

# THE BENEFICIAL EFFECTS OF THE MICROBIAL INOCULATES APPLICATION TO IMPROVE ROOT VEGETABLES PRODUCTION

**Doctoral thesis** 

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# 1. INTRODUCTION

# **1.1. Research Background**

Some plant-microbe interactions such as Plant Growth Promoting Rhizobacteria (PGPR), Arbuscular Mycorrhizal Fungi (AMF) and compost have been widely used to enhance plant growth through different mechanisms of action (TAHIRI et al. 2022). Also, microbial inoculants are easy and inexpensive to manufacture compared to chemical pesticides (ELNAHAL et al.2022). The benefits of co-inoculating phosphate-solubilizing PGPR and/or nitrogen-fixing PGPR with mycorrhiza in plants have been demonstrated (KUMAR et al. 2017). Based on the previous literatures, our study was divided into two parts; the first part was conducted as pilot research in a glasshouse of the Department of Vegetable and Mushroom Growing located at the Hungarian University of Agriculture and Life Sciences – Buda campus in 2019 to check the symbiosis between the sweet potatoes and mycorrhiza and the influence of mycorrhiza on the physical parameters of sweet potato seedlings such as, length of roots and shoots (cm), fresh weight of shoots, roots (g) and the length of the stem (cm) of the sweet potato seedlings.

Due to the corona virus in 2020, the research goals had to be changed and be involved in a field experiment by a cooperation and help from ÖMKi (Research Institute of Organic Agriculture) to be able to continue my Ph.D studies and research. This experiment carried out by ÖMKi in two years (2020+2021) at the Soroksár experimental research farm, located at the Hungarian University of Agriculture and Life Sciences. This experiment was part of the SolACE project (Solutions for Improving Agroecosystem and Crop Efficiency for Water and Nutrient Use). This study examines the effects of different microbial inoculations and their combinations on potato tubers yield (kg/m<sup>2</sup>), starch content in potato tubers (%), Total phosphorus content in potato tubers (mgP kg-1). Also, it observed the symbiosis and the mycorrhizal colonization parameters (F %: Frequency of mycorrhiza in the root system, M %: Intensity of the mycorrhizal colonization in the root system, m %: Intensity of the mycorrhizal colonization in the root fragments, a %: Arbuscular abundance in mycorrhizal parts in root fragments, A %: Arbuscular abundance in the root system).

# **1.2. OBJECTIVES**

- Highlighting the differences between two sweet potato varieties (orange and purple) in different parallel tests, such as the inoculation method used.
- Studying whether the mycorrhizal inoculum is effective in developing a symbiotic relationship with the roots of sweet potato, affecting the physiological and physical parameters of the plant; in addition, it will be investigated whether the sterilization of the substrate has an influence on mycorrhizal colonization.
- Detecting the effectiveness of mycorrhizal inoculation with a sterilized substrate on the:
  - Mycorrhizal parameters (F %, M %, m %, a %, A %).
  - Physical parameters such as, length of roots and shoots (cm), fresh weight of shoots, roots (g) and the length of the stem (cm) of the sweet potato seedlings.
- Determining the beneficial effects of the microbial inoculates to improve potato productivity with and without irrigation in field conditions.
- Observing which microbial inoculation combination yields the highest performance and improves potato production in organic farming by the measured parameters:
  - Mycorrhizal parameters (F %, M % and A %).
  - Total phosphorus content (mgP kg-1).
  - Starch content (%) in potato tubers.
  - Phenological growth stages of potato plant.
  - Tuber yield (kg/m2).

