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ORIGINAL PAPER

Inoculation and tracking of beneficial microbes reveal they can establish in field-grown potato roots and decrease blemish diseases

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Abstract

In a three-year field study, we inoculated two potato varieties with a selection of four beneficial microbial strains (i.e. *Rhizophagus irregularis* MUCL41833, *Trichoderma asperelloides* A, *Pseudomonas brassicacearum* 3Re2-7 and *Paraburkholderia phytofirmans* PsJN), alone or in combination. Plants were grown under rainfed or irrigated conditions, and potato yield and development of several diseases were evaluated. The microbial inoculants were traced in the root system at different stages of crop development via molecular markers. Whatever the water supply, the inoculants had no effect on yield. Conversely, some of the inoculants were able to lower the incidence and/or severity of several blemish diseases, namely common scab-associated symptoms (CSAS) and silver scurf/black dot-associated symptoms (SSAS). Microbial consortia were more efficient in decreasing symptoms compared to single strain inoculations. The best control was obtained with the combination of *R. irregularis* and *P. brassicacearum*, which reduced the incidence of CSAS by 22% and severity of SSAS by 21%. Root tracking revealed that *P. brassicacearum* and *P. phytofirmans* PsJN were able to establish in the root system of the potato, while only *P. brassicacearum* was detected from emergence until flowering of the plants.

Keywords Tuber blemishes · Microbial inoculants · Establishment · qPCR · Tracking

Introduction

In 2020, worldwide potato production reached 359 million tonnes. Ranking in 7th place among all food crops yields, potatoes are in 17th place in terms of cultivated surface (FAOSTAT 2020). Potato plants are susceptible to abiotic stresses like drought, high temperature, salinity and lack of nutrients. In the current context of climate change, some of these abiotic stresses are set to become more frequent (Onyekachi et al. 2019). They lead to yield losses and a decrease in tuber quality (Levy 1985; Levy et al. 1988; Naumann et al. 2020; Van Loon 1981). Biotic stresses also harm potato production. Numerous pests, including insects, bacteria, fungi and viruses, affect crop health, reducing the yield and quality of the harvest (Hooker 1981). Among these pests and pathogens, the so-called blemish diseases induce symptoms on the skin of the tubers (Hide and Lapwood 1978).

The main tuber blemishes impacting the potato fresh market are common scab, black dot, silver scurf and black scurf (Arora and Khurana 2004; Kaur and Mukerji 2004; Nærstad et al. 2012). Common scab (CS) is a disease caused by pathogenic *Streptomyces* spp. Their adaptation to a wide range of environmental conditions allows them to affect tubers in regions all over the globe. There is a lack of effective strategies to control CS, which modifies the aspect of potato tubers, thereby decreasing their market value (Dees and Wanner 2012; Kopecky et al. 2021). Silver scurf (SS) and black dot (BD) are diseases caused by *Helminthosporium solani* and *Colletotrichum coccodes,* respectively. They both induce shrinkage of the skin and water losses during storage, resulting in important economic losses (Errampalli et al. 2001; Lees and Hilton 2003). They are prevalent worldwide, but the options for their control are limited. *Helminthosporium solani* originates mainly from seed tubers and infects only potatoes, while the primary inoculum reservoir of *C. coccodes* is the soil, and its host range includes many other crops (Massana-Codina et al. 2021). Black scurf

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(BS) is also an important blemish disease. The buildup of sclerotia from the fungus *Rhizoctonia solani* will form dark brown particles on the potato tuber surface, impairing its appearance. Yield losses and misshaping of the tubers are common after infection (Anderson 1982). All of these diseases can cause yield losses, and few options are available to efficiently limit their propagation (Fiers et al. 2012).

To mitigate these various stresses, growers tend to use large quantities of fertilizer, water and pesticides. The application of these inputs has become a major concern. Novel solutions are needed to reduce their use, while maintaining productivity and reducing greenhouse gas emissions. Among the strategies to address this matter such as the selection of tolerance traits, the use of remote sensing to pursue precision agriculture or the adoption of sustainable cropping techniques (Venkateswarlu et al. 2011), there is the inoculation of microorganisms beneficial to potato. Indeed, microbial inoculants can improve plant water or nutrient absorption, strengthen their innate immunity or confer direct protection against pathogens (Lugtenberg et al. 1991). Several studies have documented the effects of potato inoculation with plant growth promoting microorganisms (PGPM) in the field. The arbuscular mycorrhizal fungi (AMF) inoculant *Rhizophagus irregularis* enhanced tuber yield in more than 85% of the 231 fields that were investigated (Hijri 2016). The inoculation with a *Pseudomonas fluorescens* strain reduced symptoms of common scab in the field (Arseneault et al. 2015). The AMF strain *Rhizophagus irregularis* MUCL41833 decreased the symptoms of *Phytophthora infestans* under low pressure of this pathogen (Alaux et al. 2018), and *Trichoderma harzianum* reduced the incidence of black scurf in organically grown potatoes (Tsror et al. 2001). However, a lack of consistency of microbial inoculants performance occurs under field conditions (Hart et al. 2017; Hoeksema et al. 2010; Kaminsky et al. 2019; Owen et al. 2015), likely because of the difficulty in achieving successful establishment of the microbes in the crop rhizosphere, rhizoplane or root endosphere. Establishing a beneficial relationship between crop and inoculants implies overcoming biotic and abiotic barriers by developing suitable formulation and parameters of application. There are a variety of abiotic barriers such as temperature, UV irradiation, rainfall and soil properties (e.g. pH, organic matter and nutrient content, texture) (Khare and Arora 2015; Malusà et al. 2016; Rilling et al. 2019). Among the biotic factors, the most important is the competition with the indigenous microbial community (Bender et al. 2019; Engelmoer et al. 2014; Kouadio et al. 2017; Verbruggen et al. 2013; Werner and Kiers 2015). As mentioned above, the formulation of microbial inoculants is also a crucial factor for the survival of microorganisms, as they should have adequate chemical and physical properties so as to allow long-term survival of the inoculated strains (Bashan et al. 2013). As it concerns the viability of commercial AMF inoculants, 21 out of 25 products were not able to allow AMF colonization (Salomon et al. 2022). In addition, the interaction of dosage and frequency of propagule application is a decisive factor regulating the outcome of inoculations (Simberloff 2009; Wittmann et al. 2014).

