# Microbe-coated Fertilizers: A Novel Technology for Agricultural Microbial Inoculation

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## List of Abbreviations

| | |
|---|---|
| PGPR | Plant Growth-Promoting Rhizobacteria |
| MCF | Microbe-coated fertilizer |
| EVL | EVL Coating® (microbial consortium) |
| EB | Era Boost Pro microbes (microbial consortium) |
| GDD | Growing degree days |
| SD | Seeding date |
| VS | Vegetative stage |
| FS | Flowering stage |
| PH | Plant height |
| LA | Leaf area |
| DB | Dry biomass |
| MSY | Marketable size yield |
| TY | Total yield |
| MST | Marketable size tubers |
| N | Nitrogen |
| WS | Water stress |
| SFW | Shoot fresh weight |
| SDW | Shoot dry weight |
| RL | Root length |
| RFW | Root fresh weight |
| RDW | Root dry weight |
| SPAD | Soil Plant Analysis Development |
| PR | Photosynthetic rate |
| SC | Stomatal conductance |
| %S | Total starch content |

## Abstract

The application of beneficial microorganisms to enhance crop plant growth has shown inconsistent results due to issues with microbial inoculation viability, growth promotion efficiency, and environmental conditions. Microbial consortia inoculants could play a key role in establishing a plant and contribute to plant health and development, particularly in unfavourable climatic conditions. Plant growth-promoting rhizobacteria (PGPR) are promising soil microorganisms due to their ability to contribute to N fixation, P solubilization, nutrient uptake, and stress tolerance, leading to enhanced growth and improved crop productivity. Sustainable methods are needed to enhance the productivity of key crops, including potato (the world's fourth most important food crop). In this project, a novel approach to microbial inoculation consisting of coating on synthetic fertilizer was used to evaluate two microbial consortia (i.e., EVL Coating® and Era Boost Pro) applied to potato (cultivar Goldrush). The microbe-coated fertilizers (MCF) were assessed for growth promotion efficiency under field and greenhouse conditions.

In a first study, a two-year field experiment was conducted in sandy soil at Sainte-Anne-de-Bellevue, QC, Canada, to evaluate the effects of the two commercial PGPR formulations: EVL Coating® (a consortium of *Bacillus*, *Pseudomonas* and *Lactobacillus* bacterial strains) and Era Boost Pro (a consortium of five *Bacillus* strains) on potato growth, development, and yield when planted at different dates. Results showed that across all environments, the two MCF formulations increased leaf area (15.3 - 23.1%), dry biomass (11.6 - 21.9%), total nitrogen concentration (3.9 - 13.8%), marketable-size (9.1 - 21.9%) and total tuber yield (9 - 20.8%), compared to non-inoculated control plants.

In a second study, the two MCF formulations were assessed for their effects on potato in the presence of water stress under greenhouse conditions. The goal was to evaluate the potential of

MCF in reducing potential negative impacts of stress, in this case mild and severe water stress, on potato. Three microbial treatments were compared: a control (uncoated NPK fertilizer formulation), NPK+EVL Coating®, NPK+ Era Boost Pro microbes, along with three levels of water treatment (fully irrigated, 25 and 50% water withhold). Plant growth and development were assessed at two phenological growth stages (vegetative and flowering) by monitoring plant height, leaf area, shoot fresh and dry weight, root length and root fresh and dry weight. Physiological data, including leaf greenness (SPAD), photosynthetic rate, and stomatal conductance (portable LiCor meter) were also monitored. This second study indicated that MCFs had positive effects on most of the variables (plant height, leaf area, shoot fresh weight, root length, root dry weight), leaf greenness and, most importantly, total tuber yield in at least one of two runs of the experiment. However, variables such as shoot dry weight, root fresh weight, and total nitrogen in plant tissues responded inconsistently to MCFs. Physiological (photosynthetic rate, stomatal conductance) and quality (total starch content) variables did not respond to MCF treatments in either experiment. Numerical values in most of the studied variables suggest higher levels for NPK+EVL than NPK+EB, particularly leaf area, shoot fresh weight, photosynthetic rate, nitrogen concentration, tuber yield and starch content. Results from field and greenhouse experiments demonstrate the potential of both microbial consortia when coated on synthetic fertilizers to promote potato growth, development, and ultimately yield, introducing a new technology for more microbe-based agriculture and sustainable potato production.

## Résumé

L'application de micro-organismes bénéfiques pour améliorer la croissance des plantes cultivées a donné des résultats irréguliers en raison de problèmes liés à la viabilité de l'inoculation microbienne, à l'efficacité de la promotion de la croissance et aux conditions environnementales. Les inoculants de consortiums microbiens pourraient jouer un rôle clé dans l'établissement d'une plante et contribuer à sa santé et à son développement, en particulier dans des conditions climatiques défavorables. Les rhizobactéries favorisant la croissance des plantes (PGPR) sont des micro-organismes du sol prometteurs en raison de leur capacité à contribuer à la fixation de l'azote, à la solubilisation du phosphore, à l'absorption des nutriments et à la tolérance au stress, ce qui permet d'améliorer la croissance et la productivité des cultures. Des méthodes durables sont nécessaires pour améliorer la productivité des cultures clés, y compris la pomme de terre (la quatrième culture vivrière la plus importante au monde). Dans ce projet, une nouvelle approche de l'inoculation microbienne consistant à enrober un engrais synthétique a été utilisée pour évaluer deux consortiums microbiens (EVL Coating® et Era Boost Pro) appliqués à la pomme de terre (cultivar Goldrush). Les engrais enrobés de microbes (MCF) ont été évalués pour leur efficacité à stimuler la croissance dans des conditions de terrain et de serre.

Dans une première étude, une expérience de terrain de deux ans a été menée dans un sol sablonneux à Sainte-Anne-de-Bellevue, QC, Canada, afin d'évaluer les effets des deux formulations commerciales de PGPR: EVL Coating® (un consortium de souches bactériennes *Bacillus*, *Pseudomonas* et *Lactobacillus*) et Era Boost Pro (un consortium de cinq souches *Bacillus*) sur la croissance, le développement et le rendement des pommes de terre plantées à différentes dates. Les résultats ont montré que dans tous les environnements, les deux formulations MCF ont augmenté la surface foliaire (15,3 - 23,1%), la biomasse sèche (11,6 - 21,9%), la teneur

en azote total (3,9 - 13,8%), la taille commercialisable (9,1 - 21,9%) et le rendement total en tubercules (9 - 20,8%), par rapport aux plantes de contrôle non inoculées.

Dans une seconde étude, les deux formulations de MCF ont été évaluées pour leurs effets sur la pomme de terre en présence d'un stress hydrique dans des conditions de serre. L'objectif était d'évaluer le potentiel de la MCF à réduire les impacts négatifs potentiels d'un stress hydrique léger ou sévère sur la pomme de terre. Trois traitements microbiens ont été comparés : un contrôle (formulation d'engrais NPK non enrobée), NPK+EVL Coating®, NPK+ Era Boost Pro microbes, ainsi que trois niveaux de traitement de l'eau (complètement irrigué, 25 et 50% de retenue d'eau). La croissance et le développement des plantes ont été évalués à deux stades phénologiques (végétatif et floraison) en contrôlant : la hauteur des plantes, la surface foliaire, le poids frais et sec des pousses, la longueur des racines et le poids frais et sec des racines. Les données physiologiques comprenant la verdure des feuilles (SPAD), le taux de photosynthèse et la conductance stomatique (compteur LiCor portable) ont également été contrôlées.

Cette seconde étude a indiqué que les MCF avaient des effets positifs sur la plupart des variables (hauteur de la plante, surface foliaire, poids frais des pousses, longueur des racines, poids sec des racines), sur la verdeur des feuilles et, surtout, sur le rendement total des tubercules dans au moins une des deux séries de l'expérience. Cependant, des variables telles que le poids sec des pousses, le poids frais des racines et l'azote total dans les tissus végétaux ont réagi de manière irrégulière aux MCF. Les variables physiologiques (taux de photosynthèse, conductance stomatique, teneur totale en amidon) n'ont pas réagi aux traitements MCF dans les deux expériences. Les valeurs numériques de la plupart des variables étudiées sont plus élevées pour NPK+EVL que pour NPK+EB, en particulier la surface foliaire, le poids frais des pousses, le taux de photosynthèse, la teneur en azote, le rendement des tubercules et la teneur en amidon. Les résultats des expériences sur le terrain et en serre démontrent le potentiel des deux consortiums microbiens lorsqu'ils sont enrobés d'engrais synthétiques pour promouvoir la croissance, le développement et finalement le rendement des pommes de terre, introduisant une nouvelle technologie pour une agriculture basée sur les microbes et une production durable de pommes de terre.

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## Contributions to knowledge

The application of beneficial microorganisms for plant growth promotion in agricultural settings has shown variable results due to the complexity of environment variables, microbial inoculation viability and growth promotion efficiency. Microbial consortia-based inoculants could play a key role in establishing a plant and contribute to the plant health and development, particularly under unfavourable climatic conditions.

In this study, ***a novel technology for microbial inoculation,*** microbe-coated fertilizers (MCF), was used to evaluate two unique microbial consortia coatings on synthetic fertilizer applied to the field and greenhouse grown potato (cv. Goldrush). The MCFs were assessed for their growth promotion efficiency in a wide range of field conditions, as well as under water stress conditions in a controlled environment.

**Industrial relevance:** SynAgri staff and personnel from the Smith laboratory have collaborated on the development of this project. With increased public awareness of environmental issues and strengthening policy around greenhouse gas emissions and volatile energy costs, there is a need to develop sustainable agricultural inputs, such as crop growth stimulators, effective at low concentrations, that can be integrated into standard agronomic practices. The development of such technologies is a major challenge for the agriculture sector.

This study's findings will significantly impact Canadian crop producers and their ability to improve yields in a genuinely sustainable way. Yield performance is the key economic benefit and the principal driver for producers; this impacts their ability to compete globally. SynAgri will benefit from enhanced understanding and full development of their current consortia-based technology, leading to improved yields and productivity for Canadian growers in a sustainable, environmentally friendly way. This will expand their commercial activities and product offerings.

**Benefits to Quebec/Canada:** The work described herein enhances our understanding of how PGPR members of the phytomicrobiome interact with plants in terms of the effects they can have on growth promotion and crop tolerance to stress. This project provides effective and sustainable crop input technology, leading to improved agricultural sustainability and output. It has the potential to mitigate issues associated with volatile energy costs, particularly those associated with the use of mineral fertilizers, and reduce the environmental footprint of Canadian crop production. The economics and wellbeing of Canadian rural communities will be improved. This technology is particularly important for Canadian agricultural producers as it contributes to improved crop growth under a range of sometimes challenging conditions, making it very applicable to Canada and potentially able to enhance crop productivity in our short-season, early-maturity zones.

In addition, as a high-latitude nation, the degree of climate change will be greater than at lower latitudes; this means more stressful conditions for crop production will occur more often and be more extreme, and low-input biological technologies that can help manage this are needed. This technology will also put Canada in a lead position with regard to the development of biologicals as agricultural inputs, an area that is now seen to have a very large potential. This work can be further commercialized in Canada and elsewhere and will contribute to a growing foundation of understanding in this promising area.

## Contribution of Authors

This manuscript-based thesis was written under the guidance and supervision of Professor Donald L. Smith. My co-supervisor, Professor Philippe Seguin, also provided insights and support throughout the thesis writing. I designed the two-year field and greenhouse experiments for the project and conducted the research. I analyzed data and wrote the initial version of all chapters/manuscripts under the supervision of Professors Donald L. Smith and Philippe Seguin.

The thesis includes five chapters. The first two chapters are a general introduction and literature review, chapters three and four present results of the two projects conducted and will ultimately be submitted for publication in peer-reviewed journals, while chapter five includes general discussion, conclusions, and future research directions.

**Chapter 1: Introduction, research objectives and hypotheses**

Mohammed Antar structured and wrote the initial version of the chapter, which was then reviewed by Donald L. Smith and Philippe Seguin.

**Chapter 2: Literature Review**

Mohammed Antar gathered relevant literature, structured and wrote the initial version of the chapter. Donald L. Smith and Philippe Seguin provided feedback and reviewed the chapter. Mohammed Antar addressed suggestions provided by them during the chapter development.

**Chapter 3: Evaluation of novel microbe-coated fertilizers applied to field-grown potato as a mean of increasing plant growth**

Mohammed Antar set up the experiment, collected data, performed data analysis, interpreted the results, and wrote the initial version of the chapter/manuscript. Donald L. Smith and Philippe

Seguin supervised and guided the scientific activities and provided the intellectual context while the experiments were implemented.

**Chapter 4: Evaluation of two microbe-coated fertilizers and assessment of potential interactions with water on growth of potato under greenhouse conditions**

Mohammed Antar set up the experiment, collected data, performed data analysis, interpreted the results, and wrote the initial version of the chapter/manuscripts. Donald L. Smith and Philippe Seguin supervised and guided the scientific activities and provided the intellectual context as the experiments were implemented. Professor Pierre Dutilleul very kindly provided extensive statistical consultation on this chapter, even using the approach to the data as an example in his Experimental Design course.

**Chapter 5: General discussion, conclusions, and future directions**

Mohammed Antar structured and wrote the chapter. Donald L. Smith and Philippe Seguin guided the scientific knowledge, provided the intellectual context, and reviewed the chapter.

# Chapter 1 : Introduction

## 1.1. Background

The growing world population is a threat to global food security. Agriculture sustainability is a key factor in meeting the food demand of nearly 8 billion people. As the population grows, increasing agricultural productivity is required to meet human food consumption and livestock feed needs. Agricultural producers tend to increase crop yield by increasing the application of synthetic fertilizers, which eventually leads to overall degradation of agricultural land, harm to associated ecosystems, and deterioration of soil properties in terms of nutrients and physical structure (El-Ramady et al., 2014; Hossain et al., 2022). In addition, agricultural producers are now being required to alter their current agricultural practices to combat global climate change. Abiotic stresses, including drought, salinity, extreme temperatures, and nutrient availability, are potential limiting factors in crop production (Raza et al., 2019).

Potato (*Solanum tuberosum*), a member of the Solanaceae family, ranks as the third most significant food crop globally, following rice and wheat. It is first domesticated in the Andes mountains of South America. Currently, there are more than 4000 varieties for human consumption. Over one billion individuals across the globe consume potatoes (Zaheer and Akhtar, 2014), with the total global crop production surpassing 300 million metric tons (Bradshaw and Bradshaw, 2021; Peralta et al., 2021).

The crop has a high need for fertilizer, specifically requiring 250 kg $ha^{-1}$ of nitrogen and 150 kg $ha^{-1}$ of phosphorus in order to get the best possible production (Aloo, 2021). Potato yield is contingent upon environmental conditions and the specific variety being cultivated (Van Oort et al., 2012). Both biotic and abiotic factors have a crucial role in determining potato output, abiotic stresses that are responsible for producing an average yield loss of up to 50% worldwide (George et al., 2017; Baligar et al., 2001). In addition, significant temperature rises can cause severe damage to the amount of tuber production, resulting in increased respiration rates, physiological wilting, decreased photosynthetic activity, and shortened life cycles (Dahal et al., 2019). The main factor contributing to the vulnerability of plants to drought is their shallow root system (Obidiegwu et al., 2015; Daryanto et al., 2017). However, research indicates that susceptibility to drought also varies depending on the specific genotype (variety) of the plant, its growth stage, and the length and intensity of the drought stress (Monneveux et al., 2013).

