severe drought stress and as reduction of irrigation) in different crops (sugarbeet, corn and tomato). This result was proven using different investigation tools, by analysing the activity of some enzymes involved in ROS scavenging and measuring ROS production, using the high-troughput phenotyping system (LLPhP) to give automatically many morphological and physiological informations, analysing plants transpiration and photosynthetic efficiency and by the conventional approach of an open-field trial. Overall, the results show an improved performance of the treated plants compared to the untreated plants in the same stressed conditions in the majority of the parameters analysed, proving the efficacy of this new product.

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LIFE Plants for Plants

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