Microbial BIOFECTORS for sustainable Plant Nutrition: technical, legal and economic aspects

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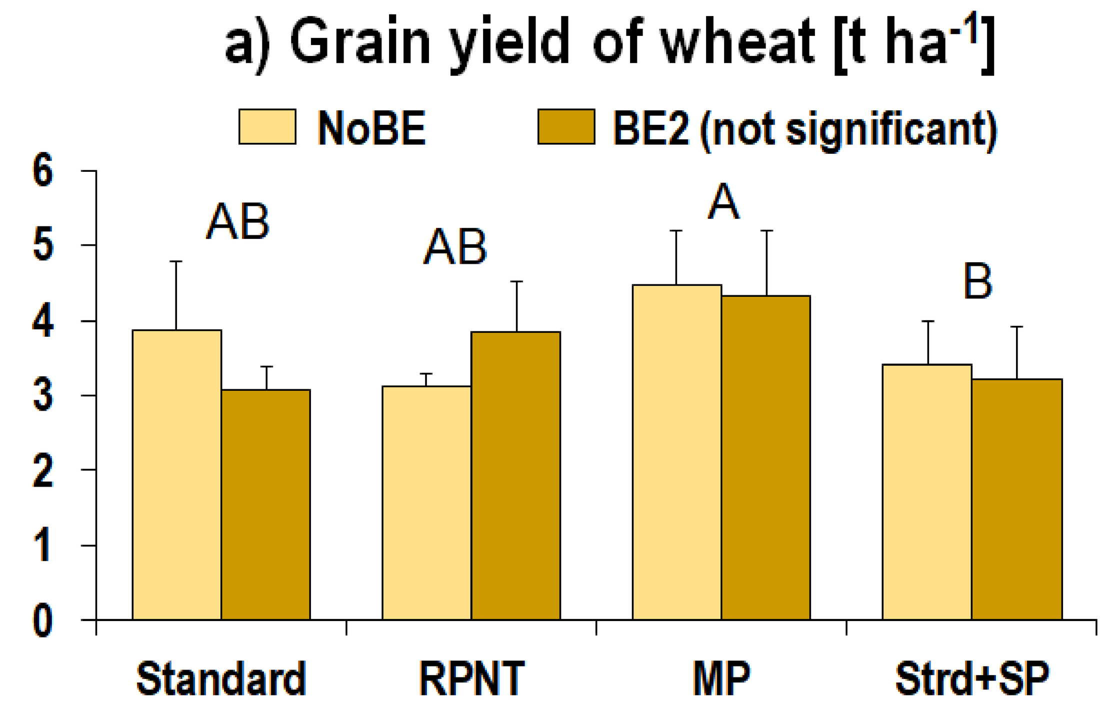
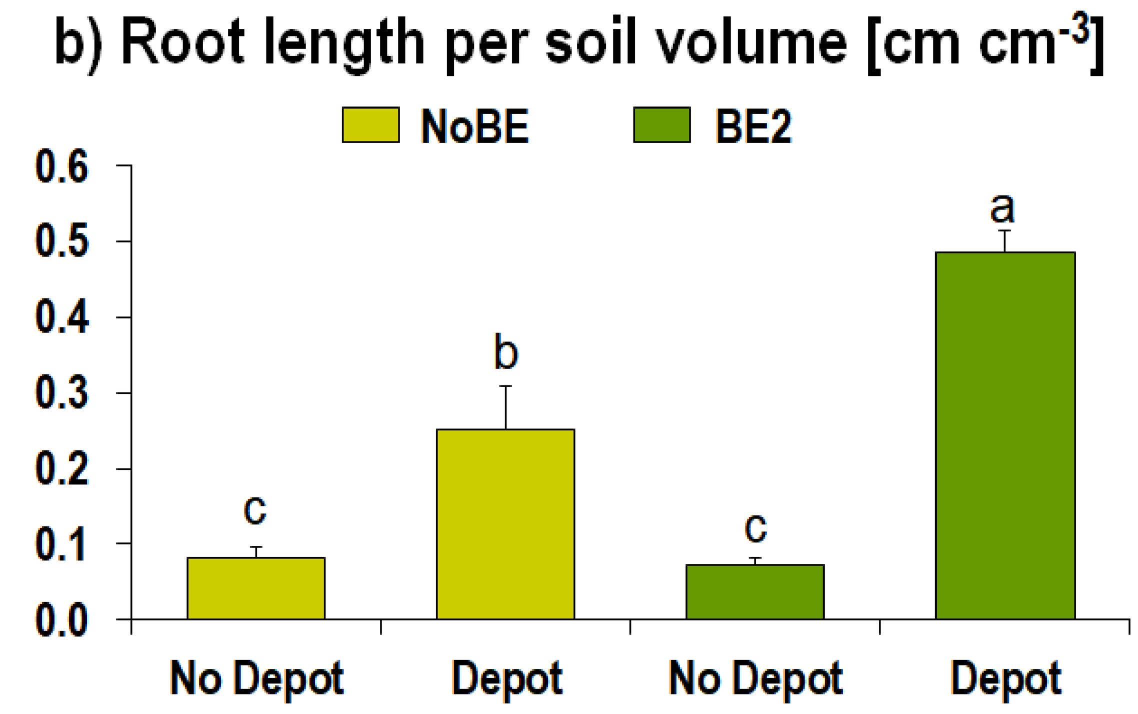
Background