In conventional row crop production, as practiced in Quebec and elsewhere in Canada, banding "starter" fertilizer during seeding is commonly used to provide readily available nutrients for young emerging plants. However, the nutrient use efficiency of fertilizer is at most 50%, and a portion of the lost fertilizer may damage soil and surrounding environments (Dimkpa et al., 2020). Plant-beneficial soil microbes can be added to plants to increase the availability of nutrients and to provide other beneficial effects to the plants. For these reasons, root-rhizosphere microbial communities (the phytomicrobiome) play a key role in establishing a plant under field conditions and contribute to plant health and development (Antar et al., 2021; Shah et al., 2021). Plants affect this community through root exudates, including specific signal compounds. Plant-beneficial microbes found in soils also play an active role in how plants absorb nutrients, either by synthesizing compounds, such as siderophores, to make the nutrients more plant-available or by solubilizing nutrients unavailable to plants into plant-available forms (Backer et al., 2018; Goswami and Suresh, 2020). Previous research results indicated that specific microbes, alone or in consortia, were more effective when the plants were experiencing abiotic stresses, such as water stress, suggesting that the variability of effects seen under field conditions may be related to variability in stress levels under field growth conditions, the result of weather/climate variability over time (Rolli et al., 2015; Joshi et al., 2020; Mandal et al., 2020; Kour et al., 2022). These studies suggest that microbial inoculation can improve crop growth and yield, particularly during times of environmental stress. Past work within the Smith laboratory demonstrated that a number of the phytomicrobiome-derived plant-beneficial microbes benefit plants experiencing abiotic stress; subsequent work was conducted to assess the potential of a wider range of beneficial microbes and materials produced by them were conducted, including the consortia used in this study (Gray and Smith, 2005; Msimbira et al., 2022; Naamala et al., 2022).

The primary focus of this project was to evaluate two novel commercial plant-beneficial microbial consortia, coated onto synthetic fertilizer beads, in order to assess their potential to affect plant growth and enhance water stress tolerance. Adding beneficial soil microbes is usually conducted using peat-based materials or direct inoculation onto seeds. In this work, we evaluated the potential of two specific microbial consortia and the ability to add them, when coated onto the fertilizers and banded into the crop site. Responses of potatoes were studied over two years in field studies and also under controlled environment (greenhouse) conditions.

## Research Objectives

### 1.1.1. General Objective

This study is primarily focused on evaluating the efficiency and commercial potential of two novel plant growth-promoting microbial consortia applied to potato as coatings on fertilizer granules (Microbe-coated Fertilizers - MCF).

### 1.1.2. Specific Objectives: The objectives of the research are to evaluate:

1. Field performance and efficacy of microbe-coated fertilizers in potato production in sandy soil. This will help determine the potential effects of the coated fertilizers on potato growth variables (in-season and yield) at two planting dates (early and recommended).

2. The efficiency and consistency of microbe-coated fertilizers in the alleviation of mild and severe water (drought) stress in potato plants grown under controlled environment (greenhouse) conditions.

## 1.2. Research Hypotheses

### 1.2.1. Field Conditions

1. Under field conditions, the microbial consortia (microbe-coated fertilizers) will promote crop growth under existing natural environmental conditions, at both early and normal planting dates, resulting in increased in-season growth variables (plant height, leaf area and dry biomass), leading to increased tuber yield.
2. Potato plants treated with microbe-coated fertilizers may alter potato plant tissue nitrogen concentration, ash content and total starch in tubers.

### 1.2.2. Controlled Environment - Greenhouse

The microbe-coated fertilizers will successfully induce the effects of beneficial microbes leading to reduced stress effects for plants grown under mild and severe water deficits. Therefore, in-season growth variables respond positively to the treatments because of the enhanced growth and improved physiological functioning, leading to increased quality and quantity in tubers.

**The importance of the study:**

1. This study will provide an improved understanding of the role of microbe-coated fertilizers in plant growth promotion.
2. It also has the potential to allow for the development of an easily applied technology that will increase agronomic variables in an economically valued and widely produced crop: potato.

# Chapter 2 : Literature Review

Potato (*Solanum tuberosum L*), an herbaceous perennial plant of the Solanaceae family, is ranked fourth in food production among the world's most important crops, and it is an important source of global human nutrition, contributing to staple food production. Zaidi et al. (2015) stated that the potato is among the most important crops in the world, based on the total annual production. However, a considerable level of fertilizer nutrient application is required for growing potatoes. Potatoes suffer from reduced productivity on almost 40% of land worldwide as their roots can have limited access to soil phosphorus (Igual et al., 2001). In recent years, some studies have demonstrated that applying bacterial strains, singly or as consortia, increases yields of potato plants. For instance, the application of *Pseudomonas putida* P13, *Microbacterium laevaniformans* P7, and *Pantoea agglomerans* P5, either alone or co-inoculated, showed a positive effect on this crop under greenhouse conditions and in field trials. The combination of *P. agglomerans* P5 or *M. laevaniformans* P7 with *Pseudomonas putida* P13 considerably increased potato growth and biomass production. Specifically, the co-inoculation of *P. agglomerans* P5 and *P. putida* P13 enhanced potato yields by 20-25% (Malboobi et al., 2009, García-Fraile et al., 2017).

## 2.1. The phytomicrobiome

The phytomicrobiome, also known as the plant microbiome, is a complex and well-orchestrated community of microorganisms that interact with plants throughout their growth. It involves all the microorganisms that establish relationships with plants during their lifecycle. Intimate associations between terrestrial plants and microbial communities have existed for on the order of 0.5 billion years (Redecker et al., 2000; Lyu et al., 2021); the phytomicrobiome and the plant have coevolved, largely for mutual benefit, since that time. The combination of the phytomicrobiome and the associated plant is referred to as the holobiont; phytomicrobiome members are present in all plant parts (Smith et al., 2015; 2017; Pandey et al., 2023). These interactions include the rhizosphere (the soil surrounding plant roots), endophytes (microbes living within plant tissues), and even the endosymbionts such as the nitrogen-fixing rhizobia (Papik et al., 2020; Lyu et al., 2021).

At least some microbes colonize plant species by releasing signals recognized by appropriate partner plants, activating several direct and indirect responses (Bukhat et al., 2020; Jian et al., 2020). The roles and functions of the phytomicrobiome included nutrient acquisition, both abiotic (e.g., drought, temperature extremes) and biotic (e.g., pathogens) stress management (Antar et al., 2021). In addition, the phytomicrobiome can regulate plant physiology, at least in part through microbe-to-plant signaling, regulating functions related to plant growth and development. Microbes also contribute to growth regulation through phytohormone production (Backer et al., 2018; Lyu et al., 2021). The phytomicrobiome is a vital resource for sustainable agriculture, influencing plant health, stress tolerance, and overall productivity (Rahi, 2017; Chouhan et al., 2021) with minimal environmental impact.

Understanding the signaling between plants and their microbiomes has begun to allow the development of technologies that can enhance plant nutrition and increase their ability to withstand stress (Smith et al., 2015). Incorporating isoflavonoids into rhizobial inoculants stimulates the expression of nodulation genes, overcoming adverse environmental conditions at the time of symbiosis establishment and improving the nitrogen-fixing symbiotic relationship between rhizobia and legumes (Shah and Smith, 2020). When plants are exposed to stressful situations, lipochitooligosaccharides (LCO) enhance plant development. In addition to isoflavonoids, jasmonates, a phytohormone, can be secreted from roots and stimulate genes that produce LCOs in specific rhizobia, improving plant ability to manage abiotic stresses (Mabood et al., 2006; Schwinghamer et al., 2014; 2015).

The phytomicrobiome plays a significant role in managing the environment, and the use of biologicals is expected to expand in the 21st century. Biologicals can enhance plant pathogen resistance, increase crop productivity, help meet growing global demands for food, fiber, and fuel, and help plants cope with stresses associated with climate change.

## 2.2. Potato and Plant Growth-Promoting Rhizobacteria (PGPR)

The effects of PGPR on plant growth and stress tolerance have been studied to some degree. The presence of these beneficial microbes can enhance plant growth and improve resistance to biotic and abiotic stresses (Lyu et al., 2021a; 2021b). PGPR enhance plant growth through the production of many substances, including phytohormone-related compounds such as auxin, cytokinin, and ACC deaminase, and also compounds such as siderophores. Siderophore substances can enhance nutrient availability and subsequent plant uptake (Pathak et al., 2017).

Moreover, PGPR have been reported to mitigate abiotic stresses (i.e. drought stress) in potato by inhibiting oxidative stress and enhancing antioxidant enzyme activity (Batool et al., 2020; Saleem et al., 2022). Various bacterial species have been recognized as potent PGPR for potato, including *Bacillus* and *Pseudomonas* species (Naqqash et al., 2020; Vishwakarma et al., 2024). PGPR have been demonstrated to promote nutrient availability and improve plant tolerance to nutrient deficits in potato growth. The application of PGPR can greatly enhance the growth of roots and shoots and have been shown to increase root length and surface area in conditions of phosphorus deprivation (Hanif et al., 2015). Inoculation of PGPR has led to encouraging outcomes in enhancing potato plant growth and boosting tuber production. In the early 1980s and 1990s, studies showed that the application of PGPR to potato plants can substantially enhance potato growth, tuber yields, and overall quality (Kloepper et al., 1980a). Vraný and Fiker (1984) demonstrated an increase in plant growth and tuber yield of 4-30% in potato tubers following PGPR inoculation prior to planting. PGPR have demonstrated promising results in experiments performed *in vitro*. In two *in vitro* studies (Frommel et al., 1991; Sturz, 1995), nonfluorescent *Pseudomonas sp*. caused growth stimulation and tuber yield enhancement in potato.

Since then, a huge number of studies have documented the potential of PGPR to benefit potato plant growth and development. Warnita et al. (2023) showed that the co-application of rhizobacteria and mycorrhizae increases tuber weight per plant. As previously reported (Vessey, 2003; Compant et al., 2005), *Bacillus*, *Pseudomonas* and *Azotobacter* are the most commonly studied genera within the PGPR, being involved in nutrient uptake, growth stimulation and phosphorus solubilization. Plant growth-promoting rhizobacterial strains can be used as single strains or consortia-based inoculants in potato production (Pathak et al., 2017). For instance, inoculating *Bacillus subtilis* strains onto potato plants resulted in a greater plant height, root length, and shoot biomass than non-inoculated control plants (Hanif et al., 2015). In addition, an *Azospirillum* strain showed the most potential in promoting potato uptake of nitrogen and subsequent growth (Naqqash et al., 2016). Moreover, lipoxygenase (LOX) associated with PGPR isolated from potato fields showed growth and tuber stimulation in potato grown in *in-vitro* and *ex-vitro* experiments (Nookaraju et al., 2011; Seleim et al., 2023).

It has been reported that *Bacillus* strains are among the more dominant types in the potato rhizosphere. In addition to *Bacillus*, more gram-positive (e.g. *Staphylococcus* and *Plantibacter*) than gram-negative (e.g. *Proteobacteria*, *Variovorax*, *Chryseobacterium* and *Agrobacterium*) bacterial genera were isolated from plant roots. Strains of some of the above-mentioned bacterial species have plant growth-promoting capabilities and are reported to be PGPR for potato plants

(Cezón et al., 2003; Pathak et al., 2017; Aloo et al., 2021; Marpaung and Susilowati, 2021; Henagamage, 2022).

Novel PGPR isolated from the potato rhizosphere have shown potential as biostimulants, biofertilizers and bioprotectants against soil-borne pathogens (Kesaulya et al., 2014). For example, isolated *Enterobacter cloacae, Bacillus cereus,* and *Achromobacter xylosoxidans* strains from the potato rhizosphere have shown growth promotion ability and have increased agronomic variables and physiological function, as well as nutrient solubilization and availability (Dawwam et al., 2013; Preeti and Shahi, 2015; Mushtaq et al., 2021).

PGPR play a crucial role in colonizing potato plant roots and enhancing plant growth. Therefore, PGPR applications as a component of potato production have benefitted plant development, ability to tolerate stress, and yield, by increasing the accessibility of nutrients, mitigating the effects of environmental stress, and promoting plant physiological functions (Calvo et al., 2010; Yasmin et al., 2020). Further studies are required to investigate underlying mechanisms by which PGPR influence potato plants and enhance their utilization in agricultural production systems. These should focus on the significance of PGPR in enhancing crop productivity and promoting development of more sustainable agricultural systems. While microbial inoculations may not replace synthetic fertilizers, they can reduce the need for chemical fertilizers like nitrogen, phosphorus and potassium. Sustainable agriculture aims to reduce fertilizer needs by utilizing naturally found soil nutrients and transforming them into accessible forms through deployment of beneficial soil microbes (Bamdad et al., 2021; Das et al., 2022).

*Table 2-1: Examples of some bacterial strains showing plant-growth promotion and biocontrol capabilities in potato.*

| PGPR | Responses | References |
|---|---|---|
| *Pseudomonas spp.* | Increased plant growth, significant increase in stolon length, early season plant growth-promotion and increased yield | (Kloepper et al., 1980) |
| *Pseudomonas gladioli*<br>*Pseudomonas viridiflava*<br>*Pseudomonas cichorii*<br>*Erwinia herbicola* | Increased root number, dry weight and secondary branching, stem length, leaf hair formation and total plant lignin content. | (Frommel et al., 1991) |
| *Pseudomonas putida*<br>*Microbacterium laevaniformans*<br>*Pantoea agglomerans* | Phosphorus solubilization and availability, increased biomass and yield, | (Malboobi et al., 2009) |
| *Erwinia carotovora* subsp. carotovora (Ecc),<br>*Bacillus sp*.<br>*Lactobacillus sp*. | Biological control to reduce the soft rot infection in tubers | (Rahman et al., 2012) |
| *Bacillus sp.*<br>*Pseudomonas sp.* | Exhibited plant growth promotion through producing IAA, the ability to solubilize phosphates, and ammonia and chitinase | (Sati et al., 2013) |

| | | |
|---|---|---|
| | production. Displayed biocontrol activities by inhibiting *Fusarium oxysporum* and *F. solani* growth due to the production of antifungal compounds. | |
| *Bacillus subtilis* | Phosphate solubilization, and growth promotion leading to increased shoot and root length and weight | (Hanif et al., 2015) |
| *Pseudomonas sp.* | Host defence response and inhibiting *Rhizoctonia Solani* by activation of plant systemic defence systems | (Velivelli et al., 2015) |
| *Bacillus amyloliquefaciens*<br>*Bacillus subtilis* | Increased plant growth and yield, inhibitory effects of soil-born disease (*Rhizoctonia solani*) on tubers | (Franco et al., 2016) |
| *Pseudomonas sp.*<br>*Azospirillum sp.*<br>*Agrobacterium sp.*<br>*Enterobacter sp.*<br>*Rhizobium sp.* | Nitrogen fixation, IAA production, increased growth and nitrogen uptake, fresh and dry weight and N contents of shoot and roots | (Naqqash et al., 2016) |

## 2.3. PGPR application in controlled environment settings (greenhouse conditions)

Potato production in the greenhouse is a useful research approach that offers several advantages, including optimizing growth conditions, such as temperature, irrigation, humidity, and light, to improve plant growth and development. The greenhouse provides a controlled environment for year-round investigation, or even for producing disease and virus-free mini tubers, which can be utilized as high-quality seed tubers (Türkmen et al., 2017; Islam et al., 202). Commercial potato production in the greenhouses, except for the seed tubers, is not common due to energy and other economic considerations. However, greenhouses are ideal for undertaking sensitive research that can not be implemented in field conditions. These include testing biological inoculants, abiotic stress-involved investigations, as well as studies on biocontrol agents for pest and disease management. A greenhouse study demonstrated the efficiency of a bacterial consortium containing *Bacillus* and *Paenibacillus* strains against bacterial wilt, *Fusarium* wilt and foot rot diseases (Thanh et al., 2009). In another study, *Fusarium* was reported to cause dry rot disease in potato, which was inhibited when tubers were inoculated with PGPR strains from *Bacillus*, *Burkholderia*, *Pseudomonas* and *Flavobacter* genera, with the antifungal activity from a *Burkholderia cepacia* strain (Recep et al., 2009). Moreover, common scab, caused by the bacterium *Streptomyces scabies*, is a significant concern in potato cultivation. Various strategies have been explored to manage this disease, including using biocontrol agents such as *Bacillus* and *Trichoderma* strains (Ma et al., 2023; Kumar et al., 2023). A study was conducted to evaluate the efficiency of a commercial product containing *Bacillus subtilis*, and it reported the inhibition of common scabies in potato tubers and increased yield in inoculated plants (Rehman et al., 2021).