#### 2. MATERIAL AND METHODS

## 2.1. Sweet Potato Pilot Study

## 2.1.1. Origin, Growing of the Experimental Seedlings

The experiment carried out from 6<sup>th</sup> march until the 23<sup>th</sup> of July 2019 at Buda campus of the Hungarian University of Agriculture and Life Sciences (MATE) in the experimental glasshouse of the Department of Vegetable and Mushroom Growing (47.28° N, 19.04° E). At the beginning of March 2019, two sweet potato varieties, orange "Norangel" and purple "Purple" characterized by mature tuber and no flowers or buds were appeared on these tubers), theses tubers were provided from Soroksár experimental research farm, located at the Hungarian University of Agriculture and Life Sciences Vegetable farming unit, they were propagated by tuber for seedlings production, and planted in individual pots, the irrigation were carried out around once a week. However, the frequency of irrigation also depended on humidity and climate avoiding to be infected by some kind of pathogens. The sprouting was waited until they reached the length of 30-40 cm. This stage was reached in the 21<sup>th</sup> of May 2019, so the seedlings were ready to be transplanted. The seedlings were grown in a plastic pot (1.5 L, 11.5  $cm \times 12 cm \times 15 cm$ ). For seedling production, non-treated ground/minced peat) was used as a growing medium from Latagro Basic Substrat KB2 type (white peat 100% (0–10 mm) with specification: pH value ( $H_2O$ ) 6.4; soluble nutrients available to the plants: Nitrogen<7 mg/L; Phosphate <7.8 mg/L; Potassium oxide < 40 mg/L).

#### 2.1.2. Roots Sampling and Staining

The roots were sampled on the 8th week after transplanting (end of July 2019). After that, the roots were washed by tap water to remove the substrate by immersing the roots in the water for 2-3 seconds and shake the roots to ensure that there is no substrate in the all parts of the root system. At the end of the pilot study, several physical parameters were measured for sweet potato seedlings for

both varieties manually by ruler such as, length of roots and shoots (cm), and the length of the stem (cm). In addition to the fresh weight of shoots and roots (g) of seedlings for both varieties were measured by electronic balance. The samples were stained at the laboratory of the Department of Vegetable and Mushroom Growing in the days following the sampling. Roots staining were carried out according to the method of PHILLIPS and HAYMAN (1970).

# 2.2. Organic Potato Experiment

## 2.2.1. Time and Place of the Experiment

This experiment was designed by Agroscope (The Swiss Confederation's center of excellence for agricultural research) and applied by ÖMKi (Research Institute of Organic Agriculture) as part of the SolACE project (Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use) at the Soroksár experimental research farm, located at the Hungarian University of Agriculture and Life Sciences organic farming units between April till August of 2020 and 2021. Organic farming methods have been practiced at the trial site for more than a decade. The site was previously planted with rye. The experiment area was open field with 1316.25 m<sup>2</sup> area divided into 64 experimental plots. A randomized complete block design was selected in the two years experiment. The size of the experimental plot was 864 m<sup>2</sup> plot with inter spacing 22.5 m<sup>2</sup>. A total of 32 experimental plots were created, in which the irrigated area was 432 m<sup>2</sup>.

# **2.2.2. Applied Treatments of Microbial Inoculates**

Seven different treatments were used with arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR) and *Trichoderma*. Each treatment had four replicates with a total of 64 plots. Each plot was planted with 12 potato tubers. The plots were separated and surrounded by at least two rows of buffers in each direction. The selected strains of the microbial inoculates were kindly supplied from other partners of SOAICE project, they already gone under selection in previous pots experiments in greenhouse and they performed the best

results. The isolates were selected for testing in the open field in previous laboratory experiments conducted within the framework of the SolACE project.

Several strains of microbial inoculants were prepared and mixtures of them were used as shown in Table 1. The treatments were conducted under both irrigated condition and another plots without irrigation. Three mixtures of inoculants were tested on potato compared to the untreated (control). The inoculants were prepared by Minigran technology (https://dcm.green/en/minigran-technology, last accessed on 2024.02.17).

**Table 1.** Treatments and types of microorganisms of inoculum mixtures used in

 the potato field trial.

Microbial inoculates strains	Applicatio	(CFU/tube	Concentratio	Quantit
	n rate/	r (for	n of	y of
	Biological	AMF:	microbial	granule
	material	g/tuber)	product	per
	need in (g)		(CFU/g)	tuber (g)
Pseudomonasbrassicacearu	7.20	2.00E+08	1.60E+10	0.75
<i>m</i> 3Re2-7				
Paraburkholderia	6.40	1.00E+08	9.00E+09	0.75
<i>phytofirmans</i> PsJN				
Trichoderma asperelloides A	0.86	1.50E+06	1.00E+09	0.75
Rhizophagusirregularis	0.3456	6.00E-04	n.d	0.75
MUCL41833				
Rhizophagusirregularis	0.3456	6.00E-04	n.d	0.75
MUCL41833+ Pseudomonas	7.20	2.00E+08	1,60E+10	
brassicacearum 3Re2-7				
Rhizophagus irregularis	0.3456	6.00E-04	n.d	0.75
MUCL41833+	6.40	1.00E+08	9.00E+09	
Paraburkholderia				
<i>phytofirmans</i> PsJN				