- Microbial bio-effectors, such as beneficial strains of *Pseudomonas*, *Bacillus* and *Penicillium* spp., can promote plant growth by multifaceted modes of action. This includes (i) improved root growth and mycorrhization, (ii) mobilization of sparingly available mineral nutrients, (iii) enhanced stress resistance, (iv) synergistic interactions with other helpful organisms, as well as (v) general or specific effects against pathogens.
- Recently, the economic interest to utilize microbial bio-effectors as alternatives or supplements to conventional pesticides or fertilizers has been growing vigorously, but a lack of adequate formulation and application technologies together with increasingly stringent legislations in the European Union concerning the registration of active agents under the plant protection or fertilizer law hampers further progress in that field.
- Aware of these challenges, the EU project "BioFector" aimed to develop comprehensive biological approaches to integrate the versatile actions of plant growth-promoting bacteria and fungi for implementation in sustainable fertilization strategies.

Results from Wheat, Maize and Tomato Experiments under Practice Conditions





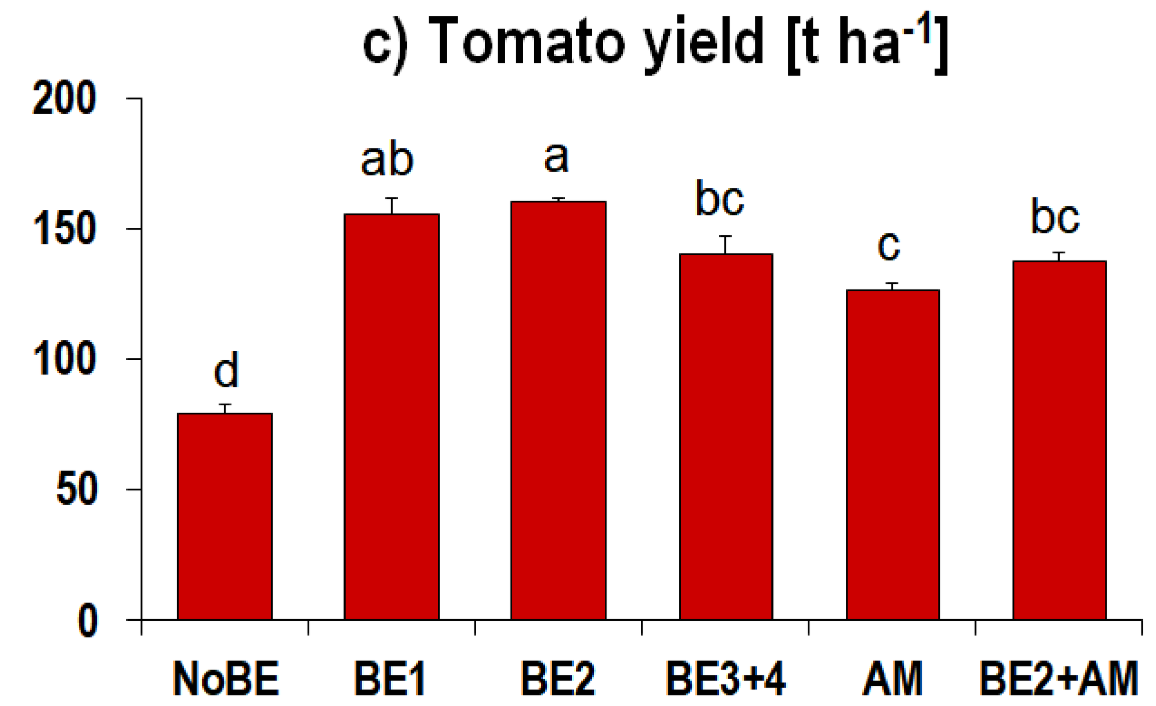


Fig. 1: (a) Application of a *Pseudomonas* product (BE2) did not clearly improve the yield of winter wheat grown under field conditions at Horb am Neckar, Germany (2017) with different fertilization strategies (Standard (Strd) = nitrate based fertilization according to the local farming practice, RPNT = rock phosphate plus stabilized ammonium sulfate (NovaTec® solub 21); MP = Manure Pellets; SP = Super Phosphate). **(b)** In maize grown at Ihinger Hof, Renningen, Germany (2014), BE2 could further increase the rooting density for the efficient exploitation of stabilized ammonium fertilizer depots in soil. **(c)** The yield and profitability of tomato production with organic fertilization under greenhouse conditions at Timişoara, Romania (2015) was increased by treatments with *Penicillium* (BE1), *Pseudomonas* (BE2), *Bacillus* (BE3, BE4), or arbuscular mycorrhizal (*Rhizophagus irregularis*, AM) products in single and combined applications. (NoBE = no bio-effector treatment; Tukey's test, $p \leq 0.05$)