PGPR strains of *Bacillus* and *Pseudomonas* have been identified for their growth promotion and enhancing the productivity of greenhouse-grown potato due to improved tolerance of environmental stresses, such as drought, as well as enhanced nutrient uptake, phytohormone production and biocontrol activity, which contribute to increased yield and quality of potato crops (Ekin, 2019: Batool et al., 2020; Naqqash et al., 2020).

The application of PGPR provides a more sustainable microbe-based approach to commercial potato production. By improving plant tolerance to adverse conditions and enhancing nutrient uptake, PGPR can help optimize the growth and development of greenhouse-produced potato plants. This is particularly important in the context of climate change, as PGPR can assist in mitigating the effects of changing environmental conditions on field crop productivity (Kabiraj et al., 2020; Shah et al., 2021).

Furthermore, using PGPR-containing biostimulants in greenhouse production can contribute to sustainable agriculture by reducing reliance on chemical inputs. They are particularly recommended under stress-inducing conditions such as prolonged drought, nutrient deficiency, salinity stress and the presence of pathogenic organisms (Jiao et al., 2021; Gupta et al., 2023). The co-administration of PGPR and other plant biostimulants, such as humic acid, is effective in enhancing the growth, yield, and nutrient uptake of various crops, including potato (Ekin, 2019). Further research is needed to optimize the use of specific PGPR strains and their interactions with other plant biostimulants, to maximize the benefits for plant production in greenhouse and field settings (Chea et al., 2021; Oswald et al., 2010; Martini et al., 2022).

Biostimulants have shown the potential to benefit many crops, including potato, by enhancing growth, yield, and quality. They promote tuber yield, improve tuber biological variables, and increase resistance to environmental conditions and pathogens (Magray, 2021). Biostimulants also improve aboveground biomass, chlorophyll content, and tuber number. While they do not affect sugar/starch content in immature tubers, they enlarge the potential photosynthetic $CO_2$ assimilation leaf area. When combined with herbicides, biostimulants increase protein content and reduce glycoalkaloid content. They also positively affect potato tuber zinc, copper, and nitrogen contents (Wozniak et al., 2020). Overall, biostimulants are a valuable resource for optimizing potato production sustainably and effectively.

### 2.4. Nutrient availability and uptake from chemical and biological sources

Various factors, including nutrient availability and uptake, influence potato production. Several studies have investigated the relationship between nutrient levels and potato productivity (Singh and Maiti, 2022). A study found that soil nutrient factors such as nitrogen (Eid et al., 2020; Sebnie et al., 2021), available phosphorus and potassium significantly influenced potato quality (Gelaye et al., 2021). Another study ranked the effects of soil moisture and nutrients on potato yield, with soil-available potassium content having the strongest correlation with potato tuber yield (Otieno and Mageto, 2021). Additionally, a study highlighted the importance of mineral nutrients for potato growth and tuber yields. In a field experiment, the response of potato plants grown under standard and slow-release fertilization programs was varied. Slow-release nitrogen fertilizers significantly increased potato yield and enhanced quality tubers with higher protein, carbohydrate ash, and fat/oil contents (Petropoulos et al., 2020).

Applying mineral fertilizers, particularly those containing humic supplements or combinations of biofertilizers, enhanced potato productivity in soils with low phosphorus and potassium contents. This suggests nutrient supplementation through fertilizers can positively impact potato yield (El-Naqma, 2020; Pyasi et al., (2020). Nutrient availability and uptake can vary depending on the crop, growing conditions, and soil properties. For example, a study on sweet potato found that phosphorus doses influenced aboveground dry matter, with higher doses resulting in increased dry matter when manure was not applied (Wang et al., 2022).

Nutrient management is crucial for optimizing potato productivity. Agricultural producers tend to use excessive amounts of fertilizers to increase the quantity and quality of potato yield. However, inappropriate fertilization could increase the risk of harming plants and reducing productivity. Therefore, Sha et al. (2021) developed a new technology to test soils in potato fields to determine the nutrient requirements, recommend fertilization based on the test, and reduce the negative impact of excessive fertilizer application. This management technology could help agricultural producers adopt new fertilization practices to improve potato yield and tuber quality while protecting against environmental hazards derived from fertilization (Sha et al., 2021). The study emphasized the need for enhanced fertilizer management methods to increase fertilizer use efficiency and minimize environmental nutrient loss (Koch et al., 2020).

Plant growth-promoting rhizobacteria are potentially important for increasing nutrient availability and uptake in plants, including potato. Through direct mechanisms, PGPR contribute to plant development by producing phytohormone compounds, siderophores, HCN and $NH_3$, fixing nitrogen, and solubilizing nutrients (P, K, Zn) for easy plant uptake (Figure 2-1). Through interactions with other microorganisms in the rhizosphere and bulk soils, some PGPR exhibit antagonism against plant pathogens, enhancing plant resistance to biotic stresses, leading to plant growth promotion (Vejan et al., 2016; Cao et al., 2023; Ramírez-Cariño et al., 2024).

One of the mechanisms by which PGPR enhance nutrient availability and use efficiency is through the solubilizing of essential nutrients and the facilitation of nutrient uptake from the soil (Adesemoye et al., 2009a; Adesemoye et al., 2009b; Adesemoye et al., 2010).

PGPR interactions have been shown to improve seed germination, root development, shoot and root weights, leaf area, chlorophyll content, hydraulic activity, protein content, nutrient uptake, and potato yields (Batool et al., 2020; Msimbira et al., 2022; Naamala et al., 2022). PGPR inoculants, such as *Azospirillum, Azotobacter, Bacillus, Enterobacter* and *Pseudomonas,* can enhance potato productivity and grain quality by increasing nutrient availability, phytohormone production and secondary metabolite production (Shahwar et al., 2023; Elmaghraby et al., 2023). In addition, PGPR inoculation of potato led to increased nitrogen, phosphorus, and potassium uptake into plant biomass. This is attributed to incremental root growth improvement and root number induced by PGPR, which allows for more efficient essential nutrient uptake from the soil. The increased nutrient content in the soil after PGPR inoculation contributes to higher biomass production by potato plants (Hafez et al., 2019; Wang et al., 2020; Ramírez-Cariño et al., 2024).

PGPR have been demonstrated to be an environmentally friendly method for enhancing crop productivity by promoting plant growth directly or indirectly through regulating hormonal and nutritional equilibrium, the induction of plant pathogen resistance, and the facilitation of nutrient solubilization for efficient plant absorption. In addition, PGPR inoculants show antagonistic interactions with potentially pathogenic microbes in the rhizosphere and beyond in bulk soil, thus providing plant protection. Although PGPR have the potential to suppress potato pathogens, such as common scab, for improved yield and tuber quality (Soares et al., 2023), potato growers should be trained to have increased awareness of potato plant diseases and their management, by choosing healthy seed tubers, practicing good on-farm management, cultivating resistant or disease-tolerated varieties, crop rotation and selecting uninfected soils for potato production (Bastas, 2023).

Numerous bacterial species function as PGPR, as shown in the literature; they are exploited because of their efficacy in enhancing plant growth. However, understanding the PGPR mode of action in plant growth simulation and altering plant functioning still requires extensive study (Khan and Mehmood, 2023; Singh et al., 2023).

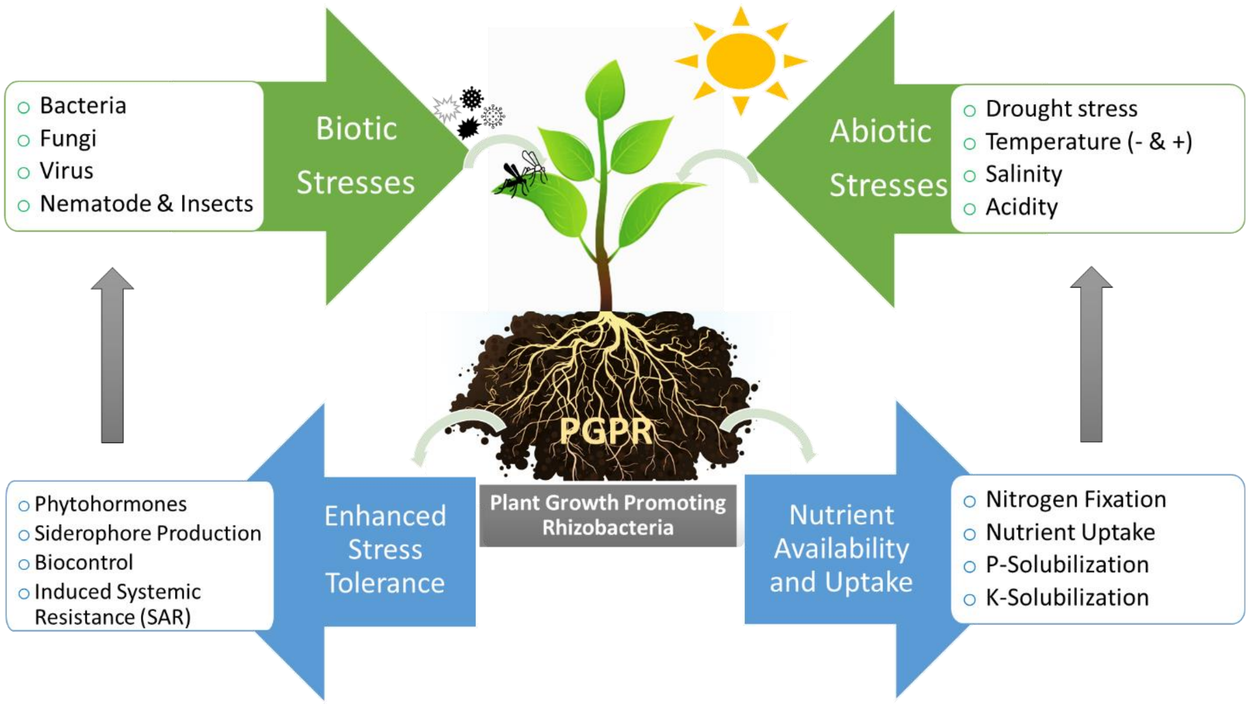


*Figure 2-1: Plant growth-promoting rhizobacteria (PGPR) and plant interactions in the rhizosphere (Shah et al., 2021).*

## 2.5. PGPR as macro- and micronutrient facilitators

Plant growth-promoting rhizobacteria play a crucial role in facilitating plant uptake of macro- and micronutrients. These beneficial microorganisms enhance plant growth and nutrition through various mechanisms. One such mechanism is the increase in root-absorptive surface area, which improves water uptake by vesicular-arbuscular mycorrhizal plants (Dietz et al., 2021). Furthermore, the study conducted by Hafez et al. (2019) illustrated the efficacy of microbial inoculations in enhancing nutrient absorption by potato plants. The microbial inoculations were found to modify the structure of the roots and increase the number of root hairs, thereby enhancing the uptake of nitrogen (N), phosphorus (P), and potassium (K) from infertile soils that lack sufficient levels of essential elements. Plant growth-promoting rhizobacteria also act through direct and indirect mechanisms to promote plant growth, including the solubilization of mineral nutrients, nitrogen fixation, and production of phytohormones (Vitorino & Bessa, 2017; Gupta et al., 2015). They can also facilitate the absorption of essential elements such as iron and non-essential elements like cadmium and lead, thereby enhancing the tolerance of host plants (Singh et al., 2011).

Through their role in nutrient uptake, PGPR can also contribute to other activities, leading to enhanced growth and development of plants. For example, potassium-solubilizing bacteria (*Bacillus cereus*) have been found to enhance nitrogen, phosphorus and potassium concentration in potato leaves (Ali et al., 2020). Furthermore, PGPR have been shown to increase nitrogen uptake from fertilizers, thereby improving plant nutrition and fertilizer use efficiency (Adesemoye et al., 2010). These microorganisms can also modulate plant morphogenesis and gene expression, particularly in relation to the absorption of iron, an essential micronutrient for plant growth (Castulo-Rubio et al., 2015).

The application of PGPR in agriculture has been recognized as a sustainable approach to enhance crop production. These rhizobacteria colonize the rhizosphere and root system of plants, increasing the root surface area for nutrient absorption and promoting better plant growth and production. PGPR can also improve the efficiency of organic matter composting processes and the availability of phosphorus (Xie et al., 2023), an essential nutrient for plant growth (Bouhia et al., 2023).

Overall, PGPR facilitate macro- and micronutrient uptake in plants. Their ability to enhance nutrient availability, solubilize minerals, and produce phytohormones improves plant growth, nutrition, and crop productivity (Figure 2-1). Applying these PGPR in agriculture systems can promote sustainable practices, reduce reliance on agrochemicals, and ensure agricultural sustainability (Buch et al., 2023).

## 2.6. PGPR and improving food quality: Biofortification

The Food and Agriculture Organization (FAO) published a report in (2015) stating that hunger is increasing; one in nine people is suffering from insufficient food. Welch and Graham (2004) showed that malnutrition is a huge challenge at the global level and that over three billion people are undernourished due to deficiencies in vitamins and nutrient elements. Based on this, Stein (2014) reported that diets are commonly deficient in elements such as Iron (Fe), (Zinc) Zn, iodine (I), selenium (Se), calcium (Ca), Magnesium (Mg), and Copper (Cu). In plants, mineral nutrition is closely associated with productivity and nutritional quality of food-products produced. Most growers select high-yielding crop varieties without considering the nutrient composition. Therefore, it is key to investigate how to enrich major food crops with essential nutrients.

Increasing micronutrient contents in staple crops has become a priority for combating nutrient deficiency. Several approaches have been shown to improve food/yield quality through agronomic, soil management practices or genetic means (Prasanna et al., 2016). Conversely, enriching major food crops with minerals and vitamins by using PGPR is also expanding and becoming more accepted by researchers and crop growers. Plant growth-promoting rhizobacteria use will significantly support the biofortification process and is an eco-friendly approach that can also reduce the costs associated with the crop production process.

It is crucial to consider the impact of PGPR on the functioning of the root system and their ability to affect the absorption of necessary nutrients (Vacheron et al., 2013). Research has demonstrated that PGPR, such as *Bacillus* and *Pseudomonas* species, show the ability to increase nutritional values in potato tubers (Naqqash et al., 2016). Moreover, PGPR inoculants have been associated with enhancing nutrient availability and uptake. It has been reported that microbial inoculants potentially contribute to nutrient absorption and biofortification in potato plants experiencing phosphorus deficiency (Chea et al., 2021). Researchers have examined the process of enriching potato tubers with vital elements such as iodine, showing the potential of biofortification methods for the enhancement of the nutritional value of potato tubers (Gonzali et al., 2017; Dobosy et al., 2020). Agriculture sustainability and its progress have greatly impacted this concern and can improve food quality and safety (Chauhan et al., 2015; Conceição et al., 2016). The promising results of PGPR application to diverse crops have been documented; they have been demonstrated to both increase crop productivity and enhance nutritional quality (Ahemad and Kibret, 2014).

## 2.7. Factors affecting PGPR efficiency and potato production

Environmental factors play a crucial role in the efficiency of PGPR in plant production. Several studies have investigated the impact of environmental conditions on the effectiveness of PGPR in promoting plant growth and development.

One of the factors is the availability of water and moisture levels in the soil, especially in the rhizosphere, which can impact the colonization and activity of PGPR and their survival. Drought conditions can limit the effectiveness of PGPR in promoting plant growth. Therefore, selecting PGPR strains which are tolerant to drought stress is crucial to maintaining optimal interactions with plants and soil microbes for enhanced plant growth through the antioxidant activity for oxidative stress suppression (Batool et al., 2020; Ahmad et al., 2022; Arora and Jha, 2023).