A promising development in microbial inoculant research lies in the application of diverse consortia rather than single strain products. Consortia improve the chance of at least one strain surmounting the obstacles to reaching its purpose (Rivett et al. 2018). In addition, it offers the possibility of a synergistic effect between the inoculated strains. If compatible, co-inoculated bacteria and AMF positively influence each other by various mechanisms like nutrient solubilization, mitigation of soil pathogens or modification of root permeability and exudates (Bashan et al. 2013; Kim et al. 1997; Thomloudi et al. 2019). Despite this encouraging approach, there is very little information on what happens after PGPM transfer to the field. In this regard, it is important to monitor the survival and persistence of strains after establishment (Malusà et al. 2016; Manfredini et al. 2021), so as to verify whether they could break down the barriers to establishment. To our knowledge, only six studies documenting the tracking of PGPM in potato are available, of which just two were done in the field (Alaux et al. 2018; Andreote et al. 2009, 2010; Garbeva et al. 2001; Loján et al. 2017; Naqqash et al. 2016). Only one of the field studies used a technique allowing discrimination of the inoculant from a naturally present fungus, but it only tested for its presence at harvest (Alaux et al. 2018).

Our study presents the first observation of fungal and bacterial inoculant establishment over time in field-grown potato plants. From emergence to late flowering, we surveyed the presence of the inoculants and their effect on potato yield and tuber blemish diseases. We hypothesized that microbial inoculants would enhance resistance of potato to water stress, while providing a reduction of potato tuber blemishes. We also expected that consortia would be more efficient in providing these services in comparison with single strain inoculants. In addition, we hypothesized that strains detected the most frequently in the roots would be more susceptible to provide the aforementioned services. Linking agronomic data with molecular tracing and disease development enabled us to identify strains that succeeded in delivering crucial services to the crop.

Materials and methods

Experimental plan

The trial was designed as a three-factor split-split-plot experiment with irrigation, inoculation and variety as plot, sub-plot and sub-subplot factors, respectively. Each subplot

contained four lines of 25 plants and was surrounded by buffer rows of potato cv. Laura to avoid border effects. Two potato varieties were used, Désirée and Pentland Dell. Désirée was selected for its regular-sized root system, while Pentland Dell was selected for its short root system, supposedly more susceptible to water stress. Sub-plots contained one line of the variety Pentland Dell and three successive lines of Désirée. While the Désirée line next to Pentland Dell was used for yield assessment, the next two lines were used as buffer row and for destructive sampling, respectively (supplementary Fig. 1). The trial contained four repetition blocks. It was conducted during three consecutive growing seasons (2019, 2020 and 2021) at the Agroscope research station (Nyon, Switzerland). Seed tubers were pre-sprouted for four weeks at 14°C with 360° pre-germination LED lamps (potatolight by Farmsupport, Heidelberg, Germany). Seed tubers were planted at the rate of one per 0.32 m and with 0.75 m row spacing. Fertilizers were applied at planting at the following rates: 120 kg/ha nitrogen (urea), 36 kg/ha phosphorus (triple superphosphate), 372 kg/ha potassium (potassium chloride) and 20 kg/ha magnesium (34.5% magnesium sulfate + 65.5% magnesium carbonate).

Seed tubers were planted on the 18th of April 2019, the 20th of April 2020 and on the 31st of May 2021. On the day of planting of each year, the soil was sampled in two horizons, 0—30 cm and 30—60 cm. Samples of each horizon were then pooled and analyzed. It revealed a similar loamy texture for the three locations. The clay proportion ranged between 22.8 and 25%, silt between 39.3 and 40.7%, and sand between 34.9 and 37.9% in all locations. The pH values were 7.7 in 2019, 7.5 in 2020 and 7.6 in 2021. Organic matter was 3.1% in 2019 and 2020, and 2.2% in 2021.

Microbial inoculants

To select the adequate strains and consortia, a greenhouse experiment was performed by the Institute of Crop Science in the University of Hohenheim (Germany). A collection of 6 strains and 10 combinations of these were screened for efficacy in improving plant growth and tuberization in potato plants exposed to water limitation (Mamun et al. 2024). Based on the measurement of several variables like shoot elongation rate, root fresh and dry weight, root length and tuber yield, consortia 1, 2 and 3 (Table 1) were selected for the field experiments.

The microbial inocula were formulated on four different test compositions of the organic soil improver MINIGRAN®, DCM, Belgium, N 3–5, P 0.3–0.6, K 1.9–2.7, which was used as a carrier for the micro-organisms. The different Minigran® compositions are referred to as formula 1, 2, 3 and 4 (Table 1). All the Minigran® types contained soy, vinasse, tobacco and peat-based materials. Minigran® 1 (NPK 3 – 0.6 – 2.7) also incorporated cocoa and vermiculite, Minigran® 2 (4 – 0.3 – 2) and 3 (4.4—0.4 – 1.9) comprised additional wheat-based materials and vermiculite,

Table 1 Concentrations and formulation of single microbial inoculants and microbial consortia

Microorganisms	CFU/tuber (for AMF: g/tuber)	Concentration of microbial product (CFU/g)	Formulation 2019	Formulation 2020	Formulation 2021
Paraburkholderia phytofirmans PsJN	1.00E + 08	9.00E + 09	not tested	Minigran® formula 2	Minigran® formula 4
Rhizophagus irregularis MUCL41833	6.00E-04	–	not tested	Minigran® formula 3	Minigran® formula 4
Trichoderma asperelloides A	1.50E + 06	1.00E + 09	not tested	Minigran® formula 3	Minigran® formula 4
Pseudomonas brassicacearum 3Re2-7	2.00E + 08	1.60E + 10	not tested	Minigran® formula 1	Minigran® formula 1
Consortium 1: *Rhizophagus irregularis* MUCL41833 + *Pseudomonas brassicacearum* 3Re2-7	see single strains	see single strains	Minigran® formula 1	Minigran® formula 1	Minigran® formula 1
Consortium 2: *Rhizophagus irregularis MUCL41833* + *Paraburkholderia Phytofirmans* PsJN	see single strains	see single strains	Minigran® formula 2	Minigran® formula 2	Minigran® formula 4
Consortium 3: *Rhizophagus irregularis* MUCL41833 + *Paraburkholderia Phytofirmans* PsJN + *Trichoderma asperelloides* A	see single strains	see single strains	Minigran® formula 3	Minigran® formula 3	Minigran® formula 4

and Minigran® 4 (5 – 0.5 – 2) contained supplementary wheat-based materials and magnesium calcium carbonate.