Experimental approaches

Winter wheat (*Triticum aestivum* L.) var. Kometus, SZ Schweiger, Germany

Soil: Rendzina on chalkstone, silty clay, pH ($CaCl_2$) 7.2; 3 mg $P_{(CAL)}$ 100 g^{-1} soil (low)
Standard (Strd): 200 kg N ha^{-1} (1 x ASN, 2 x CAN); **RPNT:** 20 kg P ha^{-1} as rock phosphate (Granuphos), 200 kg N ha^{-1} in two rates as stabilized AS (NovaTec® solub 21); **MP:** 46 kg N, 20 kg P ha^{-1} as manure pellets (Agriges) with addition of nitrification inhibitor (Entec), 154 kg N ha^{-1} (1 x ASN, 2 x CAN); **Strd+SP:** Strd plus 100 kg P ha^{-1} as super phosphate.
BE2: Proradix® WP, 1st rate (1.5×10^{15} cfu ha^{-1}) as broad cast with soil incorporation before sowing, 2nd rate as broad cast on top of the plants at vegetation start in March.

Maize (Zea mays L.) var. Colisee, KWS, Germany

Soil: Luvisol/Cambisol, silty clay loam, pH 6.9; 12.0 mg P (CAL) 100 g^{-1} soil (high)
Fertilization: 17 kg N + 35 kg P ha^{-1} as underfoot placement of MAP, 135 kg N ha^{-1} as depot of stabilized AS (NovaTec® solub 21) in furrows alternately inserted between each two maize rows at seven leaves stage.
BE2: Proradix® WP, 1st rate (1.5×10^{15} cfu ha^{-1}) as broad cast with soil incorporation before sowing, 2nd rate as broad cast on top of the plants at five leaves stage.

Tomato (*Lycopersicum esculentum* Mill.) var. Primadona, Hazera, Israel

Nursery substrate: 45 % composted cow manure, 30 % garden soil 15 % peat, 10 % sand
Greenhouse culture: Vertisol, clay loam, pH 6.7, 55 mg $P_{(CAL)}$ 100 g^{-1} soil (very high), fertilization with 100 t fresh cow manure ha^{-1} (70 % of local horticultural practice)

Application of bio-effector products in tomato production: (cfu = colony forming units)

Product name, active ingredient	1st rate at two leaves stage during the nursery phase	2nd rate at transplanting into the greenhouse soil
BE1: Biological Fertilizer, *Penicillium bilaii*	1.0×10^7 spores $plant^{-1}$	1.3×10^8 spores $plant^{-1}$
BE2: Proradix® WP, *Pseudomonas* DSMZ 13134	2.6×10^8 cfu $plant^{-1}$	3.3×10^9 cfu $plant^{-1}$
BE3: *Bacillus simplex* R41	2.0×10^8 spores $plant^{-1}$	2.5×10^9 spores $plant^{-1}$
BE4: RhizoVital®, *B.* amyloliquefaciens FZB42	2.0×10^8 spores $plant^{-1}$	2.5×10^9 spores $plant^{-1}$