Another important factor is soil salinity. Saline soils can negatively affect not only plant growth but also PGPR activity. Halotolerant and halophilic PGPR have been found to improve crop productivity in saline soils. These salt-tolerant PGPR strains can tolerate high salt concentrations and help mitigate the adverse effects of salinity on plant growth. They can enhance photosynthesis capacity, antioxidant activity, nutritional profile and root development due to phytohormone production and overall improved plant growth and development (Saghafi et al., 2019; Slimani et al., 2023; Zhang et al., 2023).

Another environmental factor that can influence PGPR efficiency is temperature. Strains of PGPR vary in terms of optimal temperatures for growth and activity. It has been found that increased temperature has varying effects on PGPR, with both positive and negative effects possible. Some strains from semi-continental climates significantly increased winter wheat root and shoot growth on light soils, while others from semi-arid climates performed well under both conditions. Some PGPR strains can grow better at high temperatures, making them particularly useful for crop plants exposed to increased temperatures. It is important to select PGPR strains that are well-adapted to temperature fluctuations to promote effective plant growth under the given temperature regime (Davies and Whitbread, 1989; Company et al., 2010; Meena et al., 2015).

Other microorganisms in the soil can also affect the efficiency of PGPR. Interactions between PGPR and other soil microorganisms, such as pathogens or competing bacteria, can influence the colonization and activity of PGPR in the rhizosphere. Understanding these interactions and selecting compatible PGPR strains can enhance their effectiveness for sustainable crop plant productivity (Castro-Sowinski et al., 2007; Antar et al., 2021).

Environmental factors such as drought, salinity, temperature, and interactions with other microorganisms can significantly impact the efficiency of PGPR in potato production. Selecting appropriate PGPR strains that can be adapted to the specific environmental conditions of effective potato production systems are crucial for maximizing their beneficial effects on potato growth and development.

## 2.8. Environmental Conditions and PGPR

Climate change results in more severe environmental conditions, including drought, salinity, and extreme temperatures. These factors can cause substantial declines in global crop output, yield, and quality (Shah et al., 2021; Chaudhry and Sidhu, 2022), resulting in significant losses for many crops. The simultaneous presence of these stresses has a detrimental effect on plant overall growth, development, and production (Harkhani and Sharma, 2023; Sati et al., 2023). Drought, salinity, acidity and other factors such as osmotic, oxidative, and ionic stress significantly impede agricultural productivity. To counteract these conditions, plants must undergo metabolic reconfiguration to fulfill the demands for anti-stress substances, such as suitable solutes, antioxidants, and proteins (Cao et al., 2023; Kumar et al., 2023; Soni et al., 2023; Msimbira and Smith, 2020; Unel et al., 2020).

The high costs and time-consuming nature of developing drought-resistant plant varieties or transgenic plants motivate the need for more sustainable and less costly technologies. The use of PGPR is a cost-effective and sustainable method to enhance plant development under conditions of environmental stress. Plant growth-promoting rhizobacteria exhibit several mechanisms able to mitigate the adverse effects of abiotic stresses, such as nitrogen fixation, phytohormone production, solubilization of macronutrients (P and K), production of exopolysaccharides, ACC-deaminase activities, antioxidant activity and microbe-to-plant signaling compounds (Gray and Smith, 2005; Mabood et al., 2014; Smith et al., 2017; Nazari and Smith, 2020). PGPR can enhance plant stress tolerance by modulating osmotic balance, ion homeostasis, phytohormone signaling, and antioxidant enzyme activity (Kang et al., 2014; Hasanuzzaman et al., 2022). They can also induce systemic tolerance to abiotic stress by activating specific genes involved in stress response pathways (Tiwari et al., 2017; Mellidou and Karamanoli, 2022).

In addition, several studies have reported the efficiency of PGPR inoculation in enhanced tolerance of plants against biotic stresses through modulating plant hormone levels, decreasing the inhibitory effects of various bacterial pathogens (Takishita et al., 2018; Marković et al., 2023), fungal pathogens (Riaz et al., 2022a,b), viral diseases (Amin et al., 2023; Kalatskaja et al. 2023) and insect pests (Katoch et al., 2023; Petrushin et al., 2024) on plant growth and development in the forms of biocontrol agents.

### 2.9. Seeding/planting date and potato production

Seeding or planting dates can indeed have an impact on potato production. Several studies have investigated the relationship between planting dates and potato yield and have found significant effects on crop growth, yield, and yield-component variables (Jones and Allen,1983; Jones, 1990; Caliskan et al., 2004; Li et al., 2021).

In Canada, studies have investigated the impact of planting dates on potato plants in changing climatic conditions. For example, British-Columbia-based research aimed to assess nitrogen fertilizer rates and planting date (typical and late) impacts on potato production, soil properties and greenhouse gas emissions in high and low-fertility fields. The authors reported that potato yield increases due to increased fertilization in high-fertility fields. However, no significant differences were noted in seeding date impact on potato production (Chizen, 2020).

It has been reported in a study conducted in a semi-arid location that potato planting date significantly affected in-season growth variables, including plant height, leaf area and NPK uptake in plant tissues and tubers when planted at a recommended date compared to a three-week delay (Sandhu et al., 2014). Another study showed the impact of planting dates (early and late) on emergence, in which late planting resulted in 50% slower emergence than those planted earlier. However, the emergence delay didn't negatively impact marketable-size tubers and yield components at the end of the season (Darabi, 2013). This shows that the weather at early planting was not optimum for accumulating growing degree days for growth promotion and eventual yield increase compared to the late seeding. Growing season length significantly impacts vegetative growth and yield; plants require sufficient heat units for optimum growth and development.

The advantages of earlier planting are a longer growing season and reduced risk of frost injury at the end of the season, especially for late plantation. In addition, early-season high temperatures offer benefits to break dormancy and increase plant growth. However, high temperatures negatively affect plants through factors such as increased irrigation requirements, early flowering, presence of diseases and most importantly, more growing degree days (GDD), which can cause reduced crop yield due to premature senescence from accelerated/forced life-cycle completion, leading to reduced crop quality (Mix et al., 2012).

The choice of planting date is crucial as it can affect the growth and development of potato, which in turn influences the yield and quality of the harvested tubers. Factors such as temperature, day length, and soil conditions can vary depending on the planting date, and these environmental factors can significantly impact potato growth and development. For example, planting potato too early in the season when the soil is still cold and wet can lead to poor germination and slow growth, resulting in reduced yields. Conversely, planting potato too late in the season may expose the crop to unfavourable weather conditions, such as high temperatures or drought, which can also negatively affect yield. Therefore, potato growers need to consider the local climate, soil conditions, and the specific requirements of the potato cultivar when determining the optimal planting date for maximizing yield and quality.

## 2.10. Microbial inoculants - from laboratory to field

Various microbial inoculants can promote the growth of plants under laboratory conditions by increasing germination rate, shoot and root length, nutrient concentration in tissues, etc. However, the positive effects associated with inoculation of plants with PGPR under controlled conditions do not always translate to field conditions. This may be due to a multitude of factors, such as the ability of the inoculant to compete with native soil microbial communities and/or the viability of microbial cells following the stresses endured during formulation, transportation, and field application. Selecting the proper type of inoculant and optimizing its large-scale production are important steps to ensure consistent results under field conditions (Herrmann and Lesueur, 2013; Antar et al., 2021).

## 2.11. Single-strain and consortia-based microbial inoculants

Currently, the majority of microbial-based products consist of living microorganisms, either in the form of a single strain or a group of strains. Agriculture has a longstanding tradition of utilizing single-strain microbial inoculants. The utilization of rhizobia spp. as microbial inoculants for improving nodulation and nitrogen fixation in legume plants began commercially in 1896, as documented by Nobbe and Hiltner. Recently, various bacterial species, including those within the genera *Bacillus*, *Pseudomonas*, *Azospirillum*, and others, have been studied for their ability to enhance plant growth. This research has resulted in the development and sale of several products. Many of the original products that were introduced contained only one PGPR strain, as the research was restricted to a small number of microbial strains that were recognized to have positive effects on plants (Tabassum et al., 2017; Bradáčová et al., 2019). Nevertheless, in field experiments, the impacts of microbial inoculants have displayed variability across years and/or locales. One source of inconsistency relates to the capacity of the inoculum to withstand environmental conditions after being applied in the field. Certain bacterial strains exhibit enhanced adaptability to specific conditions while displaying increased vulnerability to other stressors (Nelson, 2004; Khare and Arora, 2014; Ambrosini et al., 2016).

Furthermore, broad-spectrum/generalist strains will offer various advantages to a diverse array of plant species, a probable positive attribute when compared with investigated strains that specifically target a particular issue in conjunction with a certain plant species. The simpler system may be more intriguing at first. Conversely, microbial consortium-based inoculants may exhibit more resilience than single-strain inoculants if they adapt to the challenges encountered under field circumstances. This adaptability would enable them to effectively promote plant growth (Reddy and Saravanan, 2013; Liu et al., 2023). Furthermore, including multiple strains in a product will enhance the range of ecosystem functions it can perform. These functions may include the solubilization of phosphorus, the fixation of nitrogen, the production of phytohormones, or the biocontrol of pathogens (Hakim et al., 2021). By having multiple strains, each performing these functions redundantly, the product ensures that environmental stresses will not compromise any of these functions.

Nevertheless, the utilization of consortia rather than single-strain products gives rise to some concerns. An example is when multiple strains are combined, there is likely to be competition among strains, with the possible result that some will be present at high levels and others fall below the required for positive effects on plants (Borriss, 2015; Keswani et al., 2019). Furthermore, the combination of gram-negative bacteria, which exist as vegetative cells, with gram-positive or fungal strains, often present as spores, might produce inconsistent products. Combining a maximum of two strains in this case may yield optimal product consistency and efficacy (Borriss 2015), particularly if the two strains exhibit synergistic interactions. Nevertheless, it should not be presumed that two microorganisms that promote plant growth will automatically show synergistic interactions in terms of enhancing plant growth. As an example, when two *Glomus* species were simultaneously introduced, which are known to enhance plant development when used individually, it had a detrimental impact on plant growth and biomass production (Edathil et al., 1996; Jansa et al., 2008; Crossay et al., 2019). Curiously, the adverse effect was negated when additional strains were introduced into the inoculum. Subtle variations in the first stages of consortium production can result in preferential growth of one strain over another, resulting in unpredictable proportions of the combined strains and, consequently, an inconsistent final product. Future research is needed to investigate plant responses to single-strain inoculants and their combinations with others from the same or different genera to ensure their compatibility and efficiency when inoculated in agricultural systems.

## 2.12. Conclusions

The agricultural sector plays a crucial role in Canada's drive to develop its bioeconomy, which can be achieved through increasing crop productivity using sustainable approaches. The current advances in microbial biotechnology provide a sense of what the phytomicrobiome offers in terms of developing and deploying plant-associated microbes as sustainable biological inputs for crop productivity. These biologicals can be applied with synthetic fertilizers at seeding or as microbial cell-derived compounds for seed treatment and foliar spray application. Regardless of their means of application, these technologies have the potential to make Canadian crop production systems more climate change resilient by helping plants deal with abiotic stresses, especially those associated with developing climate change conditions. Therefore, we designed this study to observe if microbial inoculants contribute to plant growth promotion and improved yield when potato plants are grown in diverse environmental conditions in field experiments and controlled environment (greenhouse) settings in the presence and absence of water stress. We postulate that microbial treatments help potato plants by activating direct mechanisms, including nutrient availability and use efficiency. This unique study will further describe the importance of PGPR as crop growth-promotion agents for sustainable agriculture.

## Connecting statement between chapters 2 and 3

The previous chapter provided an overview of PGPR and their potential in plant growth and development in a sustainable way with an emphasis on potato production. It also covered environmental factors that limit potato production and the role of beneficial microorganisms in stress alleviation. The potential of PGPR to improve potato growth and productivity through direct and indirect mechanisms, including nutrient solubilization, phytohormone production and enhanced stress tolerance, was reviewed.

The first and second objectives of this study are addressed in chapter 3: to evaluate the field performance of microbe-coated fertilizers (MCF) and determine the response of agronomic variables. First, the MCF was tested in the laboratory to record EVL and Era Boost (EB) microbial consortia viability over 10 days. Subsequently, the efficiency of MCFs and their application as plant-growth promotion agents was investigated on potato plants under field conditions in two distinct growing seasons. Throughout each season, the effects of weather conditions and treatments on in-season growth variables and harvested potato tubers were measured to determine the quantity and quality of MCF-treated plants as compared to non-inoculated control plants.

Mohammed Antar designed the experiment and implemented field trials under the guidance of Professors Donald Smith and Philippe Seguin. Mohammed Antar did in-season project management, observations and data collection with some assistance from a research associate and summer students from the Smith laboratory. Marc Samoisette (chief agronomy technician) and Serge Lussier (field operations lead) from McGill's Emile A. Lods Agronomy Research Centre performed agricultural practices, including the application of pre-season fertilizers, MCFs, pesticides, hilling, and final potato harvesting at the end of the growing season. Experimental materials, including potato tubers, starter synthetic fertilizers and MCF treatments, were provided by Pierre Page, the manager of SynAgri's R&D team. The field layout, MCF preparation and application, seeding, data collection and data analysis, interpretation of results, and the initial draft writing of this chapter were all done by Mohammed Antar and then revised and edited by Professors Donald Smith and Philippe Seguin. Statistical analysis was performed by Mohammed Antar after consulting Professor Pierre Dutilleul from the Department of Plant Science.

# Chapter 3 : Evaluation of novel microbe-coated fertilizers applied to field-grown potato as a mean of increasing plant growth

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**Abstract**

The application of beneficial microorganisms for plant growth promotion in agricultural fields has shown inconsistent results due in part to issues associated with the viability of inoculated microbes and the impact of environmental conditions on plant response. Potato, as the fourth most important human food crop, needs a sustainable way to improve and increase productivity in a changing environment without excessive application of synthetic fertilizers. In this study, the field performance of two novel microbial consortia, namely EVL Coating® (a consortium of *Bacillus*, *Pseudomonas* and *Lactobacillus*) and Era Boost (Ulysse Biotech microbes: a consortium of five *Bacillus* strains) coated on synthetic fertilizers was evaluated for their potential to promote potato in-season growth and total tuber yield as well as total starch content. The microbe-coated fertilizers (MCF) were assessed over two growing seasons (2018 and 2019) at two planting dates (early and recommended). In the first growing season, planting took place on May 14, 2018, for early planting and May 24, 2018, for the recommended date. In the second growing season, the early and recommended planting dates were May 22, 2019 and June 7, 2019, respectively.

The results showed that the MCF significantly increased leaf area (15.3 - 23.1%), dry biomass (11.6 - 21.9%), total nitrogen content (3.9 - 13.8%), marketable-tuber size (9.1 - 21.9%) and total tuber yield (9 - 20.8%) across both years, compared to non-inoculated control plants. In

terms of the doses of the coating microbial consortia, there were variations in overall growth promotion. The recommended doses (1x) of EVL and double dose (2x) of EB resulted in greater growth promotion in 2018. Results for EVL (1x and 2x) were close in 2019, but EB (1x) resulted in the highest overall increase in both in-season variables and yield components. Double doses of EVL and EB did not result in any additional plant growth and yield increase in both growing seasons compared to the recommended doses. In-season growth promotion, especially in leaf area and an increase in nitrogen content at the flowering stage justify the increased yield, which is the most important economic gain for potato growers and producers.

Results demonstrate the efficiency of both microbial consortia when coated on synthetic fertilizers. This novel delivery system introduces a new method to inoculate the beneficial microorganisms in agricultural soils. The results indicate that a microbial consortium can be coated on synthetic fertilizers and banded as starter fertilizers at seeding to promote healthy growth and increase agricultural productivity.