For all the consortia formed in these experiments, the strains were fermented separately and then mixed in the formulation. The two bacterial strains were grown by overnight incubation in 10% Tryptic Soy Agar at 28°C. The *Trichoderma* was grown for 6 days on Potato Dextrose Agar before recovery of the mycelium. The bacteria and *Trichoderma* were then dried and provided as powder by the partners, at the concentrations of 9E + 09 CFU/g for *Paraburkholderia* and *Trichoderma* and 1.6E + 10 CFU/g for *Pseudomonas*. The powders were added to the Minigran® formulation and mixed to obtain the concentrations of microorganisms mentioned in Table 1. The AMF *Rhizophagus irregularis* MUCL41833 was mass-produced on plants of Zea mays L., following the protocol described in Calonne-Salmon et al. (2018). Inoculated maize plants roots were collected, dried and ground to obtain fragments between 200µm and 500µm. This size allowed to preserve inoculum potential while permitting integration into the Minigran® formulations.

The dose of inoculum varied for each microorganism. It corresponded to 2×10^8 colony forming units (CFU) per tuber for *Pseudomonas brassicacearum* 3Re2-7 (*P.brass*), 10^8 CFU per tuber for *Paraburkholderia phytofirmans* PsJN (*P.phyt*), 1.5×10^6 CFU per tuber for *Trichoderma asperelloides* A (*T.asper*), and 6×10^{-4} g of dried and ground AMF-colonized maize roots per tuber for *Rhizophagus irregularis* MUCL 41833 (*R.irreg*). In addition to the four single strains, three different microbial consortia were used: (i) a consortium of *R.irreg* and *P.brass*, (ii) a consortium of *R.irreg* and *P.phyt*, and (iii) a consortium of *R.irreg*, *P.brass* and *T.asper* (Table 1). In consortia, the aforementioned amount of each microorganisms was included.

After seed tuber placing in the field, 0.75 g of microbial inoculum was mixed with approximately 200 ml of field soil. Operators were equipped with two plastic containers, one to fill with soil and one with microbial inoculant. The 200 ml volume of soil and the volume corresponding to 0.75 g of microbial formulation were highlighted on the respective containers with a marking line. After filling the first container with soil up to the mark, the inoculum formulation was added. The opening of the container was closed and the content was shaken three times then the mix was laid under the tuber. After inoculation of the tubers, a hilling machine (Haruwy, Romanel-sur-Lausanne, Switzerland) was used to cover the tubers by forming potato hills.

Water content of the soil was monitored using two probes (Drill and drop 60 cm, Sentek) connected to a data logger (DS3, Sensorscope). One probe was installed in an irrigated plot and the second one in a rainfed plot. Before the soil water content (SWC) reached the onset of stress for the potato plants, irrigation was performed in the unstressed plots via a drip-by-drip system avoiding irrigation of the rainfed lines. Onset of stress was avoided by maintaining the SWC above two thirds of the field capacity (FC). FC was determined empirically by recording the SWC one to two days after an excess irrigation, once the SWC within the rooting zone had stabilized.

Solar radiation for each day and year as well as precipitation were obtained from an automatic weather station (CR1000, Campbell scientific) located on the research station less than two kilometres away from the fields (46°23′52.073″N 6°13′52.061″E). The data were retrieved from the "agrometeo" website (https://www.agrometeo.ch/meteorologie?stations=3).

Plant analyses

During the growing season, we used the *Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie* (BBCH) scale to identify the phenological stages of plants (Hess et al. 1997). Three stages of potato plant development were recorded for each plot: (i) emergence (potato BBCH 009), (ii) full flowering, when 50% of flowers in the first inflorescence opened (BBCH 635) and (iii) onset of senescence (BBCH 901).

In order to monitor the N content of the plants in a non-destructive way, a chlorophyll meter was used at two time-points during the growing season. The SPAD-502 from Konica Minolta (Osaka, Japan) was used by clamping the device on leaf tissue. The reading is an index significantly correlated with chlorophyll content, with no influence of environmental light or irrigation events (Mielke et al. 2010). The average of five measurements was recorded in newly unfolded and intact leaves of the main stem of each plant. Five plants per plot were analyzed.

Stomatal conductance measurements were done with an AP4 porometer from Delta-T devices (Cambridge, United Kingdom), according to the recommendations of the manufacturer. Special care was taken to avoid cloudy weather and to perform calibration (less than 5% error on the curve was a prerequisite) before each measurement session. Five plants per plot were measured, on two replicates of the trial. Measurements were taken only during the second year of trial.

After harvest, yield was measured for the following tuber sizes: < 35 mm, 35 to 42.5 mm, 42.5 to 55 mm, 55 to 70 mm, and > 70 mm, using a mechanical potato grader (Allround Vegetable Processing, Andijk, NL). In addition, a subsample of six tubers was taken from each plot and sent to Szent István university in Gödöllö (Hungary), where the phosphorus concentration in tubers was determined using the NaOH-EDTA method originally used for soil by Bowman and Moir (1993).

Sampling and DNA extraction from roots

Potato roots were sampled in the four repetition blocks each year. With the help of a garden fork, plants were uprooted and roots were gently shaken to remove attached soil. As many roots as possible were sampled and placed in Falcon® tubes. The stolons were not harvested. Root samples were refrigerated during the sampling time, then frozen at -20°C and kept until processing. No more than 2 h passed between uprooting and freezing of the plant material. In 2019, 2020 and 2021, samples were taken at emergence (BBCH 009) and full flowering, when 50% of flowers in the first inflorescence opened (BBCH 635). Sampling stages were intensified in 2021 by taking supplementary samples when first leaf of the main stem unfolded (BBCH 101), first individual buds (1–2 mm) of first inflorescence appeared (BBCH 501) and first flowers in the blocks were observed (BBCH 600).