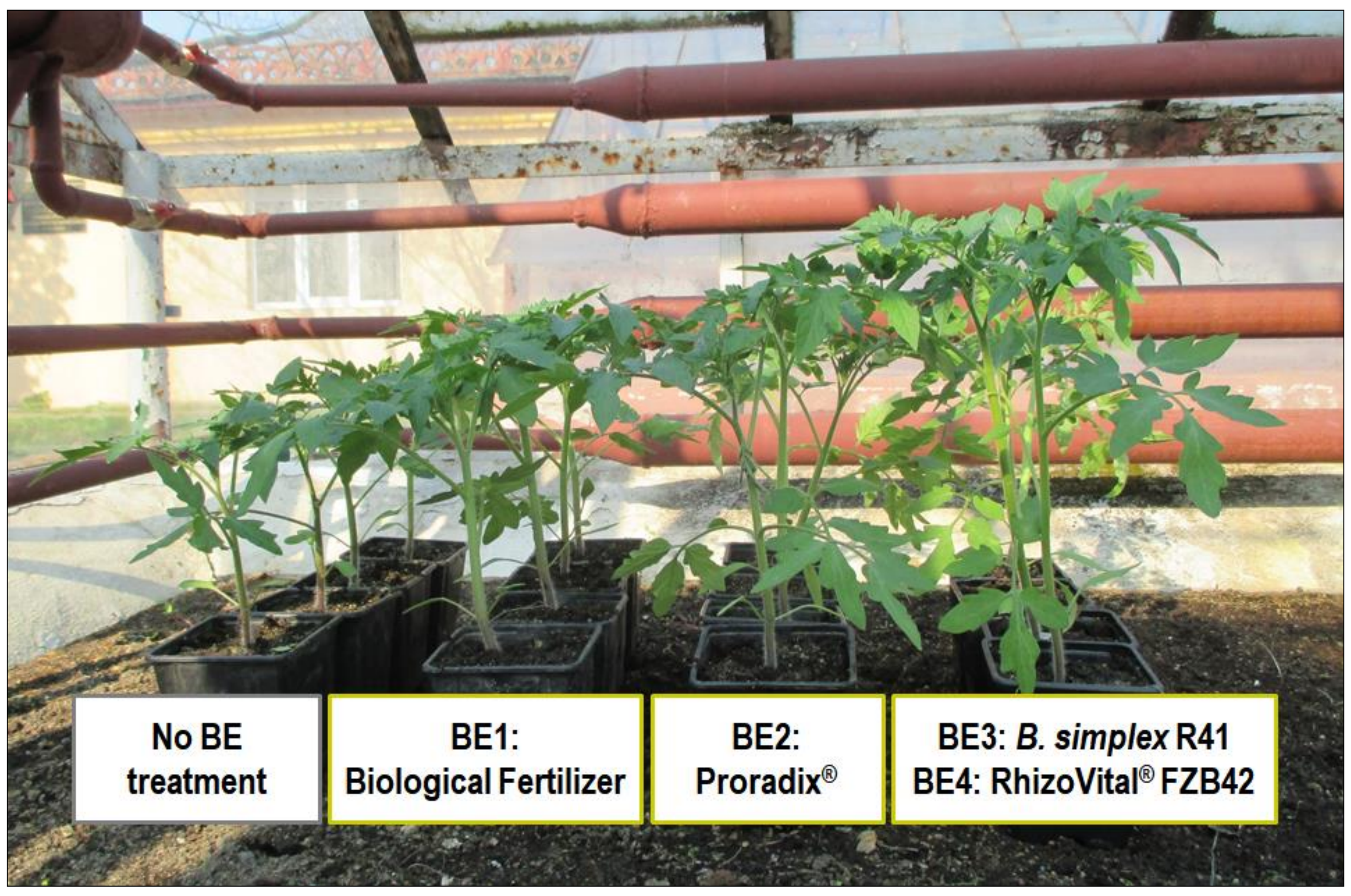


Fig. 2: Already during nursery in small pots, tomato plantlets showed improved shoot growth in response to BE treatments.

Conclusions

- In tomato production reproducible yield increase of 70% on average, with an average economic benefit of 35200 € ha^{-1} were achieved.
- It will be a great challenge to develop adapted application strategies for the successful use of microbial bio-effectors in wheat and maize crops.
- A one-sided functional classification of these agents as bio-pesticides would disregard many of their other beneficial traits that could be reasonably used in integrated strategies for sustainable plant nutrition.

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