## 3.1. Introduction

Potato (*Solanum tuberosum* L.) is the largest vegetable crop and fourth largest agricultural crop worldwide in total production (Aksoy et al., 2021). Potato yield varies greatly across regions, averaging 45 t $ha^{-1}$ in the US, Germany, and France, and 35 t $ha^{-1}$ in Canada, while the average global yield is about 20 t $ha^{-1}$ (Nyiraneza et al., 2021). Potato yield is impacted not only by nutrient availability but also by biotic (fungal diseases) and abiotic (drought and temperature) stresses that can considerably limit yield (Koch et al., 2019; Chakrabarti et al., 2022). In Canada, the average yield increased by 10.1% in 2021, over 2020, due to increased seeding of higher-yielding varieties and favourable growing and environmental conditions (Statistics Canada 2021). Growers must use effective agronomic management strategies to address adverse growing conditions and negative environmental impacts. One of the main factors to maintain a high yield is ensuring adequate nutrient management to supply sufficient mineral nutrients to the crop. Due to recent increases in fertilizer prices as well as concerns about the impact of synthetic inputs on the environment, there have been attempts to reduce the use of synthetic fertilizers, and several agricultural companies have been actively looking for efficient natural products that can promote plant growth and reduce the need for synthetic fertilizers (Antar et al., 2021). The application to crops of beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR) that can help with nutrient solubilization and improve acquisition of soil nutrients under conditions of insufficient nutrients and improve tolerance of stressful climate conditions could represent a sustainable option (Shah et al., 2021).

The use of single strain or consortia of PGPR are gaining attention as an approach to reduce excessive use of synthetic fertilizers. These inoculants are categorized as biofertilizers or plant growth regulators depending on whether their effect is associated with nutrient availability or phytohormone production (Franzoni et al., 2022). Both types can be used either as an alternative to or simultaneously with synthetic fertilizers to improve overall plant growth and productivity (Shahrajabian et al., 2021). In today's agricultural market for microbial inoculants, several commercial products containing PGPR have emerged for plant growth promotion and biostimulation, and many of these include *Bacillus* strains (Umesha et al., 2019; Santos et al., 2019). Some of these PGPR inoculants can significantly affect plant growth and, ultimately yields. For example, it has been reported that a combination of potassium fertilizer with a *Bacillus* strain increased potato plant height, shoot dry weight, and total tuber yield by 15, 26, and 21% over non-inoculated control plants, respectively (Ali et al., 2021). Also, the use of a rhizosphere-derived microbial consortium containing *Bacillus subtilis* and *Trichoderma harzianum* increased potato yield at two field sites by 22 to 32% (Wang et al., 2019). In addition, inoculated potato plants with *Bacillus subtilis* reduced water stress effects and caused increased growth, leaf area, dry matter, tuber number and weight compared to uninoculated plants. They also resulted in improved physio-chemical traits such as higher photosynthetic rate, chlorophyll content, and enzymatic activities (Batool et al., 2020). More recently, a study conducted by Liu et al. (2022) showed that the combination of a *Bacillus licheniformis* strain and biochar amendment improved potato growth and water use efficiency under a deficit irrigation condition. Moreover, the study demonstrated that this PGPR strain improved photosynthesis rate, stomatal conductance and transpiration rate at early growth stages.

The efficiency of microbial consortia inoculation in agricultural systems was found to be species-dependent and strain-specific because bacterial strains use various mechanisms to promote plant growth, shape microbial community composition, and regulate mechanisms that could, directly and indirectly, affect potato plant development. Diverse microbial consortia could lead to diverse responses by plants, suggesting an explanation for the higher efficiency observed in consortia containing several microbial strains (Mondal et al., 2020; Kalozoumis et al., 2021; Khan). *Bacillus*-based microbial consortia could lead to sustainable potato production under field conditions when the climatic conditions in the air and soil are not optimal (Uysa and Kantar, 2020; Jabnoun-Khiareddine et al., 2023; Mamun et al., 2024).

In the two-year research study described here, two novel microbial consortia from two biostimulant companies, namely EVL Coating® Inc. and Era Boost Pro (Ulysse Biotech microbes), were evaluated for their commercial potential and field performance when they were coated on synthetic fertilizers to be applied as banded fertilizer at seeding. The microbial consortia coatings used in this study were *bacillus* dominant. The makeup of the EVL Coating® is two

*Bacillus* strains, a strain of *Pseudomonas* and *Lactobacillus,* while five *Bacillus* strains are the composition of the Era Boost Pro microbes.

The overall objective of this work was to understand the efficiency of microbial consortia as applied to potato, particularly under more challenging conditions such as lower temperatures at earlier plantings (at alternate seeding dates). Understanding the efficiency of microbial consortia allows the development of new products containing new microbial strains with commercial potential. Through the findings of this study, we present specific biological inputs and provide information that could guide crop producers in the region on how the products work and the conditions that will make them most effective and profitable.

The hypothesis of the study is that inoculation of potato plants with microbe-coated fertilizers, will result in greater potato plant biomass and yield a more balanced nutritional composition due to improved nutrient availability and alleviation of the environmental stresses by the microbial consortia.

## 3.2. Materials and Methods

### 3.2.1. Site description

Field experiments were conducted at the Emile A. Lods Agronomy Research Center (45°25'29"N 73°56'17"W in 2018 and 45°25'22"N 73°56'35"W in 2019) in Sainte-Anne-de-Bellevue, Quebec, Canada. Potato (*Solanum tuberosum* cv Goldrush) was grown from May to September 2018 and May to October 2019. Potato was planted in sandy soils of the St. Amable series (Humic Gleysol) with a pH of 7, containing from 20 to 45 g soil organic matter $kg^{-1}$, with an average 137 kg P $ha^{-1}$ and 197 kg K $ha^{-1}$ (Mehlich-3 extraction). In the year before potato planting, the fields were in alfalfa/corn and soybean/corn rotation.

During the growing season, the average temperature was 17.1 °C with 418 mm of precipitation in 2018 and 16 °C with 621 mm of rainfall in 2019 (Table 3-1). The weather conditions were distinct between growing seasons; therefore, accumulated growing degree-days (GDD) were calculated for each growing season and seeding date compared to the historical GDD average (1994 to 2020). Maximum and minimum temperature values were obtained from Environment Canada. The base temperature of 4.4 °C was used to calculate GDDs (Hartz and Moore, 1978).

*Table 3-1: Weather conditions during the study. Data from Environment Canada (2023).*

| | ***Temperature (°C)*** | | | ***Precipitation (mm)*** | | |
|---|---|---|---|---|---|---|
| *Month* | Average of 2018 | Average of 2019 | Average of 1994-2020 | Sum of 2018 | Sum of 2019 | Sum of 1994-2020 |
| ***May*** | 15.0 | 11.5 | 13.5 | 56.7 | 103 | 90.9 |
| ***June*** | 18.2 | 17.6 | 18.5 | 91.6 | 85.9 | 107.9 |
| ***July*** | 23.4 | 22.8 | 21.1 | 80.2 | 60.3 | 98.6 |
| ***August*** | 22.2 | 19.7 | 20.0 | 65.2 | 77.2 | 93.8 |
| ***September*** | 17.2 | 15.2 | 15.8 | 98.0 | 96.4 | 87.3 |
| ***October*** | 6.7 | 9.5 | 8.8 | 79.8 | 198 | 101.8 |
| *Growth season average* | **17.1** | **16** | **16.3** | **472** | **621** | **580.5** |

### 3.2.2. Micro-coated fertilizer: Coating viable microbes on synthetic fertilizer

EVL Coating® (EVL) and Era Boost ® (EB) microbial consortia were used as treatments to prepare microbe-coated fertilizers. EVL Coating® is a microbial consortium commercialized by EVL Inc. (Saint-Hyacinthe, Quebec, Canada), which contains four bacterial strains (two in the genus *Bacillus*, one *Lactobacillus*, one *Pseudomonas*) and a fungal strain (*Saccharomyces*). EB is a microbial consortium commercialized by Ulysse Biotech (Trois-Rivière, Quebec, Canada), which contains five *Bacillus* strains (Table 2). Both consortia were provided as ready-to-use products containing suppliers' bioactive fermentation products.

Synthetic NPK (10.9-15.2-16.3) fertilizer was provided by SynAgri (Saint-Hyacinthe, Quebec, Canada) as a control fertilizer and carrier of the EVL and EB microbes. Both microbial consortia were superficially spray-coated onto fertilizer granules in an industrial mixer at the manufacturer's recommended rate (1x) and twice the recommended rate (2x) (Table 3-2). Then, microbe-coated fertilizer was air-dried and applied to the field within 24 h. Synthetic NPK fertilizer without microbial coating was the control. All treatments were applied simultaneously and banded into the soil as a starter fertilizer at seeding.

*Table 3-2: Summary of the treatments, active substances, and fertilization rates of the investigated products*

| Treatments | Description | Active substance | Rate per hectare |
|---|---|---|---|
| **Control** | NPK Fertilizer (no microbes) | 922 kg fertilizer/ha x 10.9 %<br>**N = 100 kg N ha$^{-1}$**<br>922 kg fertilizer/ ha x 15.2%<br>**$P_2O_5$ = 140 kg $P_2O_5$ ha$^{-1}$**<br>922 kg fertilizer/ha x 16.3 %<br>**$K_2O$ = 150 kg $K_2O$ ha$^{-1}$** | 922 kg ha$^{-1}$ |
| **NPK+EVL (1x)** | EVL Coating® (Biostimulants) | *Bacillus amyloliquefaciens*<br>*Bacillus subtitles*<br>*Lactobacillus helveticus*<br>*Pseudomonas putida*<br>*Saccharomyces cerevisiae* | 2 L t$^{-1}$ ha$^{-1}$<br>(7 × 10$^{8}$ cfu mL)<br>700 million viable cells mL |
| **NPK+EVL (2x)** | | *Bacillus amyloliquefaciens*<br>*Bacillus subtitles*<br>*Lactobacillus helveticus*<br>*Pseudomonas putida*<br>*Saccharomyces cerevisiae* | 4 L t$^{-1}$ ha$^{-1}$ |
| **NPK+EB (1x)** | Era Boost Pro (Probiotic biostimulants) | *Bacillus licheniformis* U35<br>*Bacillus megaterium* U48<br>*Bacillus megaterium* U49<br>*Bacillus velezensis* U47<br>*Bacillus velezensis* U50 | 3.5 L t$^{-1}$ ha$^{-1}$<br>(4 × 10$^{8}$ cfu g$^{-1}$)<br>400 million viable cells g$^{-1}$ |
| **NPK+ EB (2x)** | | *Bacillus licheniformis* U35<br>*Bacillus megaterium* U48<br>*Bacillus megaterium* U49<br>*Bacillus velezensis* U47<br>*Bacillus velezensis* U50 | 7 L t$^{-1}$ ha$^{-1}$ |

### 3.2.3. Experimental design, fertilization, and standard agronomic practices

Fields were conventionally tilled agroecosystems (fall tilling with moldboard plowing to a depth of 17 cm, spring cultivation with disk harrows to 7-8 cm). In addition to the disk harrows, fields in both years were spring cultivated with a field cultivator at the same 7-8 cm depth as disk harrowing. Each spring, plots were arranged in a randomized complete block design (RCBD) with five microbe-coated fertilizers: EVL (1x), EVL (2x), EB (1x), EB (2x) and uncoated NPK fertilizer as the control. Each plot was six rows (5 m long) with a 90 cm inter-row spacing and tubers planted with a 25 cm spacing within the row, equivalent to nearly 44,500 plants ha$^{-1}$. Treatments were replicated (n = 4) in four blocks. We planted 2 fields following this design each growing season at early or late seeding dates. Seeding dates were on 14 May 2018 and 24 May 2018 in the first year and on 22 May 2019 and 7 June 2019. Immediately before planting the potato, microbe-coated fertilizer was machine-banded as a starter fertilizer in the row at a depth of 10-12 cm. The potato was planted by hand, 5 cm below the soil surface. All plots received 100 kg N ha$^{-1}$, 140 kg $P_2O_5$ ha$^{-1}$ and 150 kg $K_2O$ ha$^{-1}$ in the form of synthetic NPK applied as starter fertilizer. In addition, plots receiving EVL (1x) had about $7\times10^8$ CFU.mL$^{-1}$ of *Bacillus*, *Lactobacillus* and *Pseudomonas* strains, while the EVL 2x had double the CFUs of this consortium. The EB (1x) had approximately $4\times10^8$ CFU.g$^{-1}$ of five *Bacillus* strains, and there was double the amount in the EB 2x treatment.

Potato fields were managed according to conventional practices, which included topdressing additional synthetic fertilizer (180 kg N ha$^{-1}$ from urea and ammonium nitrate, Amidas, Saint-Hyacinthe, Quebec, Canada) at the hilling stage when plants were 15-20 cm tall, approximately 5-6 week after emergence. The field plots were not irrigated. Weeds were initially controlled manually and then with the herbicide Sencor® 75DF (Bayer Crop Science Inc. Canada), which was applied pre- or post-emergence at the rate of 550 g ha$^{-1}$. Colorado Potato Beetles (CPB) were controlled physically in the first year of the study, whereas in the second year, an insecticide, Matador® 120EC (Syngenta, Canada), was applied at the rate of 125 mL $ha^{-1}$ and Success® (Corteva, Canada) with the concentration of 167 mL $ha^{-1}$ were used to control the CPB.

### 3.2.4. Plant sampling and harvest

Data were collected daily on the number of emerged seed tubers until the full stand was established. Five plants were sampled per treatment in each plot at two phenological stages: vegetative and flowering. Values were recorded for plant height, and leaf area was measured using an LI-300 area meter (LI-COR Inc, Lincoln, Nebraska, USA). Then, the plant shoots were oven-dried at 60 $^{o}$C for 2-3 days for biomass determination.

The potato tubers were harvested at the end of the season when all plants reached maturity (first season: 20 August 2018, second season: 24 September 2019). At this stage, the plant loses its leaves, tuber growth ceases, and the shoots turn yellow and then die. Data was collected from five randomly selected plants in each plot and assessed for yield components. The first three plants within the row were excluded to avoid border effects. All the tubers harvested from each plant were grouped into marketable size (3-6 cm diameter), below marketable size (< 3 cm diameter) and above marketable size (> 6 cm diameter) ranges. Data was recorded on the total number of tubers per plant, weight and grading sizes. For total yield, data was collected on the weight of marketable size tubers, plus the weight of tubers greater and lower than the marketable size.

### 3.2.5. Nitrogen concentration and Ash Content in Potato Plant Tissues and Tuber Starch Analysis

Total Nitrogen (N): Potato aboveground plant tissue was collected from five plants per plot per treatment at two phenological stages, mid-vegetative and mid-flowering. Plant tissues were oven-dried at 50 °C for 48 h and then ground for 2-3 min using a 1-mm sieve grinder (Model 4

Wiley Mill, Thomas Scientific - New Jersey USA). After grinding, 60-70 mg of samples were weighed and encapsulated in a tin (Elemental Microanalysis, Isomass Scientific Inc. - Alberta, Canada) for the total N analysis using an elemental analyzer (FLASH 2000 Series Organic Elemental Analyzers, Thermo Scientific - Milan, Italy).

Ash analysis: Plant tissue ashing was conducted in a muffle furnace (Thermolyne Largest Tabletop Muffle Furnace, Thermo Scientific - Milan, Italy) where 1 g of ground tissues was placed in crucibles and ashed in the furnace at 500 ˚C for four h to determine the residuals of inorganic minerals after combusting of organic matter in the potato plant tissue (Harris and Marshall, 2017).

Estimating total starch in potato tubers: After harvesting potatoes, 3-4 tubers were randomly selected from each plot, washed, peeled and cut into ~2-3 cm slices for a total of 10 g (Velásquez-Herrera et al., 2017). The prepared slices were immediately freeze-dried to be used to estimate the total starch. The total starch content in potato tubers was estimated using a total starch assay kit (K-TSTA, Megazyme, Wicklow, Ireland) following the AOAC method 991.11 (2019). The estimated starch content was expressed as total starch percentage and calculated following the method described in Mccleary et al. (1997).