In 2019 and 2020 samples were rinsed under tap water to eliminate soil particles, frozen with liquid nitrogen and then manually ground into powder with mortar and pestle. Then, DNA was extracted using the innuPREP Plant DNA kit (Analytik Jena, Germany) following the manufacturer's instructions. In 2021, different grinding and extraction protocols were used in order to improve efficiency and allow bigger sample size. After rinsing the roots with tap water, 1 ± 0.2 g samples were ground in 12×15 cm Universal extraction bags (Bioreba, Reinach, CH) containing 6 ml 3% CTAB solution, using the semi-automated homogenizer Homex 6 (Bioreba, CH). The CTAB extraction solution and DNA extraction protocol used were identical to that described in Mahillon et al. (2022).

Detection of strains

Detection of the microbial inoculants in DNA extracts was performed by amplification of four sets of primers, using real-time PCR. All of the amplification/detection experiments were performed on a CFX-96 Touch Real-Time PCR Detection System (Bio-rad, Hercules, US). Prior to amplification, every DNA sample was subjected to quantification using Qubit™ dsDNA BR Assay (Thermo Fischer Scientific, US), following the manufacturer's instructions. Each sample was then diluted to a concentration of 4 ng/µl and stored at 5°C until real-time PCR was performed on 96-well plates with sealing film. Probe-based detection experiments were conducted using GoTaq® Probe qPCR kit and dye-based ones using GoTaq® qPCR (both from Promega, Madison, US). For all experiments, standard curves were created using serial dilutions of pure gDNA of the strains, and used to determine PCR efficiencies which ranged from 90 to 110%. Standard curves, PCR efficiencies and absolute quantification of the target sequences were achieved using the CFX Maestro Software (Bio-rad, Hercules, US).

The primers and probes used for detection of the four strains are detailed in Table 2. They were all synthesized by Microsynth AG (Balgach, Switzerland). Detection of *R. irregularis* MUCL41833 was performed with the primer set BF6/BF8 targeting the Mitochondrial Large SubUnit (mtLSU) and cycling conditions published in Buysens et al.

Table 2 Primers used for the detection of microbial inoculants

Organism	Gene product	Primers and probes sequences (5′→3′)	Reference
Paraburkholderia phytofirmans PsJN	transcription termination factor Rho (rho)	FWD: AAAAACGAGCCAAAA CGGGC RWD: CACCAGCGCGAAATA ACG FAM- AAACCTCGTACCTCG CCAGC-BHQ	Sheibani-Tezerji et al. (2015)
Rhizophagus irregularis MUCL 41833	mitoribosome large subunit (mtLSU)	FWD: AAGTCCTCTAGGTCG TAGCA RWD: ACAGGTATTTATCAA ATCCTTCCC	Buysens et al. (2017)
Pseudomonas brassicacearum 3Re2-7	nucleotidyltransferase family protein (NFP)	FWD: CCAGAAACCGGCCAA TAACA RWD: AAAGCGTGGCGTGAG ATATG PROBE: FAM- AAACCTGCT TCTGCCTCGTCACTC-BHQ	developed by the Austrian Institute of Technology (2020)
Trichoderma asperelloides A	translation elongation factor EF-1alpha (tef1)	FWD: ACCCAATTCCCCAAG CACC RWD: AAATTGACACCCCAC TAAAAGCCA	developed by De Ceuster Meststoffen (2021)

(2017). Each reaction well contained 10 µl GoTaq® mix, 0.4 µM of each primer and 8 ng DNA template, and was completed with PCR grade water from the kit to a final volume of 20 µl. Detection of *P. brassicacearum* 3Re2-7 was done with a pair of primers designed to amplify a genomic region situated on the single circular chromosome of the strain, between 928,322 and 928417bp. This region partly codes for a nucleotidyltransferase family protein (NFP, coding DNA sequence QEO76763.1, NCBI locus tag ELZ14_RS04075). Each reaction well contained 10 µl GoTaq® probe mix, 0.4 µM of each primer, 0.2 µM of the probe and 8 ng DNA template, and was completed with PCR grade water from the kit to a final volume of 20 µl. Thermocycling parameters were as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles at 95°C for 5 s and 60°C for 25 s. Detection of *P. phytofirmans* PsJN was performed using a pair of primers designed to amplify the transcription termination factor Rho (Bphyt_1824). The amplified fragment is situated on chromosome 1 between 2046634 and 2047902 bp. Primers and probe sequences were developed by Sheibani-Tezerji et al. (2015). Each reaction well contained 10 µl GoTaq® probe mix, 0.5 µM of each primer, 0.35 µM of the probe and PCR grade water from the kit to reach the final volume of 20 µl. Thermocycling parameters were as follows: 95°c for 2 min, followed by 40 cycles of 95°C for 5 s and 59°C for 20 s. Detection of *T. asperelloides* A was done using a pair of primers (Table 2) amplifying a 315bp fragment located on the tef1 gene, coding for the translation elongation factor EF-1alpha. The primers were used at concentrations of 0.4 µM. The reaction also contained 8 ng template DNA and 10µl GoTaq® mix and PCR grade water from the kit, for a final reaction volume of 20 µl. Thermocycling parameters were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 64°C for 30 s and 60°C for 30 s.

Quantification of blemish diseases

Symptoms of infection by *R. solani*, *Streptomyces* spp., *C. coccodes* and *H. solani* were recorded during the three growing seasons. After randomly taking 25 tubers from each row and washing them in water, we scored the blemishes by estimating the percentage of tuber surface that was infected (0, 20, 40, 60, 80 or 100%). As they are not distinguishable with the naked eye, symptoms of *H. solani* and *C. coccodes* were scored together.