Rhizophagus irregularis	0.3456	6.00E-04	n.d	0.75
MUCL41833+	6.40	1.00E+08	9.00E+09	
Paraburkholderia	0.86	1.50E+06	1.00E+09	
phytofirmansPsJN+				
Trichoderma asperelloides A				
Control treatment	n.d	n.d	n.d	n.d

## 2.2.3. Total Phosphorus Content in the Potato Tubers

The Total phosphorus content of the potato tubers measured at the soil lab in Godollo campus located at the Hungarian University of Agriculture and Life Sciences, Godollo. Potato samples were cut into small pieces, placed in 1-1 Petri dishes and dried in a drying oven (LP-321 (200 L) (Labor-Mix Laborszerviz, Hungary). After drying, the samples were ground, homogenized and filled into small paper packets. The total phosphorus content was determined according to the MSZ 21470-50:2006 standard (Environmental testing of soils- Determination of soil toxic element, heavy metal and chromium(VI) content, Hungarian standards institution, Budapest-Hungary) (HEGEDUS et al. 2017)

## 2.3. Statistical Analysis

#### 2.3.1. Sweet Potato Pilot Study Data

Data analysis was performed with IBM SPSS 25 software version 25.0. Armonk, NY: IBM Corp (2017). Shoot and root fresh weight (g) and root and stem lengths were analyzed using the two-way MANOVA model with the factor's diversity (orange and purple) and treatment levels [(L+SYM). S], F, (L +SYM). The normality of the residuals was tested using the ShapiroWilk method (K (74) > 0.95; p > 0.05). After having an overall significant MANOVA result, we performed subsequent univariate ANOVA tests with Bonferronis correction. In some cases, the homogeneity of variances was slightly violated (Levenes 0.05 > p > 0.02), so pairwise comparisons of treatments were performed using the Games-Howell post hoc test.

#### 2.3.2. Organic Potato Experiment

Data analysis was performed using SAS software version 9.4 (2013). Total phosphorus was analyzed using the one-way three-factor Anova model; year, microbial inoculation treatment and water treatment. Prior to ANOVA, descriptive statistics were generated for all measurements to monitor the distribution of the data and normality using a general linear model (p-value > 0.05). Means were separated at a significance level of 0.05 using Tukey's test.

# 3. RESULT

#### **3.1.** Physical Parameters of Sweet Potato Seedlings

Two-way MANOVA resulted in significant diversity and treatment effects (Wilks lambda = 0.48, p <0.001; Wilks lambda = 0.18, p <0.001) with significant interaction (Wilks lambda = 0.64, p <0.05). Subsequent univariate ANOVA was for both shoot fresh weight and stem length for both cultivar and treatment (F (1.38 >6.48; p <0.05)) and in the case of total root fresh weight for the treatment effect (F (4.38)= 7.90; p <0.01) as well as for their interaction in the case of shoot fresh weight and stem length (F (4.38) > 4) significant. 88; p<0.01). The variety influence was not significant in the case of fresh weight of total roots and length of roots ((F (4.38) = 0.08) and the treatment effect was not significant in the case of length of roots (F (4.38) = 3.13; p= 0.06). Means and standard deviations of the four physical parameters and post-hoc test results are summarized in Table 2.