### 3.2.6. Statistical Analysis

The data collected in both growth seasons was subjected to a normal distribution determination, and field sites within the year were tested for homogeneity of variances and the data from sets of sites were pooled as a result. Each year, the data from early and late seeding dates were pooled/combined and analyzed separately with the PROC GLM model using SAS statistical software, Version 9.4 of the SAS System for Windows (SAS Institute, Inc, Cary, NC, USA). The mean of treatments, seeding dates and interactions between treatments and seeding dates were calculated to determine the statistical significance for both factors.

The Least Significant Difference (LSD) multiple comparison test was used to determine whether differences between control and treatments were statistically significant at $P < 0.05$. The values in the results section are the means $\pm$ standard error of the studied treatments. Tables and graphs of mean values were generated using Microsoft Excel (Microsoft 365 for Enterprise Version 2401).

## 3.3. Results:

Microbial inoculations can potentially improve the agronomic variables of potato plants due to their contribution to nutrient availability and uptake. Synthetic fertilizers have been extensively used in crop production to feed plants with nutrients and improve plant productivity. Therefore, microbe-coated fertilizers can be a significant resource for plant growth by establishing a beneficial association between plants and their rhizosphere. This leads to improved nutrient uptake and increases the tolerance to harsh environmental stresses impacting plant growth and development from seeding to harvesting.

### 3.3.1. Weather Conditions and Growing Degree Days (GDD)

In spring 2018, weather conditions were favourable for the first seeding on May 14th and the second seeding on May 24th. The first seeding is considered an early seeding to test the efficiency of the MCF treatments when applied in cooler temperatures. In contrast, second seeding is considered a recommended date in the region. On the other hand, the weather in spring 2019 delayed the first (early) seeding until May 22nd and the second seeding on June 7th. In both years, the aim was to establish two adjacent experiments 10-15 days apart. The temperature and precipitation at the beginning of the experiments were close to optimal for potato tuber sprouting and subsequent growth. The summers in 2018 and 2019 were distinct in that summer 2018 conditions were hot and dry, leading to drought stress by the end of the season when harvesting occurred. The weather in the summer of 2019 was colder and humid, leading to a longer growing season due to the late seeding compared to 2018. Because of the differences in the environmental conditions in growing seasons, accumulated growing degree-days (GDDs) were calculated for each growing season and seeding date (Table 3-3) to determine whether potato plants accumulated sufficient GDDs to complete the season compared to the available historical data. In 2018, the GDDs of early and late-seeded potato were close, but the accumulation of GDDs throughout the season compared to the historical average was relatively high (144 and 151 GDDs, respectively). This was due to the dry and hot weather in the summer of 2018, when plants reached maturity (full tuberization) within 96-99 days. The GDDs in 2019 were considerably higher than in 2018 in both seedings. Potato plants took between 117 and 126 days to reach maturity (full tuberization) due to the wet and cooler weather leading to a longer growing season. GDDs in early and late-seeded potato plants were very close to each other and the historical average. Based on the weather conditions and GDDs, the data for seeding dates within each year were pooled, while the two years' data were analyzed separately.

*Table 3-3: 2018, 2019 and historical growing days for potato growth in Sainte-Anne-de-Bellevue.*

| *Year* | *Growing Degree Days (GDD)* | |
|---|---|---|
| | First Seeding | Second Seeding |
| *2018* | **1524.4** | **1578.4** |
| *Historical Average 1994-2020* | 1380.9 | 1427.8 |
| *2019* | **1708.2** | **1661.0** |
| *Historical Average 1994-2020* | 1731.5 | 1647.7 |

### 3.3.2. Response of the agronomic variables to the microbe-coated fertilizers

Throughout two growing seasons, data on plant height (PH), leaf area (LA) and dry biomass (DB) were collected at the vegetative (VS) and flowering stages (FS) to test if the two studied microbe-coated fertilizers (MCF) were effective in improving agronomic variables as well as yield components. There was an apparent effect and increase in growth variables in both years, growth stages and seeding dates (SD), but not always with statistically significant differences between treatments (Tables 3-4, 3-5).

In 2018, there was no statistical significance between treatments, whereas SD was significantly different for PH at both growth stages and LA at the VS (Tables 3-4). In 2019, treatments were significantly increased PH at the FS and, LA and DB at the VS. On the other hand, SDs were statistically significant for PH and LA at the FS (Table 3-5). There were no interaction effects between treatments and seeding dates for all variables in both growing seasons.

*Table 3-4: A summary of analysis of variance (p-values) and mean values of agronomic variables studied during the **2018 growing season**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

***First growing season 2018***

| *Factors* | **Plant Height (cm)** | | **Leaf Area (cm2)** | | **Dry Biomass (g/5plants)** | |
|---|---|---|---|---|---|---|
| *Treatments* | Vegetative Stage | Flowering Stage | Vegetative Stage | Flowering Stage | Vegetative Stage | Flowering Stage |
| *Control (NPK)* | 52.3 a | 56.1 a | 2511 a | 3176 a | 142 a | 183 a |
| *NPK+EVL (1x)* | 51.4 a | 57.4 a | 2725 a | 3376 a | 152 a | 206 a |
| *NPK+EVL (2x)* | 49.5 a | 56.7 a | 2744 a | 3299 a | 156 a | 210 a |
| *NPK+EB(1x)* | 50.6 a | 57.1 a | 2567 a | 3328 a | 148 a | 197 a |
| *NPK+EB(2x)* | 52.3 a | 57.3 a | 2860 a | 3253 a | 156 a | 197 a |
| ***Seeding Date 2018*** | | | | | | |
| *14-May* | **47.3 b** | **59.3 a** | **2518 b** | 3212 a | 149 a | 201 a |
| *24-May* | **55.1 a** | **54.5 b** | **2845 a** | 3361 a | 152 a | 196 a |
| ***p-values*** | | | | | | |
| *Treatments* | 0.232 | 0.802 | 0.419 | 0.781 | 0.440 | 0.216 |
| *Seeding Date* | **<.0001** | **0.0001** | **0.018** | 0.381 | 0.646 | 0.649 |
| *Interaction T*SD* | 0.566 | 0.647 | 0.517 | 0.810 | 0.705 | 0.801 |
| *Standard Error* | 0.9 | 1.1 | 137.7 | 184.5 | 7.4 | 10.6 |
| *Coefficient Var.* | 5.1 | 5.3 | 14.5 | 15.9 | 13.9 | 15.1 |

*Table 3-5: A summary of analysis of variance (p-values) and mean values of agronomic variables studied during the **2019 growing season**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

| *Factors* | **Plant Height (cm)** | | **Leaf Area (cm2)** | | **Dry Biomass (g/5plants)** | |
|---|---|---|---|---|---|---|
| | ***Second growing season 2019*** | | | | | |
| *Treatments* | Vegetative Stage | Flowering Stage | Vegetative Stage | Flowering Stage | Vegetative Stage | Flowering Stage |
| *Control (NPK)* | 54.4 a | 57.8 a | **2641 b** | 3185 a | **155 b** | 177 a |
| *NPK+EVL (1x)* | 54.6 a | 60 a | **3056 ab** | 3410 a | **173 ab** | 188 a |
| *NPK+EVL (2x)* | 55.8 a | 60.7 a | **3084 ab** | 3470 a | **176 ab** | 191 a |
| *NPK+EB (1x)* | 55.5 a | 61.1 a | **3250 a** | 3452 a | **189 a** | 189 a |
| *NPK+EB (2x)* | 54 a | 59.7 a | **3044 ab** | 3268 a | **174 ab** | 181 a |
| | ***Seeding Date 2019*** | | | | | |
| *22-May* | 54.5 a | **58.7 a** | 2976 a | 3419 a | 167 a | 177 a |
| *07-Jun* | 55.2 a | **61 b** | 3039 a | 3295 a | 180 a | 193 a |
| | ***p-values*** | | | | | |
| *Treatments* | 0.604 | **0.041** | **0.022** | 0.550 | **0.018** | 0.330 |
| *Seeding Date* | 0.456 | **0.026** | 0.659 | 0.584 | 0.136 | 0.080 |
| *Interaction T*SD* | 0.580 | 0.927 | 0.939 | 0.882 | 0.958 | 0.926 |
| *Standard Error* | 1.1 | 1 | 157.3 | 248.2 | 9.1 | 9.7 |
| *Coefficient Var.* | 5.4 | 4.8 | 14.8 | 20.9 | 14.8 | 14.8 |

#### *3.3.2.1. Plant height (PH)*

The response of potato PH to microbe-coated fertilizers ranged from -5.4 to +5.6%, where plants were higher at the FS in both years 2018 and 2019, compared to the VS in which MCF-treated plants showed poorer performance. In 2018, the greatest PH at the VS was for non-treated plants, while NPK+EVL (1×) treated plants recorded the greatest PH at the FS, which was 2.4% greater than the untreated control (Table 3-4). In 2019, plants were taller at both measure stages, for plants treated with NPK+EVL (2x); at the VS and NPK+EB (1x), increased PH by 2.7 and 5.6%, respectively (Table 3-5). Despite the slight increase in MCF-treated plants, there were no significant differences between treatments in both the 2018 and 2019 growing seasons, except for the FS in 2019, for which the *p-value* was 0.041. PH was significantly affected by SD, in which early seeded plants at the VS ($p < 0.0001$) and FS ($p < 0.0001$) were shorter than plants seeded later in 2018, whereas SD was only significant at the FS ($p = 0.026$). There was no interaction between MCF treatments and SD in 2018 and 2019 (Tables 3-4 and 3-5).

#### *3.3.2.2. Leaf area (LA)*

Plant leaf area is an important variable for plant growth, determining the capacity of plants to capture sunlight and balance water and gas exchange for vigorous plant development. In this study, the response of potato LA to the MCF treatments was positive and ranged from 2.3 to 23.1% at the VS and 2.6 to 8.9% at the FS. The increase was consistently higher at the VS than at FS in both growing seasons. All treated plants had greater LA due to the MCF treatments than the control; however, the considerable increase did not show any statistical significance for the treatments except for the NPK+EB (1x) treatment ($p = 0.022$) at the VS in 2019. Conversely, SD was not a considerable factor in both growth stages and growing seasons, except for a significant difference at the VS ($p$ = 0.018) in 2018. There was no interaction effect between MCF treatments and SD in both growing seasons (Tables 3-4 and 3-5).

#### *3.3.2.3. Dry Biomass (DB)*

Dry biomass was one of the variables measured for each treatment at the two phenological sampling stages in the 2018 and 2019 growing seasons. An increase in DB indicates healthy growth and plant development in response to the MCF treatments. In this study, the MCF treatments consistently increased potato DB. There were considerable differences in the degree of increase, but statistical significance was absent for most treatments in both years. In 2018, the increase ranged from 4 to 9.9% at the VS and 7.5 to 14.8% at the FS. In 2019, there was an opposite pattern in which DB increase ranged between 11.6 and 21.9% at the VS, whereas the increase was lower at the FS, ranging from 2.4 to 7.7%. Despite the increase in all treated plants, treatments were only significant ($p$ = 0.018) at the VS in 2019, where NPK+EB (1x) was the most effective treatment ($p$ = 0.0185). Conversely, SD did not have a clear effect at either growth stage or growing seasons, showing no significant differences. In addition, there was no interaction effect between MCF treatments and SD for both growing seasons (Tables 3-4 and 3-5).

### 3.3.3. Nitrogen concentration and ash content in potato plant tissue and total starch in tubers

The potato plant tissue analysis for total nitrogen and ash contents was used to determine nitrogen concentration and ash content in response to the microbe-coated fertilizer treatments compared to non-treated plants. Total starch in potato tubers was also quantified for all treatments.

*Table 3-6: A summary of the analysis of variance (p-values) and mean values of total nitrogen concentration and ash content from plant tissues and total Starch (%) from potato tubers in response to microbe-coated fertilizers in **2018**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

| ***First growing season 2018*** | | | | | |
|---|---|---|---|---|---|
| ***Factors*** | **N (mg/g)** | | **Ash (mg/g)** | | **Starch%** |
| ***Treatments*** | Vegetative S. | Flowering S. | Vegetative S. | Flowering S. | Harvest |
| *Control (NPK)* | **32.96 b** | 23.6 b | 236 dc | **236 c** | 59.4 a |
| *NPK+EVL (1x)* | **34.54 a** | 26.25 a | 238 bc | **253 ab** | 59.8 a |
| *NPK+EVL (2x)* | **32.84 b** | 24.51 b | 241 a | **246 bc** | 59.9 a |
| *NPK+EB (1x)* | **32.95 b** | 26.28 a | 235 d | **260 a** | 59.9 a |
| *NPK+EB (2x)* | **34.46 a** | 26.86 a | 240 ab | **258 ab** | 59.9 a |
| ***Seeding Date 2018*** | | | | | |
| *14-May* | 32.94 a | 26.03 a | 238 a | 258 a | 59.4 a |
| *24-May* | 34.16 b | 24.94 a | 238 a | 243 b | 60.1 b |
| ***p-values*** | | | | | |
| *Treatments* | **0.0514** | **0.0115** | **0.0002** | **0.0049** | 0.665 |
| *Seeding Date* | **0.0155** | 0.0815 | 0.9552 | **0.0009** | **0.002** |
| *Interaction T*SD* | 0.1465 | 0.2368 | 0.3029 | 0.1059 | 0.174 |
| *Standard Error* | 0.5 | 0.6 | 4 | 0.8 | 0.2 |
| *Coefficient Var.* | 4.2 | 7.0 | 0.9 | 4.5 | 1.0 |

*Table 3-7: A summary of the analysis of variance (p-values) and mean values of total nitrogen concentration and ash content from plant tissues and total Starch (%) from potato tubers in response to microbe-coated fertilizers in **the 2019 growing seasons**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

| ***Second growing season 2019*** | | | | | |
|---|---|---|---|---|---|
| ***Factors*** | **N (mg/g)** | | **Ash (mg/g)** | | **Starch%** |
| ***Treatments*** | Vegetative S. | Flowering S. | Vegetative S. | Flowering S. | Harvest |
| *Control (NPK)* | 33.24 b | **23.5 c** | 237 a | 239 a | 59.8 b |
| *NPK+EVL (1x)* | 33.78 ab | **24.44 bc** | 238 a | 246 a | 60.6 a |
| *NPK+EVL (2x)* | 34.56 a | **26.31 a** | 236 a | 240 a | 60.1 ab |
| *NPK+EB (1x)* | 33.45 ab | **26.48 a** | 235 a | 247 a | 60.4 a |
| *NPK+EB (2x)* | 33.31 ab | **24.95 b** | 237 a | 252 a | 60.3 ab |
| ***Seeding Date 2019*** | | | | | |
| *22-May* | 33.75 a | 23.82 a | 236 a | 251 a | 60.3 a |
| *07-Jun* | 33.59 a | 26.46 b | 238 a | 239 b | 60.1 a |
| ***p-values*** | | | | | |
| *Treatments* | 0.4124 | **0.0027** | 0.6412 | 0.4888 | 0.3033 |
| *Seeding Date* | 0.467 | **<.0001** | 0.249 | **0.028** | 0.447 |
| *Interaction T*SD* | **0.044** | 0.071 | 0.565 | 0.531 | 0.488 |
| *Standard Error* | 0.5 | 0.5 | 5.7 | 1.3 | 0.3 |
| *Coefficient Var.* | 4.4 | 5.5 | 1.6 | 6.5 | 1.3 |

#### *3.3.3.1. Plant tissue nitrogen concentration/content*

Plant tissue analysis measures the complete elemental composition of the above-ground biomass of plants or specific plant components, such as leaves with or without stems. Plant tissue analysis in crops can be used to verify the effectiveness of a fertilizer program. It can be used as a diagnostic method to identify a nutrient deficiency, aiding in identifying elemental limitation problems. Plant nutrient levels can fluctuate based on the specific stage of plant growth. Hence, the growth stage of the plants at sampling plays a crucial role in collecting plant samples for analysis to assess the effectiveness of fertilization. The plant tissue samples were taken at two distinct growth stages (VS and FS) for accuracy, because the plant might not have started to uptake applied fertilizers in larger amounts at the early stages. In contrast, at the end of the season, potato plants die back, and above-ground parts start drying due to senescence, resulting in inaccurate nitrogen concentration determination. Regarding plant tissue, all the above-ground parts were used in total nitrogen determination because stems and petioles contribute to nutrient transportation to the leaves for accumulation; therefore, a mixture of all the parts would provide better cumulative nitrogen status in potato plants.