Statistical analysis

All analyses were performed using R Statistical Software (R Core Team 2023) (v4.2.1). A mixed model was used to test the combined effect of the fixed factors irrigation, inoculation and variety, in addition to the random factor blocks. The years were analyzed separately. The model was fitted using R with the software Rstudio (Allaire 2012) and the package agricolae (Mendiburu and Yaseen 2020), using the ssp.plot function. After the analysis of variance, significant differences between the treatment groups were explored using Fisher's protected least significant difference test (LSD test). Correlation between variables was tested using Pearson's correlation coefficient. Varietal susceptibility of cv. Pentland Dell and cv. Désirée to the monitored diseases has been quantified in previous studies (Dowley 1969, 1972; Hilton et al. 2000; Pasco et al. 2005; Zhang et al. 2014) and is not in the scope of this study. Hence, in the analysis of the external defaults, years were considered as an explanatory variable but the varieties were presented independently. Results of the detection of the microbial inoculants were plotted using packages ggplot2 (Kahle and Wickham 2013) and ggforce (Pedersen et al. 2020). Correlation graphs were built with the additional help of the ggpubr (Kassambara 2020) package.

Results

Yield and plant growth assessments

Yield over the three growing seasons averaged 37.3 t/ha. Overall, cv. Pentland Dell yielded less (34,93 t/ha) than cv. Désirée (39.67 t/ha). Nonetheless, during the second year of the trial, there was no difference between the averaged yield of the two varieties, cv. Pentland Dell performing better under water stress, whereas cv. Désirée showed higher yield in irrigated conditions (Table 3). There was an impact of irrigation on yield in the two first years of the study. The rainfed lines yielded 16.8% less than irrigated plots in the first year, and 18.6% in the second. The third year of trial suffered heavy rainfall. From planting day to haulm killing, there was an average of 5 l/m^2 rainfall per day while the 1st and 2nd years averaged 2–3 l/m^2 per day (supplementary Fig. 2). No yield loss in rainfed plants was recorded during the third year.

Stomatal conductance of potato plant leaves was affected by irrigation, with a lower conductance in rainfed lines during the second year for both varieties (no data for 1st and 3rd year). The stomatal conductance of Désirée averaged 218 mmol/m^2/s in irrigated lines and 159.89 mmol/m^2/s in rainfed plots. This variable was not affected by the inoculation treatments.

During the first year, only consortia of inoculants were applied. In this growing season, the irrigated plots showed a significantly higher yield compared to rainfed lines ($p = 0.044$). In addition, the cv. Désirée showed higher yield in comparison with cv. Pentland dell ($p < 0.001$). No effect of inoculation treatment on yield ($p > 0.05$) was detected.

Table 3 Average potato tuber yield in metric tons per hectare

Treatment	1st year—2019	2nd year—2020	3rd year—2021
Irrigation			
Irrigated	49.6 ± 7.6 a[a]	43.5 ± 8.1	34.3 ± 5.1
Rainfed	41.3 ± 8.1 b	35.3 ± 6.5	34.2 ± 5
Variety			
Désirée	51 ± 7.8 a	39.5 ± 9	37.2 ± 4.7 a
Pentland Dell	39.9 ± 5.7 b	39.3 ± 7.8	31.2 ± 3.1 b
Inoculation			
Not inoculated	46.0 ± 8.7	38.2 ± 9.0	33.6 ± 5.8
P.brass	–	38.4 ± 7.3	35.2 ± 4.4
P.phyt	–	41.1 ± 9.0	33.9 ± 4.7
R.irreg	–	37.1 ± 8.2	33.8 ± 5.1
T.asper	–	42.7 ± 8.2	33.5 ± 4.6
R.irreg + P.brass	45.1 ± 8.9	37.4 ± 9.5	34.2 ± 6.1
R.irreg + P.phyt	44.4 ± 8.6	40.2 ± 7.3	34.9 ± 5.4
R.irreg + P.phyt + T.asper	46.2 ± 9.8	40.2 ± 8.5	34.7 ± 4.6
Mixed Model			
irrigation	*	ns	ns
inoculation	ns	ns	ns
irrigation x inoculation	ns	ns	ns
variety	**	ns	***
variety x irrigation	ns	*	ns
variety x inoculation	ns	ns	ns
variety x irrigation x inoculation	ns	ns	ns

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

[a]Data are means ± SD of 4 blocks (25 plants per block)

The interaction between the aforementioned factors was not significant ($p > 0.05$).

The yield of the second year of trial was not influenced significantly by the inoculation treatments ($p > 0.05$), the irrigation ($p = 0.09$) or the variety ($p > 0.05$). However, the interaction between variety and irrigation was significant ($p < 0.05$).

In the third year of trial, cv. Désirée yielded significantly more than cv. Pentland Dell ($p < 0.001$). Again, no inoculation effect was observed ($p > 0.05$). The interactions between the factors impacted yield in a non-significant way ($p > 0.05$). Recording of the phenological stages (emergence, flowering, onset of senescence) revealed that they were not influenced by the application of inoculants. The same was true for the chlorophyll content index of potato leaves.

Phosphorus content

Tuber P concentration was determined but was not significantly influenced by any of the explanatory variables. Variation between samples was high, with an average of 0.31% of P with an SD of 0.09. While both varieties had similar tuber P content (0.31% for cv. Désirée and 0.30% for cv. Pentland Dell), the biggest variation between groups was found between inoculation treatments. During the 1st year, the control averaged 0.33%, while the tubers originating from plants inoculated with *R.irreg* + *P.phyt* contained 0.27% P. This was also the case during the 2nd year, where control tubers averaged 0,34% P while tuber inoculated with R.irreg + P.phyt + T.asper contained 0.28% P.

External defaults; cv. Désirée

Silver scurf and common scab-associated symptoms, as well as *R. solani* sclerotia were observed on tubers of cv. Désirée, one month after harvest. The incidence of these diseases was highly variable on this cultivar: *R.solani* successfully colonized 3.1% of the Désirée tubers, whereas common scab associated symptoms (CSAS) and silver scurf associated symptoms (SSAS) were observed in 44.2% and 82.1% of the tubers, respectively (Fig. 1).

CSAS were significantly higher during the second year of the trial. In addition, the consortium *R.irreg* + *P.phyt* + *T. asper* significantly lowered CSAS incidence by 19.9% on cv.