The highest mean of shoot fresh weight for orange sweet potato seedlings was found with the (L+SYM) treatment (11.43 g). There was no significant difference between treatment [(L+SYM). S] and (F), therefore we concluded that mycorrhizal inoculum could increase shoot weight over time, while sterilization had no increased effect on shoot weight (g) in treated orange sweet potato seedlings. The highest fresh shoot weight in purple sweet potato seedlings was

under treatment (L+SYM) with a mean of (15.81 g) while [(L+SYM).S] treatment gave the lowest mean (3.80 g), the same conclusion previously, the mycorrhizal inoculation also increased shoot fresh weight in the purple sweet potato seedlings, whereas sterilization had no effect on shoot fresh weight. The highest root weight was found in orange sweet potato seedlings, with the highest mean at [(L+SYM). S] treatment (4.89 g), while the lowest mean was found in the control treatment (F) with a mean of (1.09 g). This means that mycorrhizal inoculation and sterilization had a great impact on the root weight of orange sweet potato seedlings. For the root weight of purple sweet potato seedlings, the highest mean was with the (L+SYM) treatment (5.89 g), while the lowest mean was with the (F) treatment with a mean (3.44 g). This is because mycorrhizal inoculation had a stimulatory effect on fresh root weight of purple sweet potato seedlings. PATI et al. (2024) described the phenological growth stages of sweet potato for the first time using the extended BBCH scale. From the previous results, it can be refer to the extended BBCH scale stage 4 which describe the development of the tubers. According to the BBCH scale, code (400) Tuber initiation: swelling of first stolon tips to twice the diameter of subtending stolen. At (BBCH 405), half of the last tuber mass comes during the inflorescence, and 95% of the last tuber development is completed after the plant reaches maturity (BBCH 408). The highest root length of the orange sweet potato variety was measured in seedlings treated with Symbivit in sterilized substrate (peat moss) [(L+SYM).S] treatment gave a mean of (35.52 cm). However, there was no significant difference between the mean values when comparing the three stages of treated orange sweet potato seedlings. Therefore, mycorrhizal inoculation with sterilization had no effect on root length of orange sweet potato seedlings. The highest mean root length (36.13 cm) was measured in the purple sweet potato seedlings propagated in a sterilized Latagro peat moss with Symbivit [(L+SYM).S] treatment. However, the differences between the treated seedlings were not significant. The highest mean stem length in orange sweet potato seedlings was measured with the (L+SYM) treatment (75.32 cm), while the lowest mean occurred with the (F) treatment (20.00 cm). Accordingly, the mycorrhizal inoculation could increase the length of the stems in the orange sweet potato seedlings.

**Table 2**. Sweet potato seedlings means and standard deviations of the four physical parameters fresh weight of shoots (g), fresh weight of total roots (g), length of roots (cm) and length of stem (cm) together with the post hoc tests results (Games–Howell's, p < 0.05). The different letters are for significantly different groups (lower case: comparison of treatments for fixed varieties-read vertically), upper case: comparison of varieties for fixed treatments-read horizontally).

Varie	ty			Orange		Purple			
parameters	treatment	Mean	Std. Dev	comparison of treatments <sup>1</sup>	comparison of varieties <sup>2</sup>	Mean	Std. Dev	comparison of treatments	comparison of varieties
FW of shoots (g)	[(L+SYM).S]	4.76	0.88	А	А	3.80	2.99	a <sup>1</sup>	A2
	F	2.83	1.73	А	А	9.42	3.04	b <sup>1</sup>	B2
	L+SYM	11.43	3.85	В	А	15.81	4.32	c <sup>1</sup>	A2
FW total roots (g)	[(L+SYM).S]	4.89	1.14	В	А	3.88	2.38	a <sup>1</sup>	A2
	F	1.09	0.89	А	А	3.44	1.87	a <sup>1</sup>	B2
	L+SYM	4.79	1.87	В	А	5.89	2.97	a <sup>1</sup>	A2
	[(L+SYM).S]	35.52	6.26	А	А	36.13	13.58	a <sup>1</sup>	A2
Length of roots (cm)	F	23.17	8.69	А	А	32.74	6.54	a <sup>1</sup>	B2
	L+SYM	25.90	7.44	А	А	30.83	8.30	a <sup>1</sup>	A2
Length of stem (cm)	[(L+SYM).S]	35.56	12.89	А	В	14.69	5.80	a <sup>1</sup>	A2
	F	20.00	11.21	A	А	29.37	7.83	b <sup>1</sup>	A2
	L+SYM	75.32	36.27	В	А	47.69	14.72	c <sup>1</sup>	A2

<sup>1</sup> comparison of treatments for fixed varieties – read vertically; <sup>2</sup> comparison of varieties for fixed treatments – read horizontally.