In this study, potato above-ground plant tissues (shoot: stem and leaf) were collected at two growth stages to determine the total nitrogen in the tissues in response to the MCF treatments. One of the components of the MCF treatments was nitrogen fertilizer, which was applied with phosphorus and potassium in the presence of microbial consortia coatings, except for the control, which was not coated with microbes. There were considerable increases in the total nitrogen concentration, specifically at the FS, in both growing seasons. In 2018, the response range was from -0.4 to 4.8% at the VS and 0.2 to 4% at the FS. In 2019, the increase ranged between 3.9 and 13.8% at the VS from 4 to 12.7% at the FS. The results of this study showed significant differences between treatments at both sampled growth stages. Among the treatments in 2018, NPK+EVL (1x) and NPK+EB (2x) treatments resulted in the highest total nitrogen concentration in plant tissues at the VS. The pattern was slightly different at the FS in which all treatments, except for the NPK+EVL (2x), were significantly different compared to the non-MCF-treated control plant tissues (Table 3-6). In 2019, the MCF treatments showed significant differences at the flowering stage in which all treatments accumulated more nitrogen than control plants, specifically NPK+EVL (1x) and NPK+EB (2x) (Tables 3-7). In addition to the MCF treatment, seeding dates were also significant across all the stages in both growing seasons, except for the VS in 2019, which showed a significant interaction effect between MCF treatments and the seeding date ($p$ = 0.0044). Results for the VS in 2019 showed that neither treatments nor seeding dates significantly affected the nitrogen accumulation in the potato plant tissue. This might be due to the variation in the collected samples and the intervention of unknown factors during sampling at the VS (Tables 3-6 and 3-7).

#### *3.3.3.2. Plant tissue ash content*

Ash residue, an important quality attribution for plant biomass (shoots: stem and leaf), was measured at the VS and FS to determine ash content, reflecting the inorganic elements in the plant tissue samples in response to the MCF treatments. The results show significant differences between treatments at both growth stages in the 2018 growing season only. The increase ranges were considerable in 2018, specifically at the FS in both growing seasons, ranging from 4.4 to 10.2%, while the range was between -0.3 to 2.3% at the VS. In 2019, the ash content was either lower than the control or slightly higher. In 2018, treatments were significant at both VS and FS ($p$ = 0.0002 and 0.0049, respectively). Among the treatments in 2018, NPK+EVL (2x) and NPK+EB (2x) at the VS resulted in the highest ash content compared to the rest of the treatment and control plants. At the FS, NPK+EB (1x) and NPK+EB (2x) treatments led to considerable increases in ash content (Table 3-6). In 2019, there were no significant differences between the MCF treatments at both growth stages. The seeding date significantly affected ash content only at the FS in both growing seasons. There were no interaction effects between the MCF treatments and SD either in 2018 or 2019 (Tables 3-6 and 3-7).

The data show no consistent effect of treatments on ash content in two growing seasons. However, significant treatment effects in 2018 and 2019 indicated that the warmer season positively affected potato plants in terms of nutrient uptake, as demonstrated by the increase in ash content, illustrating the amount of inorganic minerals in the potato plant tissue residue. This might explain the MCF treatment effects on growth promotion and enabling the potato root systems to access more nutrients, translate trends to the below-ground level, and contribute to potato tuber quality (Tables 3-6 and 3-7).

#### *3.3.3.3. Total starch in potato tubers*

Starch is an important quality index contributing to potato tuber taste and nutritional value. Environmental factors such as heat, drought and precipitation, nutrient availability in soil, and overall plant health impact potato starch quality. This study quantified total starch as a percentage of dry matter in response to the MCF treatments. In both years, the starch content was slightly more than the control, in which the increased range was between 0.8 and 0.9% in 2018 and 0.6 to 1.5% in 2019, leading to no significant difference between any of the MCF treatments and the control (Tables 3-6 and 3-7). However, multiple comparisons show that NPK+EVL (1x) and NPK+EB (1x) treatments differed significantly in 2019. In terms of SD, there was a significant difference in 2018 but not in 2019. There was no interaction between treatments and seeding dates for both growing seasons (Tables 3-6 and 3-7).

### 3.3.4. Effect of the microbe-coated fertilizers on potato yield and tuber numbers

The most important variable to evaluate the efficiency of the MCF treatments was yield, particularly marketable-size tuber yield (MSY), total yield (TY), marketable-size tubers (MST) and total number of tubers (TT). Yield is the variable most crucial to understanding how above-ground organs responded to the treatments and proportionally accumulated more weight in tubers. The importance of yield is related to gaining agricultural producers' acceptance and promoting the use of such products. The overall performance of the MCF treatments in the 2018 and 2019 growing seasons is summarized in tables 3-8 and 3-9.

*Table 3-8: A summary of the analysis of variance (p-values) and mean values of marketable size yield (MSY), total yield (TY), number of marketable size tubers (MST) and the total number of tubers (TT) in the **2018 growing season**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

***First growing season 2018***

| ***Factors*** | **MS Yield** | **Total Yield** | **MS Tubers** | **Total Tubers** |
|---|---|---|---|---|
| ***Treatments*** | (t $ha^{-1}$) | (t $ha^{-1}$) | No $plant^{-1}$ | No $plant^{-1}$ |
| *Control (NPK)* | 18.3 c | 19.2 b | 5.9 b | 7.1 b |
| *NPK+EVL (1x)* | 21.9 a | 22.6 a | 6.4 a | 7.6 a |
| *NPK+EVL (2x)* | 21.1 ab | 21.8 a | 6.4 a | 7.6 a |
| *NPK+EB (1x)* | 21.5 ab | 22.6 a | 6.3 a | 7.5 a |
| *NPK+EB (2x)* | 20 bc | 21 a | 6.3 a | 7.5 a |
| ***Seeding Date 2018*** | | | | |
| *14-May* | 18 a | 19.5 a | 5.9 a | 7.4 a |
| *24-May* | 23 b | 23.4 b | 6.7 b | 7.5 a |
| ***p-values*** | | | | |
| *Treatments* | **0.036** | **0.023** | 0.370 | **0.014** |
| *Seeding Date* | **<.0001** | **<.0001** | **<.0001** | 0.728 |
| *Interaction T*SD* | 0.684 | 0.824 | 0.179 | 0.960 |
| *Standard Error* | 0.7 | 0.7 | 0.1 | 0.2 |
| *Coefficient Var.* | 9.4 | 9.7 | 5.5 | 6.0 |

*Table 3-9: A summary of analysis of variance (p-values) and mean values of marketable size yield (MSY), total yield (TY), number of marketable size tubers (MST) and total number of tubers (TT) in the **2019 growing season**. Means in the same column with the same letter are not significantly different according to the LSD test at p<0.05. **Bold** values are significant p-values.*

| ***Second growing season 2019*** | | | | |
|---|---|---|---|---|
| ***Factors*** | **MS Yield** | **Total Yield** | **MS Tubers** | **Total Tubers** |
| ***Treatments*** | (t $ha^{-1}$) | (t $ha^{-1}$) | No $plant^{-1}$ | No $plant^{-1}$ |
| *Control (NPK)* | **18.7 b** | **20.3 b** | 7.1 a | 9.0 a |
| *NPK+EVL (1x)* | **22.9 a** | **24.5 a** | 7.8 a | 9.7 a |
| *NPK+EVL (2x)* | **21.9 a** | **23.5 a** | 7.7 a | 9.4 a |
| *NPK+EB (1x)* | **22.7 a** | **24.5 a** | 7.5 a | 9.5 a |
| *NPK+EB (2x)* | **21.1 a** | **23.4 a** | 7.5 a | 9.6 a |
| ***Seeding Date 2019*** | | | | |
| *22-May* | 21.525 a | 22.89 a | 7.75 a | 9.28 a |
| *07-Jun* | 21.38 a | 23.585 a | 7.29 a | 9.57 a |
| ***p-values*** | | | | |
| *Treatments* | **0.017** | **0.011** | 0.472 | 0.676 |
| *Seeding Date* | 0.778 | 0.166 | 0.145 | 0.322 |
| *Interaction T*SD* | 0.590 | 0.591 | 0.599 | 0.279 |
| *Standard Error* | 0.6 | 0.5 | 0.3 | 0.3 |
| *Coefficient Var.* | 7.4 | 6.4 | 12.6 | 9.5 |

#### *3.3.4.1. Marketable size (MSY) and total yield (TT) of potato tubers*

In this study, potato tubers were harvested at the end of growing seasons at the full tuberization stage to record the MSY and TY. In response to the MCF treatments, the MSY and TT were significantly increased in both years. In 2018, the increased range for the MSY was 9.1 to 19.4%, whereas the TY was increased by 9 and 17.8%. There were significant differences between MCF-treated plants and non-treated control plots for MSY and TY ($p$ = 0.036 and 0.023, respectively). NPK+EVL (1x) and NPK+EB (1x) treatments cause the highest MSY and TT values among MCF-treated plots (Table 13). Seeding dates were also significant for MSY and TY without negatively impacting the performance of MCF treatments. There was no interaction between MCF treatments and SDs in the 2018 growing season (Table 3-8).

In 2019, a similar pattern was observed for the MCF treatments. The increase in MSY ranged from 12.7 to 21.9%, whereas the TY increased between 15.5 to 20.8%. The MCF treatments resulted in significantly greater TY levels than the non-treated control plots for MSY and TY ($p$-= 0.017 and 0.011, respectively). Similar to 2018, NPK+EVL (1x) and NPK+EB (1x) treatments resulted in the highest MSY and TY values among MCF treatments (Table 3-9). In 2019, there were no significant differences between SDs or the interaction effect of MCF treatments and SDs (Tables 3-8 and 3-9).

These results indicate the efficiency of the MCF treatments under field conditions compared to the control treatment in both growing seasons. Despite the difference in seeding dates, especially in 2018, the treatments increased MSY and TY consistently. However, the efficiency was greater when plots were treated with the recommended dose of the microbial consortia (NPK+EVL (1x) and NPK+EB (1x)).

#### *3.3.4.2. Marketable-size (MST) and total tuber numbers (TT)*

The MST and TT were considerably increased by MCF in both years. In 2018, the increases for the MST were 6.3 to 8.4%, whereas the TT increases were 6.4 and 7.8%. There was a significant difference between MCF-treated plots compared to non-treated control plots for TT (*p-value*=0.014). NPK+EVL (1x) resulted in the greatest MST and TT among MCF treatments (table 16). The seeding date was only significant for MST without a negative impact on the performance of MCF treatments in terms of MST increase. No interaction between MCF treatments and SDs was observed in the 2018 growing season (Table 3-8).

In 2019, there was an increase in MST, ranging from 6.4 to 10.6%, whereas the TT increased between 4.7 and 8.7%. Among the MCF-treated plots, the NPK+EVL (1x) treatment resulted in the greatest MST and TT levels (Table 16). Despite these considerable increases in both variables, there were no significant differences between the MCF treatments and SDs or the interactions between the MCF treatments and SDs (Table 3-9).

*Table 3-10: An overview of the studied variables' percent change in each treatment compared to control plants in the 2018 and 2019 growing seasons. VS = Vegetative stage; FS = Flowering Stage;* ***PH*** *= plant height;* ***LA*** *= leaf area;* ***DB*** *= dry biomass;* ***N*** *= plant tissue nitrogen concentration;* ***Ash*** *= plant tissue ash content;* ***MSY*** *= marketable-size yield;* ***TY*** *= total yield;* ***MST*** *= marketable-size tubers;* ***TT*** *= total tubers;* ***%S*** *= total starch in potato tubers*

| **Variables** | **2018 growing season** | | | | **Treatments** **2019 growing season** | | | |
|---|---|---|---|---|---|---|---|---|
| | **NPK + EVL (1x)** | **NPK + EVL (2x)** | **NPK + EB (1x)** | **NPK + EB (2x)** | **NPK + EVL (1x)** | **NPK + EVL (2x)** | **NPK + EB (1x)** | **NPK + EB (2x)** |
| **PH VS** | -1.9 | -5.4 | -3.3 | -0.1 | 0.4 | 2.7 | 2.1 | -0.8 |
| **PH FS** | 2.4 | 1.1 | 1.8 | 2.1 | 3.8 | 5.0 | 5.6 | 3.3 |
| **LA VS** | 8.5 | 9.3 | 2.3 | 13.9 | 15.7 | 16.8 | **23.1** | 15.3 |
| **LA FS** | 3.8 | 5.0 | 5.6 | 3.3 | 7.1 | 8.9 | 8.4 | 2.6 |
| **DB VS** | 7.2 | 9.9 | 4.0 | 9.7 | 11.6 | 13.8 | **21.9** | 12.6 |
| **DB FS** | 12.4 | **14.8** | 7.5 | 7.5 | 6.3 | 7.7 | 6.7 | 2.4 |
| **N VS** | 4.8 | -0.4 | 0.0 | **4.6** | 1.6 | **4.0** | 0.6 | 0.2 |
| **N FS** | 11.2 | 3.9 | **11.3** | **13.8** | 4.0 | **12.0** | **12.7** | **6.2** |
| **Ash VS** | 0.9 | 2.3 | -0.3 | 1.7 | 0.3 | -0.4 | -0.8 | 0.0 |
| **Ash FS** | 7.3 | 4.4 | **10.2** | **9.1** | 3.2 | 0.7 | 3.7 | 5.5 |
| **MSY** | **19.4** | **15.5** | **17.3** | 9.1 | **21.9** | **16.7** | **21.1** | **12.7** |
| **TY** | **17.5** | **13.3** | **17.8** | **9.0** | **20.8** | **15.9** | **20.8** | **15.5** |
| **MST** | **8.4** | **7.2** | **6.3** | **6.3** | **10.6** | **9.6** | **6.7** | **6.4** |
| **TT** | **7.8** | **7.1** | **6.7** | **6.4** | **8.7** | **4.7** | **6.1** | **7.0** |
| **%S** | 0.8 | 0.9 | 0.9 | 0.8 | **1.5** | 0.6 | **1.1** | 0.9 |

## 3.4. Discussion

### 3.4.1. The impact of weather conditions on potato growth

The results of the currently reported two-year experiments show inconsistency in variables studied at the vegetative and flowering stages when compared between growth seasons (Tables 3-4 and 3-5). The weather data were considerably different between the 2018 growing season, which was hot and dry, and the 2019 growing season, which was close to optimal. Differences in weather conditions mean the seeding dates differed for each growing season, resulting in variation between the two years in accumulation growing degree days (GDD), which has important effects on potato growth and development over the growing season. In both years, the data from two seeding dates were pooled and analyzed separately for each year. In 2018, the cumulative GDDs for the two seeding dates were less different (Table 3-3), and plants had almost the same GDDs at the end of the season, meaning that aspect of the climate had little impact on plants. Despite the hot and dry weather negatively impacting potato growth and development, above-ground agronomic variables were considerably increased, at least for one of the microbe-coated fertilizer (MCF) treatments, at both growth stages, except for the plant height at the VS (Tables 3-4 and 3-5). The positive effects of the MCF on the in-season variables increased potato yield (Tables 3-8 and 3-9). A similar trend was observed in the 2019 growing season in which the GDD difference between seeding dates was minimal (Table 3-3) despite the late seedings compared to 2018. However, the close-to-optimal weather conditions and the microbe-coated fertilizer application resulted in taller plants, greater leaf area, and increased dry biomass and nitrogen concentration in plant tissues (Table 3-4). Enhancement of agronomic variables related to plant growth during the 2019 season translated to increased tuber yield (Table 3-5).