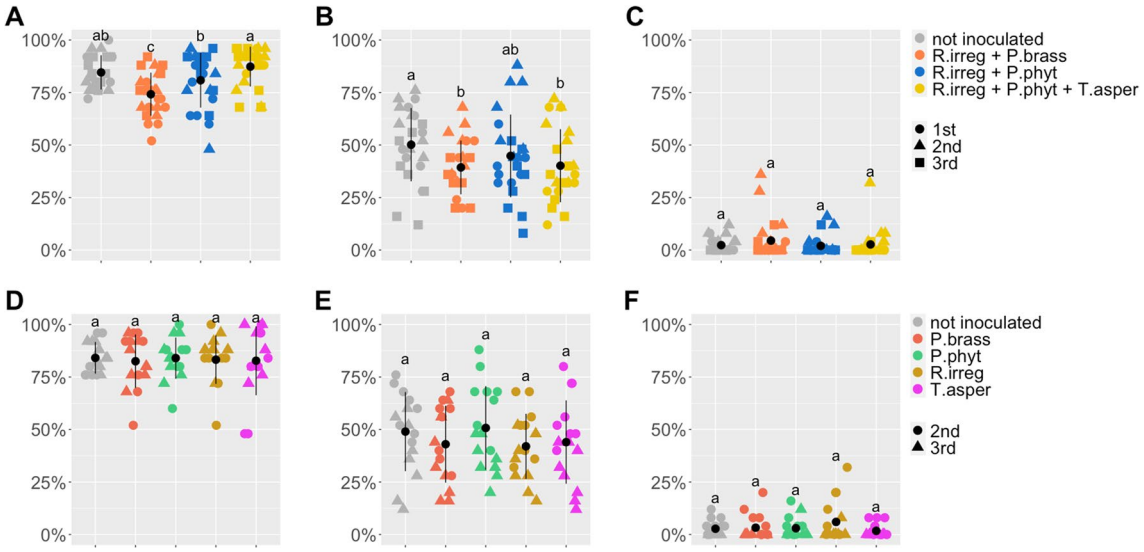

Fig. 1 Incidence of silver scurf-associated symptoms (**A**, **D**), common scab-associated symptoms (**B**, **E**) and *Rhizoctonia solani* symptoms (**C**, **F**), in the variety Désirée. Each inoculum is presented by a color. Black dots represent means and vertical bars show the standard deviation. Values followed by different letters are significantly different according to Fisher's LSD test ($P < 0.05$)

Désirée (Fig. 1B). This was also the case for the consortium *R.irreg* + *P.brass*, which generated a 21.6% decrease in the number of infected tubers (Fig. 1B). The severity of CSAS was also impacted by year, but not by the inoculation treatments ($p < 0.05$, supp Fig. 3B, E).

SSAS showed a very high occurrence in cv. Désirée, and were more frequent in irrigated plots ($p < 0.05$). SSAS were also impacted by inoculations ($p < 0.001$), significantly in plots inoculated with the consortium *R.irreg* + *P.brass* which showed 11% fewer infected tubers (Fig. 1A). Severity of the SSAS was also 21.2% lower after inoculation with *R.irreg* + *P.brass* (Fig. 1B), and was significantly higher in irrigated plots, and during the first year of trial. Lastly, incidence and severity of *R. solani* symptoms (RSS) were low in every season. Less than 5% of the tubers were infected in 2020, and less than 2% in 2019 and 2021 (Fig. 1C, F), while severity always remained under 5%, (supp. Fig. 3C, F).

External defaults; cv. Pentland Dell

Incidence of CSAS (37,8%) and especially SSAS (35.7%) were lower for cv. Pentland Dell, while RSAS were similar (2,4%) to cv. Desiree (Supp. Fig. 4). In cv. Pentland Dell, CSAS incidence was higher during the first year of the trial, averaging 51.4%, while incidence values in the 2nd and 3rd years were 30.3% and 38.5%, respectively. Inoculation with single strains or with consortia did not influence the CSAS ($p > 0.05$). However the severity of these symptoms was significantly impacted by the interaction between the inoculation of consortia and the year of trial ($p < 0.01$). SSAS severity on cv. Pentland Dell was influenced by inoculation ($p < 0.05$), which was not the case for the incidence ($p = 0.075$). Single strain inoculation provided an overall decrease of the symptoms ($p < 0.05$), in a significant way for *P.brass* and *T.asper*, which lowered incidence by 25.6% and 34.5% (Supp. Fig. 4D). As in the case of cv. Désirée, RSS symptoms were almost absent on cv. Pentland Dell (Supp. Fig. 4C, F), and their severity remained below 5% (Supp. Fig. 5C, F).

Microbial strain establishment

R.irreg was not detected in the samples of the 2nd and 3rd year, while it was detected in several samples in the first year. However, several non-inoculated control samples of the same year showed amplification of *R.irreg* as well, therefore we cannot conclude that the detection was a result of inoculation. *P.phyt* was detected in several samples during the 1st and 3rd year, while it was absent in the samples of the 2nd year. *P.brass* was the only strain that was detected every year. In addition, it was detected in every timepoint during the final year, from emergence to full flowering. *T.asper* could not be detected in any of the samples analyzed (Table 4).

In the first year of trial, *P.brass* and *P.phyt* were detected in 10.5% and 12.5% respectively of all the samples collected. While *P.brass* DNA was present at emergence (8% of the samples) and flowering (17% of the samples) in comparable quantities (~ 10E-6 ng/ng DNA extract), *P.phyt* was only detected at emergence (Table 4).

Detection of microbial DNA in potato roots harvested during the second year revealed the presence of *P.brass* in 19 of the 48 samples analyzed. Of of these 19 samples, nine

Table 4 Summary of microbial strains detection

2019	*R.irreg*	*				*
	P.phyt	● 21%				○
	P.brass	● 8%				● 17%
	T.asper	○				○
2020	*R.irreg*	○				○
	P.phyt	○				○
	P.brass	● 38%				● 42%
	T.asper	○				○
2021	*R.irreg*	○	○	○	○	○
	P.phyt	● 6%	● 25%	○	○	○
	P.brass	● 100%	● 100%	● 75%	● 87.5%	● 50%
	T.asper	○	○	○	○	○

Black circles indicate that the strain was present, and the associated value represents the percentage of samples in which the DNA of the strain was detected. *cases where amplification occurred in non-inoculated samples

were taken at emergence (38% of the samples) and 10 at full flowering (42% on the samples). The quantification showed that the nine emergence samples averaged 3.25E-06 ng/ng DNA extract while the 10 samples taken at flowering averaged 3.39E-06 ng/ng DNA extract.