# 3.2. Total Phosphorus Content in the Potato Tubers

**Table 3.** Means of phosphorus content (mgP kg<sup>-1</sup>) in potato tubers by microbial inoculants in irrigated and non-irrigated conditions within two years experiment.

	Total phosphore	us in the tubers	Total phosphor	us in the tubers		
	2020		2021			
	Ι	С	Ι	С		
Pseudomonas brassicacearum 3Re2-7	0.32 ±0.012	0.32 ±0.010	0.62 ±0.044	0.56 ±0.053		
Paraburkholderiap hytofirmans PSJN	0.32 ±0.012	0.34 ±0.008	0.68 ±0.036	0.58 ±0.022		
Trichoderma asperelloides A	0.33 ±0.010	0.32 ±0.006	0.64 ±0.037	0.64 ±0.045		
Rhizophagus irregularisMucL41833	0.31 ±0.014	0.32 ±0.013	0.54 ±0.051	0.57 ±0.034		
Rhizophagusirregul aris MucL41833+Pseudomo nas brassicacearum PSJN	0.31 ±0.007	0.30 ±0.016	0.63 ±0.029	0.54 ±0.018		
Rhizophagusirregul aris MucL41833+ Paraburkholderiaphytof irmans PSJN	0.31 ±0.012	0.32 ±0.005	0.68 ±0.053	0.66 ±0.038		
Rhizophagusirregul aris MucL41833+ Paraburkholderiaphytof irmans PSJN+ Trichoderma asperelloides A	0.32 ±0.014	0.32 ±0.011	0.63 ±0.014	0.54 ±0.025		
C (control)	0.32 \±0.018	0.35 ±0.010	0.69 ±0.031	0.50 ±0.030		

The total phosphorus content in the tubers is shown in Table 3. The results show nonsignificant differences in both years under both irrigated and non-irrigated conditions. The highest value was recorded for the *Trichoderma asperelloides* A treatment under irrigation conditions and for the control treatment without irrigation treatment. In the second year (2021) there was an apparent increase in phosphorus levels in almost all irrigated treatments, however there was no significance difference within microbial inoculates in the irrigated and non-irrigated treatments separately. The highest was seen in the control treatment with irrigated treatment and in the *Rhizophagus irregularis*MucL41833+ *Paraburkholderia phytofirmans*PSJN treatment without irrigation.

# 4. **DISCUSSION**

#### 4.1. Sweet Potato Pilot Study

From our results on fresh shoots and root weights, it appears that mycorrhizal inoculation could improve the physical parameters of sweet potato seedlings. Several other studies have already shown the positive effect of arbuscular mycorrhizal fungi on physical parameters. In maize, it has been demonstrated that *Glomus intraradices* can enhance dry weight of shoots and roots (ORTAS 2011). KAKABOUKI et al. (2021) examined the effect of *Rhizophagusirregularis* on cannabis seedlings and found significantly increased root length, significantly increased stem dry weight, and an improvement in survival rate and phosphorus content. Another study also confirmed that arbuscular mycorrhizal fungi increased the fresh weight of sweet potato sprouts and roots in sweet potato varieties PROC 65-3 (white-fleshed) and Tainung 57 (orange-fleshed) (NEUMANN et al 2009). In addition, in the study of SAKHA and JEFWA (2019), two sweet potato cultivars, Kemb-10 and Bungoma, were examined with and without arbuscular mycorrhizal fungi inoculation, focused on physical parameters, namely branch number, vine length and yield; They found that mycorrhizal inoculation improved yield and growth (REDDY et al 2018).

The yield of storage roots correlates positively with the vegetative properties and the correlation with the number of leaves per plant is significant (MARTIN 2013). Regarding the length of the roots, we did not find any positive effect of mycorrhizal inoculation, while the length

