FORMULATION OF ARBUSCULAR MYCORRHIZAL FUNGI: FIRST RESULTS

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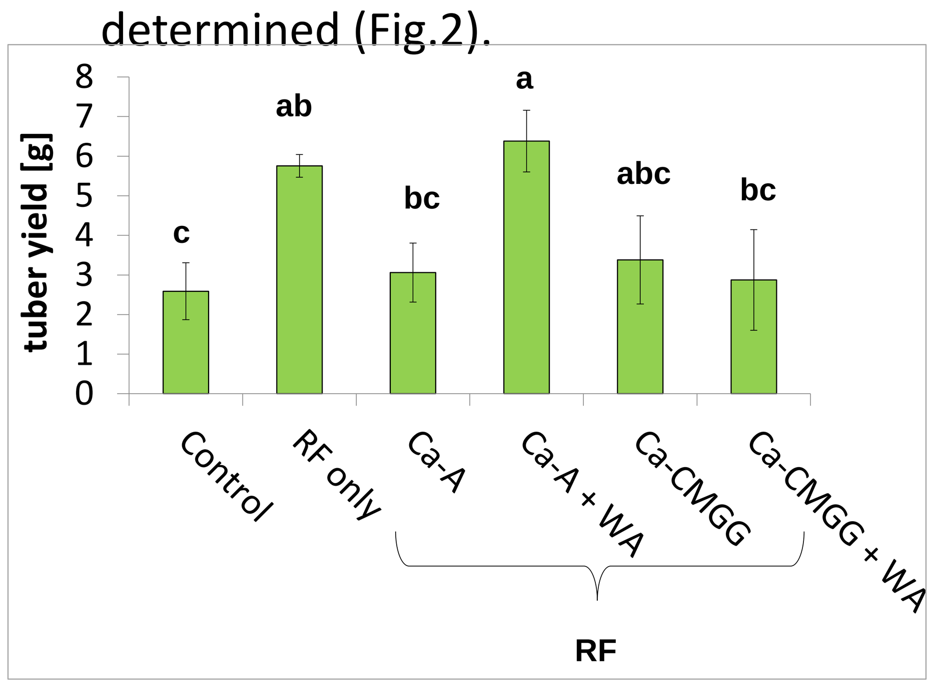
State of the Art

- Arbuscular mycorrhizal fungi (AMF) are known to establish symbiotic interactions with 80% of todays plant families, which leads to enhanced nutrient and water uptake for the plants. Furthermore "non nutritional" effects of mycorrhizal fungi are known as soil stabilization and alleviation of plant stress through biotic and abiotic factors (Smith and Read, 2008).
- Application of microorganisms as biofertilizers is a well known practice nevertheless a formulation with a reliable effect is still a bottleneck for the wider utilization (Malusá *et al.*, 2012).

Immobilized AMF in Ca-alginate beads

- Droplets consisting of 2% sodium alginate or 5% carboxymethylguar gum, and 10% root fragments containing AMF were cross-linked in 2% $CaCl_2$ solution. As an additive, wooden ash was used in a final concentration of 5%.
- Potato *in vitro* plantlets were inoculated with 15 moist beads per treatment in non-sterile field soil.
- As a positive control, 20 mg mycorrhizal root fragments were utilized.
- Plants were harvested 8 weeks after inoculation and tuber yield [g] was determined (Fig.2).



- ➢ The highest tuber yield was obtained with Ca-alginate beads containing AMF and wooden ash.
- ➢ The results suggest the potential of in Ca-alginate encapsulated AMF with wooden ash as an eliciting additive for the use as a novel biofertilizer.

Figure 2: Influence of in Ca-alginate (Ca-A) and Ca-carboxymethylguar gum (Ca-CMGG) immobilized root-fragments (RF) containing AMF, with or without wooden ash (WA), on tuber yield (in g) of potato in vitro plantlets. Different letters above bars indicate significant differences according to Waller-Duncan Baysian K-ration test at p<0.05.

Problem

- AMF development is strongly inhibited by the presence of high amounts of phosphate in soil (Breuillin, *et al.*, 2010), which is a common soil condition nowadays due to the extensive use of mineral fertilizers.
- As commercial AMF products on the market are often of low quality and give inconsistent results the agricultural application of microbial inoculants as biofertilizers is not widely accepted by farmers (Vassilev *et al.*, 2015).