In the third year, the sampling was intensified to include new developmental stages. In roots inoculated with *P.brass* alone, the DNA of the strain was detected in every sample, at every stage, except for one sample taken at the first bud stage. The highest quantity of *P.brass* NFP DNA was detected at the emergence stage, with an average of 0.004 ng/ng DNA extract. This value was increased by the concentration of one sample containing a very high number of the marker gene copies, reaching 0.014 ng/ng DNA extract (Fig. 2, first sample from the left, value only partially shown in the zoom area). Thereafter quantities of NFP DNA were comparable for the first leaf, first flower and full flowering stages. The first bud stage averaged significantly lower amounts, and one of the samples did not show any amplification (Fig. 2). Since *P.phyt* was only detected in the two first developmental stages and in a minority of the samples (3 out of 52), no graphical representation of the quantification is shown.

Discussion

Introduction of PGPM in the field has a long history, with the first commercial patent dating as far back as the end of the nineteenth century, when pioneers inoculated legumes with rhizobia (Santos et al. 2019). This practice has evolved and diversified to include new plant species, microbial

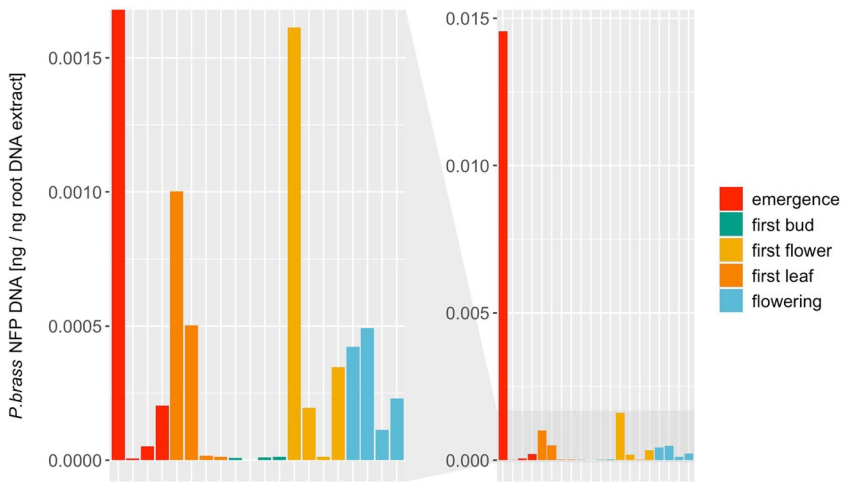

Fig. 2 ng *P.brass* NFP DNA per ng root DNA extract at each of the five timepoints, in the last year of trial (2021). Each bar represents the amount of nucleotidyltransferase family protein (NFP) DNA specific to *Pseudomonas brassicacearum* 3Re2-7 quantified in one root sample. The sample furthest left (in red), is only partially shown in the zoom on the left panel

inoculants, carrier formulations and delivery techniques (de-Bashan et al. 2020). In addition to atmospheric N_2-fixing rhizobia, it now benefits from a whole range of microorganisms to improve crop growth and health (Nadeem et al. 2013).

In the present study, we inoculated strains with diverse plant growth- or health-promoting effects. *Paraburkholderia phytofirmans* PsJN produces 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD) to prevent ethylene synthesis, alleviating plant stress (Onofre-Lemus et al. 2009). It can also synthesize Indole-3-Acetic Acid (IAA), which is a plant growth-promoting hormone (Naveed et al. 2015), as well as siderophores, providing iron to plants and protecting them from phytopathogenic infections (Esmaeel et al. 2018). *Rhizophagus irregularis* strains are able to take up and translocate minerals such as inorganic P to plant roots (Ezawa and Saito 2018), and to activate local and systemic resistance in plants (Gallou et al. 2011; Pozo and Azcón-Aguilar 2007). *Trichoderma asperelloides* is also able to produce ACCD (Viterbo et al. 2010) and has been shown to induce the expression of genes involved in salt tolerance, biotic resistance and plant growth responses (Brotman et al. 2013). *Pseudomonas brassicacearum* 3Re2-7 genome mining revealed the presence of genes related to phosphate solubilization, the antibiotic 2,4-Diacetylphloroglucinol (2,4-DAPG), ACCD and antipathogenic compounds like chitinase and exoprotease and genes involved in iron acquisition (Nelkner et al. 2019). Small volatile organic compounds released by this strain are able to lower the mycelial growth of *Rhizoctonia solani* (Kai et al. 2007).

All of the strains selected for this study have strong abilities to stimulate potato growth and resilience to biotic and abiotic stresses. However, these effects are strictly conditioned by the successful establishment of the microorganisms in the roots or the rhizosphere of the target crop. Our results show no effect of microorganisms inoculated alone or in consortia on tuber yield, whether under rainfed or irrigated conditions, and for both varieties. Similarly, many studies report the absence of any yield effect after field inoculations of plant growth-promoting microbes (Buysens et al. 2017; Dal Cortivo et al. 2017; Mayer et al. 2010). Explanations often reside in the ability of the strains to colonize the target niche, in our experiment, the potato roots or rhizosphere. In addition, the service provided by the strain could be masked by the environmental conditions. As an example, it is possible that high P availability of the field soil did not favor the accommodation of phosphate-solubilizing strains (Jansa et al. 2006) or did not stimulate the AMF to transport P to the plant (Smith and Read 2010). In our trials, this phenomenon could explain the absence of *R. irregularis* from the analyzed root samples, along with the competition with native AMF communities. Amplification in non-inoculated samples during the 1st year probably resulted from the inability of the primer pair to distinguish between closely related strains, as exposed in the reference article, Table 2 (Buysens et al. 2017). Similarly, *T. asperelloides* was not detected in the root samples. However, it is possible that *T. asperelloides* exerted an effect in the potato rhizosphere without having colonized the roots of the crop. This could explain the lack of detection in the roots, even though reduction in the severity of SSAS was observed after inoculation with this fungus. Similarly, the lower CSAS incidence recorded after co-inoculation with *R. irregularis* and *P. phytofirmans* could be attributed to rhizospheric *T. asperelloides*. This hypothesis could be tested by monitoring the presence of *T. asperelloides* in soil DNA extracts. Unfortunately, not enough rhizospheric material was harvested in this study to be able to answer this question.