of the stem increased. The effect of the sterilization treatment was small; the reason for this could be that the microbial population in the autoclaved growth medium was lower than in the nonautoclaved growth media. This is consistent with the results of KÖHL et al. (2016). Nevertheless, the defined influence of arbuscular mycorrhizal fungi on plant growth and development is not stable due to the complex relationship between arbuscular mycorrhizal fungi, the inoculation method and environmental conditions (PERNER et al. 2006). Our results showed that although the highest colonization rates were observed in sweet potatoes grown on sterilized peat, the treatment did not perform better in terms of physical parameters compared to non-sterilized inoculated treatments. Furthermore, this can be confirmed by other studies showing that plants with high mycorrhizal colonization rates can be maintained on peat-based substrate, but that plants may not consistently benefit from mycorrhizal symbiosis growth under these conditions (BAUM et al. 2015). However, the interaction between arbuscular mycorrhizal species may differ between sweet potato varieties (WIPF et al. 2019). In our experiment, the mycorrhizal inoculum (Symbivit) enhanced the fresh weight of shoots (cm), fresh weight of total roots(g). Also, the length of roots and stem(cm) in both sweet potato varieties, orange and purple.

## 4.2. Organic Potato Experiment

The total phosphorus content in potato tubers increases with time and with the microbial inoculants used. Nevertheless, there is no significant difference between the treatments and irrigation conditions in our study. The control treatment in the second year (2021) gave the highest total phosphorus content under irrigation conditions (0.69 mg kg<sup>-1</sup>), followed by the mixed treatment of the microbial inoculated plants *Rhizophagus irregularis* MucL41833 and *Paraburkholderia phytofirmans*PSJN (0.68 mg kg<sup>-1</sup>). This can correspond with a study carried out by MA et al. (2021) showed that the mycorrhizal colonization and activity can be affected by several factors which may lead to decrease the arbuscular mycorrhizal colonization such as; high nutrient content in the soil , high temperature and precipitation can also affect negatively the development of mycorrhiza, high phosphorus supply decreases root colonization, and root cadmium content decreases the root mycorrhizal colonization, Furthermore; other study by AVIO et al. (2013) showed that intensive soil-tillage also affects negatively the development of arbuscular mycorrhizal fungi spores. Among other possible reasons; the interaction between arbuscular mycorrhizal fungi and other microorganisms might have an antagonistic effect on the

mycorrhizal colonization and development, there was no soil solarization during the experiment, therefore there was a variation of the microorganisms which might affect negatively the arbuscular mycorrhizal fungi activity, and the influence of the soil and environmental conditions during the experiment application.

Mixing the inoculation with different species could have an antagonistic effect or no effect, according to studies. For the mixture of plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi, inoculation of a mixture of the microbial inoculates such as plant growth promoting rhizobacteria species; Azospirillum with Pseudomonas had no effect on maize plant growth (VAZQUEZ et al. 2000). Furthermore, inoculation of Pseudomonas and Trichoderma reduced the activity of other inoculated microorganisms. Colonization with arbuscular mycorrhizal fungi can eliminate the effect of *Trichoderma* on grapevine growth (WASCHKIESET al. 1994). Inoculation of just one microorganism in the plant can have a significant positive effect on the plant. However, inoculation with other microorganisms, especially arbuscular mycorrhizal fungi, can lead to a weakening of the effect of other inoculations. This could be explained by the qualitative change in root exudate caused by arbuscular mycorrhizal fungi colonization (COX et al.1975). In our research, the results show that the treatments showed no significant difference in most measurements in both years of study. There was no significant difference between the 2020 and 2021 results for both inoculation treatments. The non-irrigated plants showed better results in terms of arbuscular mycorrhizal fungi colonization, higher starch content and higher total phosphorus content in the non-irrigated samples compared to the irrigated ones.

#### **5. NEW SCIENTIFIC RESULTS**

- Our work proved that the sweet potato seedlings for both varieties, purple (Purple) and orange (Norangel) can establish a symbiotic relationship with the Symbivit mycorrhizal inoculum. Mycorrhizal inoculation with a sterilized substrate performed better results on root length (cm) among the studied sweet potato varieties.
- 2. We demonstrated that the inoculation of tested sweet potato seedlings with Symbivit inoculum can be functional, which presumably improves plant growth.
- 3. Colonization of arbuscular mycorrhiza is higher in arid climate in organic potato cultivation in case of Desiree variety, under non-irrigated environment.

- Starch content in potato tubers increasing by the water supply not by the applied microbial inoculates (Arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria and Trichoderma).
- 5. There is no influence of the water supply and the microbial inoculates applied together (Arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria and Trichoderma) on the yield of potato tubers

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# 7. PUBLICATIONS

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