Idea

Formulation strategies can contribute to:

- Protection the AMF from biotic and abiotic stress
- Development of mixed inocula with synergistic microorganisms and eliciting agents to further improve the AMF performance
- Controlled release
- Exploitation the potential of different application strategies (Fig.1)

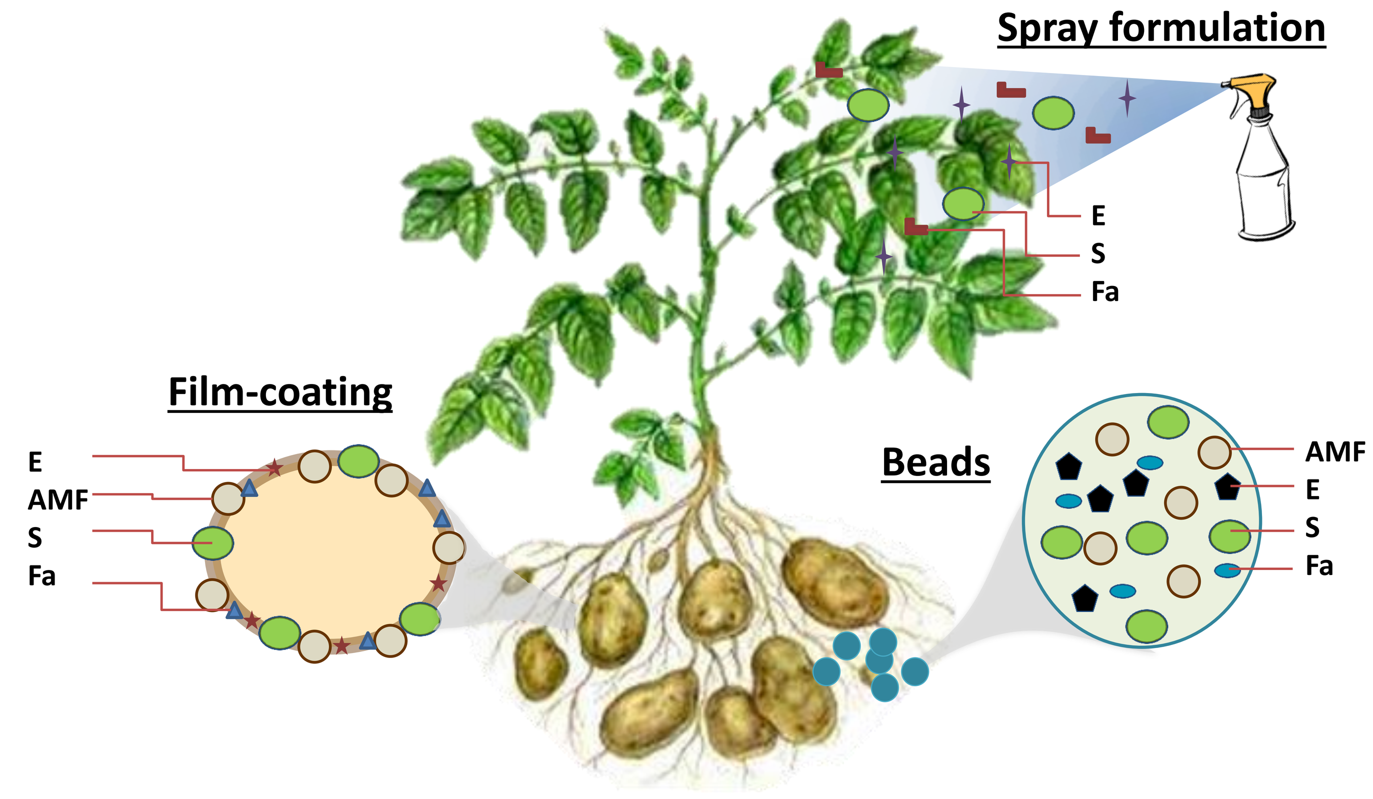


Figure 1: Formulation and application strategies for AMF as novel biofertilizer
AMF: arbuscular mycorrhizal fungi; E: elicitors; S: synergists; Fa: formulation additives

Ongoing experiments

Influence of bead composition on the colonization of potato plants

Bead preparation

- Hydrogel beads were prepared according to Tab. 1

Table 1: Composition of beads.
RF: Root fragments 8mg/g; Y: baker's yeast 5%:; S:maize starch: 10%

	Ca^{2+}	Cu^{2+}	Zn^{2+}
Na-Alginate 2%	RF + S +Y	RF + S +Y	RF + S +Y
	RF	RF	RF
	S + Y	S + Y	S + Y
	-	-	-
Pectinamide (P) 2% + Meypro-Film (MF) 2%	RF + S +Y	RF + S +Y	RF + S +Y
	RF	RF	RF
	S + Y	S + Y	S + Y
	-	-	-

- Beads were tested in a greenhouse trial using potato *in-vitro* plantlets with a dose of 20 beads/L mixed into sterile sand.
- Plants will be harvested after 8 weeks, AMF colonization, plant growth, weight and tuber yield will be evaluated.

AMF viability after encapsulation

- Viability of the immobilized mycorrhizal propagules was evaluated after 6 weeks with INT staining (Fig.3)
- ➢ Vital mycorrhizal propagules were observed for all beads containing RF excluding formulations cross-linked with Cu^{2+}.