The two inoculated bacteria (i.e., *P. brassicacearum* and *P. phytofirmans*) were detected in the potato root samples. They can both behave as endophytes (Compant et al. 2005; Frommel et al. 1991), meaning that they are able to colonize the internal tissues of a plant host without causing infection or lesions (Compant et al. 2008). Inoculation with *P. brassicacearum* resulted in a decrease of the symptoms of potato blemishes, namely CSAS and SSAS. Moreover, *P. brassicacearum* provided protection against pathogens to both potato cultivars. This effect was observed after inoculation of the single strain, in the case of the severity of SSAS on cv. Pentland Dell tubers. Co-inoculation of *P. brassicacearum* with *R. irregularis* reduced the incidence of CSAS and SSAS in cv. Désirée. The AMF strain was not detected in the root system of cv. Désirée at emergence or later developmental stages. This does not preclude interaction between the two microorganisms during the root initiation phase, potentially resulting in a plant protective effect. The co-inoculation of *R. irregularis* MUCL41833 and *Pseudomonas* sp. R41805 was described as a potential activator of the systemic defense of potato plantlets, which was not the case for single inoculations of these strains (Velivelli et al. 2015). Detection and quantification of *P. brassicacearum* exposed its ability to establish and survive in cv. Désirée roots. In the three years of this trial, *P. brassicacearum* colonized the crop root system in most of the samples analyzed, suggesting that it was well suited to the environmental conditions and was compatible with the cv. Désirée. In addition, it could overcome the competition with native microorganisms to establish in the root system of potato, potentially providing a defense against phytopathogenic infections by *Streptomyces* spp. and *C. coccodes* or *H. solani*. Our study revealed that the establishment of *P. brassicacearum* depends on the phenological stage of the plant. At the first bud stage (BBCH 501), very few copies of *P.brass* DNA were detected in two samples out of the four analyzed. The third sample also showed amplification, but the quantification value was beneath the lowest standard in our protocol, indicating absence or an extremely low

concentration, while the fourth sample resulted in no amplification at all (Fig. 2). This low presence or absence may be due to plants selecting for particular microbial communities at different stages of growth, promoting the development of other microorganisms instead of *P. brassicacearum*. This selective process has already been observed in many crops (Cavaglieri et al. 2009; Houlden et al. 2008), including potato (Andreote et al. 2010). Inoculation with *P. phytofirmans* did not provide any significant effect on the extent of blemishes. This strain was detected in year 1 and 3 of the trial, but only in 10% and 6% of the samples analyzed, respectively. However, as part of a consortia with *R. irregularis* and *T. asperelloides*, it may have played a role in the reduction of CSAS incidence (Fig. 1B).

As expected, consortia were more effective at lowering incidence or severity of the symptoms than strains inoculated alone. However, the incidence of SSAS was decreased after inoculation with single strains *P. brassicacearum* and *T. asperelloides* in Pentland Dell (Supp. Fig. 4D). In some cases, incidence but not severity of the symptoms was lowered. This was the case for the symptoms of CSAS on cv. Désirée inoculated with consortia, or symptoms of CSAS on cv. Pentland Dell inoculated with single strains. On the contrary, inoculation of Pentland Dell with consortia provided a reduction in the severity of SSAS but no decrease in incidence. These variables are known to be correlated, but not necessarily in a linear way (Seem 1984). Calculation of the Pearson correlation coefficient between the two variables for the whole dataset reveals a strong correlation, with $R = 0.83$ for SSAS and 0.88 for CSAS and RSS (Supp. Fig. 6). In addition, the scoring of SSAS includes two pathogens (*H. solani* and *C. coccodes*) without discrimination between the two, and these pathogens are favored by different environmental conditions. Potato plants grown during humid seasons are more prone to infection by *C. coccodes* (Hide et al. 1994), while warm and dry conditions would favor *H. solani* (Massana-Codina et al. 2021). This could result in seasonal prevalence of one or the other pathogen, potentially influencing the relationship between incidence and severity for these symptoms. Binocular observations on a random selection of tubers showing SSAS revealed the presence of both pathogens in most of the cases (data not shown).

In Switzerland, incidence and severity of potato blemishes like silver scurf determine the acception by the consumer market. A maximum of 75% coverage of the tuber surface by SSAS on 6% of tubers is tolerated. Rejection of potato batches based on these criteria results in a loss of 80% of the harvest value, as the batches cannot be used for human consumption and are then sold at a lower price for animal feeding. In order to avoid such dramatic losses for the farmer, we provide the basis for the development of novel control strategies based on the application of microbial inoculants.

Conclusion

While we demonstrated a synergy between PGPM, crop genotype and environmental conditions, it must be noted that progress still needs to be made in the formulation of the products or in the timing of application of the strains, in order to maximize the benefits associated with the inoculants. In an effort to lighten up the "black box" that follows field inoculation of PGPM, this study provides a dynamic observation of strain establishment and survival, and provides a link between crucial agronomic results and the presence of beneficial microorganisms in the potato root system.

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Author contributions G. Darbon, B. Dupuis and S. Declerck conceived and planned the experiments. G. Darbon, G. Riot carried out the field experiments, while G. Darbon and M. Doubell carried out the lab work. G. Darbon and G. Riot contributed to sample preparation. G. Darbon, B. Dupuis and S. Declerck contributed to the interpretation of the results. G.Darbon took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Data availability The data that support the findings of this study are available on request from the corresponding author, GD.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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