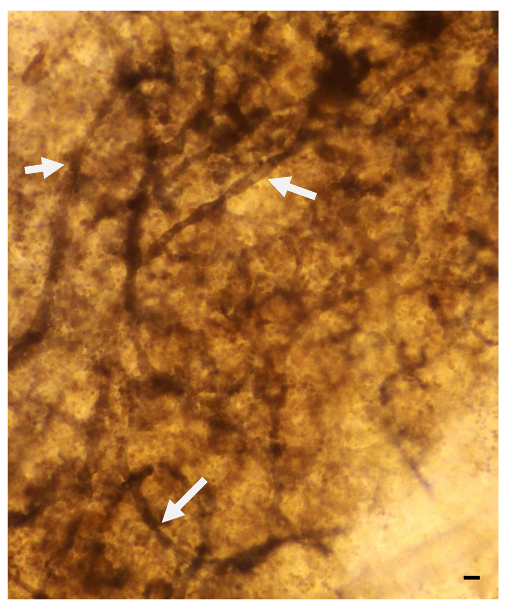


Figure 3: Vital mycorrhizal hyphae (arrows) growing in the bead matrix. Micrograph taken at 200x magnification. Scale bar: 10 µm.

Bead persistence

- Bead persistence was measured in moist, sterile sand with aid of a caliper rule and stored at 18 °C.
- Selected results are displayed in Fig. 4.

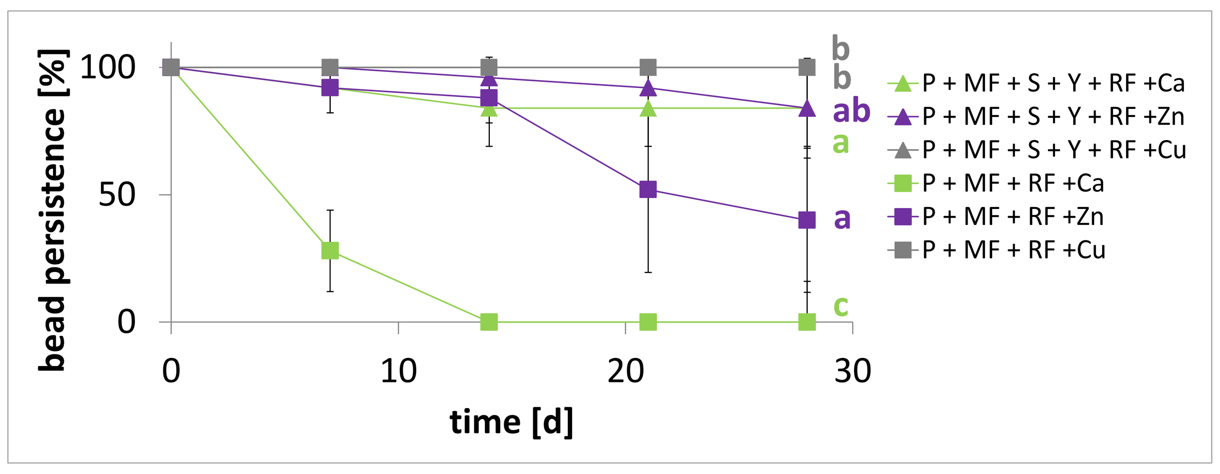


Figure 4: Influence of time and treatment on the persistence of beads in moist, sterile sand. Letters indicate significant differences of treatments according to RM-ANOVA and pairwise Tukey post hoc test at P<0.05.

- ➢ The degree of degradation changed significantly over time ($F_{5,138}$ = 85.45; P<0.001) and between treatments ($F_{1,138}$ = 84.52; P<0.001)
- ➢ P+MF bead type formulations containing RF start to degrade after 7d, alginate beads remain persistent even after 28d as well as P + MF beads with Cu^{2+}.
- ➢ The microbial community located on the non-sterile RF are able to actively degrade the P+MF beads

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Conclusions and Outlook

- ➢ In Ca-alginate immobilized root fragments were able to enhance tuber yield in potato plants.
  - Ca-alginate is a promising carrier for root fragments applied as biofertilizers.
- ➢ Stable bead formation was accomplished for all tested counter ions.
  - An additional beneficial effect for plant and fungus may be considered in future studies.
- ➢ Vital mycorrhizal propagules were observed for beads containing RF excluding formulations with Cu^{2+}.
  - Formulations using Zn^{2+} or Ca^{2+} as counter ions are promising for the immobilization of RF .
- ➢ The bead persistence can be controlled through the choice of polymer.
  - The microorganisms involved in the degradation process should be explored in detail.

References:

- Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., Druege, U. & Reinhardt, D. (2010). Phosphate systemically inhibits development of arbuscular mycorrhiza in Petunia hybrida and represses genes involved in mycorrhizal functioning. The Plant Journal, 64(6), 1002-1017.
- Malusá, E., Sas-Paszt, L., & Ciesielska, J. (2012). Technologies for beneficial microorganisms inocula used as biofertilizers. The scientific world journal, 2012.
- Smith, S. E., & Read, D. J., *Mycorrhizal symbiosis*. (Academic press. 2010)
- Vassilev N, Vassileva M, Lopez A, Martos V, Reyes A, Maksimovic I, Eichler-Löbermann B, Malusà E. 2015. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Applied microbiology and biotechnology 99: 4983–4996.