Crop management (seeding date, water availability and fertilization), weather conditions (temperature and precipitation) and genetics (variety choice) are the factors that can affect potato yield (Ojeda et al., 2021). The effect of diverse weather conditions on potato yield in North America is a multifaceted issue influenced by various environmental and agronomic factors. Potato yield is associated with the growing degree days due to seeding date and environmental factors such as daily temperature and precipitation. It is reported that in-season precipitation at specific growth stages and water loss at the tuberization stage are critical factors for potato yield determination (Zarzyńska et al., 2023; Jun et al., 2018). This finding is supported by other studies, which concluded that the impact of seeding dates and, precipitation amounts and events on potato yield were meaningful (Singh, 2020; Biazin et al., 2021).

Moreover, the year-to-year variation in the cumulative growing degree days from May to July potentially influences potato yield (Crosby and Wang 2021). Overall, the outcomes of these studies collectively show the potential of diverse impacts of environmental conditions on potato yield. With expected changes in climatic conditions, it is crucial to understand the influences and adapt agricultural practices to mitigate these environmental factors and enhance the resiliency for an optimal potato yield in the region.

A case study was conducted to determine the effects of extreme weather (wet start or end to the growing season) on potato yield (Van Oort et al., 2012), reporting that rainfall (Dalla Costa et al., 1997) and temperature (Kooman et al., 1996) cause significant variability in potato yield. The influence of weather extremes on agricultural productivity can be substantial, but exact definitions of their effects are lacking, and their interactions with other yield-affecting factors can obscure their effects (Ojeda et al., 2021, 2020). Overall, potato yield variability because of diverse weather conditions was shown in many studies. Variability in year-to-year potato yield in response to microbial inoculations or biological inputs is less well understood, largely due to a lack of field studies. Although our findings show that the year-to-year variability in weather conditions, measured in-season growth variables and yield components affected but generally did not remove the overall increases resulting from the use of microbe-coated fertilizer (MCF) treatments for potato plant growth promotion and mitigation of unfavourable weather effects. The findings we present here are in agreement with Ali et al. (2021), who found that a combination of synthetic fertilizer and *Bacillus* strains increased plant height, shoot dry weight, and total tuber yield by 15, 26 and 21%, respectively, compared to non-inoculated controls plants. Furthermore, variability in field-grown potato yield was reported in two field sites where application of a microbial consortium of *Bacillus* and *Trichoderma* strains increased potato yield by 21.8 to 31.5% (Wang et al., 2019). It is evident that the MCF treatments could assist potato plants in mitigating environmental factors presented during the growing seasons and promote growth, leading to better crop productivity.

The inconsistent results of microbial inoculation in promoting plant growth in the field can be attributed to a range of factors, such as the viability of inoculants, environmental conditions, and soil variability, including soil biology. It is crucial to understand these factors to optimize the use of microbial inoculants in agricultural practices. Further investigations need to be undertaken to elucidate the specific mechanisms underlying the variability in the efficiency of microbial inoculants and to develop strategies for maximizing their effectiveness in different agricultural contexts.

### 3.4.2. Microbe-coated fertilizers to mitigate environmental stresses

In this study, the positive effects of the MCF treatment on most of the studied variables were consistent across two growth stages and two years except for the plant height, total nitrogen concentration and ash content at the VS. These numerical effects were not always statistically different. The variability could be due to variations in climatic conditions. However, potato plants benefited from microbial inoculations to overcome the stressful conditions in 2018, in which yield was significantly increased. The close-to-optimal weather conditions in 2019 could help potato plants perform better and translate the in-season more optimal growth to higher yield. It seems likely that microbial inoculation technologies will continue to be improved and developed to promote plant growth under unfavourable environmental stresses. This could be due to enhanced nutrient availability resulting from interactions between plants and beneficial microorganisms involved in the production of phytohormone and phytohormone analogue molecules, solubilizing essential nutrients, nitrogen fixation, siderophore production, and many more benefits that could result from this interaction. When weather conditions are more favourable, plants most likely do not initiate an interaction with at least some of the potentially beneficial organisms in the rhizosphere, and the contribution of the microbial inoculations to the enhancement of plant growth and development would be minimal. However, microbial inoculation in cool and wet growth seasons could significantly help with the availability of nutrients and uptake by preserving the nutrients from leaching and releasing them later in the season when more challenging conditions occur (Guo et al., 2020; Riseh et al., 2023).

The application of beneficial microorganisms for plant growth promotion in the agricultural fields has shown contradictory and diverse results (Caradonia et al., 2022; Overbeek et al., 2021; Rosyidah et al., 2020; Saini et al., 2021 and Antar et al., 2021). A range of factors impact the viability and growth promotion efficiency of the microbial inoculations (single strains or consortia of strains) before or after application. One of the factors could be environmental effectors. The microbial consortia efficacy can be compromised at any time, from their production at the laboratories to inoculation into agricultural systems and subsequent rhizosphere colonization for plant growth promotion (O'Callaghan et al., 2022; Mahmud et al., 2021; Romano et al., 2020 and

Yin et al., 2022). Studies report that environmental stresses are key to initiating plant and microbe interactions for plant-growth promotion because specific microbes associate with the plant when a higher level of stress exists, and these are either not present or not active in the rhizosphere under the optimal environmental conditions (Antar et al., 2021; Shah et al., 2021). In addition, edaphic factors such as soil structure and composition can also determine the activity and effectiveness of microbial inoculation for plant growth promotion in agricultural fields. The literature provides information regarding the impact of edaphic factors on microbial applications and their potential implications for potato production (Naqqash et al., 2016; Batool et al., 2020). Considering the factors mentioned above, plants are expected to respond to microbial inoculations with variation, making the plant growth promotion efficiency of the microbial consortia under field conditions variable from site to site and year to year.

It has been reported that microbial inoculations in the rhizosphere mitigate environmental stresses, such as water deficit, in potato plants by modulating antioxidant enzyme activities and oxidative stress suppression (Batool et al., 2020). It has also been reported that the application of microbial inoculations, including *Bacillus, Pseudomonas* and *Azospirillum,* and their presence and activity in the rhizosphere improve potato plant growth and development due to phytohormone (e.g. indole acetic acid - IAA) production, signal exchange compounds (Backer et al., 2018) and nutrient (nitrogen and phosphorus) availability and uptake (Diallo et al., 2011). In addition, the effectiveness of microbial inoculations in enhancing nutrient uptake by potato plants has been highlighted by Hafez et al. (2019), in which microbial inoculations altered root morphology and expanded root hairs, improving N, P, and K uptake in infertile soils with minimal essential elements.

The efficiency of microbial consortia inoculation in agricultural systems was species-dependent and strain-specific because bacterial strains possess various characteristics to manage unfavourable environmental conditions, promote plant growth, shape microbial community composition and regulate plant mechanisms that could, directly and indirectly, affect potato plant development. Therefore, taxonomically diverse microbial consortia could lead to diverse responses by potato plants, suggesting higher efficiency due to the specific microbial strains present in the consortia (Kalozoumis et al., 2021).

### 3.4.3. The role of microbial inoculations in potato plant growth promotion

In this study, the contribution of microbe-coated fertilizers to enhanced potato yield was significant due to the efficacy and compatibility of the microbial consortia when coated on synthetic fertilizer. The growth promotion trend was observed in both growing seasons despite the variability in weather conditions. At least one of the microbial treatments at one of the growth stages showed a significant increase in PH, LA, DB, plant tissue total nitrogen (N) and ash content, and most importantly, yield (MSY and TY). The findings of this study are consistent with previously reported studies in which microbial inoculants were applied to promote growth and enhance potato yield. Plant growth-promoting rhizobacteria may influence plant phytohormones and photosynthesis, which could explain gains in dry matter weight and earlier and more intense tuberization of inoculated plants (Oswald and Calvo 2009). Inoculation of potato with a bacterial consortium also increased the concentration of NPK in shoots, root dry weight, and tuber weight (Yasmin et al., 2017). In another study, a consortium of *Pseudomonas putida* and *P. fluorescens*, which were isolated from high-yield potato fields, were tested for their efficiency under field conditions on a set of potato cultivars. The PGPR treatments significantly increased yield up to 1.37-fold over non-inoculated controls (Howie and Echandi 1983). Moreover, a study showed that the inoculation of field-grown potato plants with a combination of *Bacillus subtilis* and *Bacillus amyloliquefaciens* significantly reduced the time required for emergence, improved plant growth, and increased tuber yield by 9% compared to the non-inoculated control plants. The study also showed that tuber yield was higher in the warmer growing season than in the cooler season. This might be due to the nature of the beneficial microorganisms when the influence of the stressed conditions results in higher activity in the rhizosphere, leading to greater growth promotion than uninoculated controls in the above-ground tissue and mass accumulation below-ground in the tubers (Uysa and Kartar 2020). The selection of microbial inoculants that possess the ability to sustain their activity under adverse conditions presents a promising opportunity for improved productivity by potato. All the studies mentioned earlier clearly support this study's findings regarding the efficiency of specific microbe treatments, in this case, in the from of MCF. The diverse composition of the two microbial consortia used in this study may support the advantage of using consortium-based microbial inoculants rather than a single inoculum. The Era Boost Pro microbes from Ulysse Biotech were tested and selected based on their ability to solubilize some nutrients. For example, *Bacillus velezensis* U47, *Bacillus megaterium* U48, and *Bacillus megaterium* U49 can solubilize organic phosphorus and calcium; *Bacillus velezensis* U50 showed the ability to produce enzymes involved in the solubilization of organic phosphorus; *Bacillus velezensis* U47 produces a siderophore to chelate iron; and *Bacillus megaterium* U48 produces phytohormones, specifically IAA (Personal communication from industrial partner). The consortium included in EVL Coating® is believed to possess characteristics generally similar to those of the Ulysse material after being tested and examined for efficiency using *in-vitro* assays and field trials. Three studies in 2022 have been conducted on the EVL Coating® strains using Cell-Free Supernatant (CFS) as the treatment. The microbial cell-free supernatant derived from the EVL *Lactobacillus* strain was assessed under salinity stress. Results showed that the CFS-derived form of the *Lactobacillus* strain significantly increased potato plant fresh weight and physiological variables such as photosynthetic rate and leaf greenness. Also, the CFS treatment enhanced germination percentage in soybean seeds and radical length in maize. The authors concluded their findings by recommending *Lactobacillus* CFS as a potential biostimulant technology and product for growth enhancement in potato, corn and soybean (Naamala et al., 2022)

In another study, the microbial cell-free supernatants derived from EVL *Bacillus* and *Lactobacillus* strains were evaluated under low pH stress. The results revealed that both EVL strains enhanced seed germination rate and seedling growth, leading to greater length and volume of potato roots in tomato and maize (Msimbira et al., 2022). Most recently, *Bacillus subtilis* and *Lactobacillus helveticus* CFS treatments showed promising results for physiological (photosynthetic rates and stomatal conductance) and agronomic (root and shoot fresh weights) variables in potato grown under low pH levels (Msimbira et al., 2022). The results of these studies show the efficiency of the products derived from EVL Coating® strains, especially in alleviating abiotic stresses, including salinity and acidity, which was the case in the mentioned studies. These results support our findings that EVL microbial-consortium coating on synthetic fertilizer positively affected potato plant in-season growth variables and increased yield.

A diverse range of beneficial organisms have the capability to enhance plant growth in controlled environment settings, including laboratories, growth chambers and greenhouses (Msimbira et al., 2022; 2023; Naamala et al.., 2022; Yaghoubian et al., 2022). However, the beneficial impacts linked to microbial inoculants on plants in controlled environments do not consistently transfer to uncontrolled open-field conditions where several factors can influence their efficiency. This might potentially be attributed to the capacity of the microbial inoculants to effectively maintain the viability of the microbial cell between their production in the laboratories, transportation, and application when coated on synthetic fertilizers. In addition, diverse weather conditions and the interaction between the microbial inoculants with indigenous soil microbial communities and their survival, as a result, would be other factors determining the ability of microbial inoculants in plant growth promotion (Antar et al., 2021; Shah et al., 2022). In order to achieve consistent outcomes under field conditions, it is imperative to carefully choose the appropriate form of inoculants and effectively optimize their large-scale manufacturing. These measures play a crucial role in achieving desired results. Microbial-based products typically consist of live microbes in either single strains or consortia. Single-strain inoculants have a long history in agriculture, most notably with the $N_2$-fixing rhizobia associated with legumes. It was the first commercially used strain in the 1890s (Khan 2022 and Bradáčová et al. 2019). However, field studies have shown inconsistent effects due to the inoculum's ability to resist environmental stresses. Microbial consortium-based inoculants may be more robust and provide more ecosystem functions, such as phosphorus solubilization (Lin et al., 2023), potassium solubilization (Yousef et al., 2023), nitrogen fixation (Smith et al., 2015), phytohormone production (Miransari and Smith (2014), or biocontrol of pathogens (Riaz et al., 2022). However, this concept could show contrary results for plant growth promotion due to the concentration and quality variability in the consortia. For example, microbial consortia with two plant-beneficial microbes with similar shelf lives could be more effective and potentially act synergistically than a single strain. Therefore, it is essential to note that synergistic interactions between plant growth-promoting microbes may not always be necessary for optimal results (Borriss 2014).

### 3.4.4. Microbe-coated fertilizers affect plant tissue nitrogen concentration and potato tuber starch content

There were considerable increases in the total nitrogen concentration, specifically at the flowering stage in both growing seasons. The results of this study show significant differences between treatments at both growth stages. Our findings are supported by a previously conducted two-year field study in which a significant increase in nitrogen, phosphorus and potassium concentrations in potato leaves was reported in response to treatment with the potassium-solubilizing bacteria *Bacillus cereus* (Ali et al., 2021). Further studies have reported increased nutrients in potato biomass. It has been reported by Elkholy et al. (2012) that a combination of potassium-based synthetic fertilizer and a microbial consortium containing a *Bacillus circulans* strain application resulted in increased concentrations of macronutrients (N, P and K) and micronutrient concentration (Cu, Fe, Mn and Zn) in potato shoots. A similar study reported consistent results of the same synthetic and microbial fertilizer combination for enhanced N, P and K uptake by potato plants (Elkhatib et al., 2019). All the mentioned studies concluded that microbial inoculants have the potential to improve potato productivity by enhancing nutrient availability, increasing nutrient uptake and accumulation in plant tissues, and boosting potato yield.

In this study, we reported numerical increases in the total starch content in response to the MCF treatments. The total starch data shown here are similar to Liu et al. (2007), where three potato cultivars are grown in New Brunswick, Canada, and tested for physicochemical properties. The results showed variability in total starch in which AC Stampede Russet potato, Russet Burbank potato and Karnico contained 70.5, 71.6 and 72.4% starch, respectively. The authors indicate that the variability was due to potato cultivars, individual tubers of the same cultivar, and phosphorus content. In a similar Canadian study, significant differences in total starch content, ranging between 67.2 and 79.4%, were observed among thirteen potato cultivars growing in two provinces, namely Alberta (Lethbridge Research Center-AAFC) and New Brunswick (Fredericton Potato Research Center-AAFC). The authors linked the source of variability in higher total starch content in New Brunswick to soil chemical components, specifically phosphorus content (Lu et al., 2011). Another study conducted in Canada shows that growing regions and potato cultivars are variable factors for total starch content. For example, the total starch of cultivars growing in New Brunswick, including Innovator, Russet Burbank and Shepody, was 74.2, 69.8 and 68%, respectively, while the same cultivars growing in Manitoba had 68.2, 66.2 and 63.3% starch contents, respectively. This trend indicates that environmental conditions (i.e. temperature and precipitation) and edaphic factors (i.e. soil type and biological composition) are important determinants of the total starch content in potato tubers (Chung et al., 2014). Cooler growing seasons and summer temperatures are ideal for potato growth (Western Potato Council 2003; Agriculture and Agri-Food Canada 2015). The examples above explain our results, where total starch was higher in the 2019 growing season than in 2018, which had a dry and hot summer, which would have excreted a general effect, regardless of the application of the MCF treatments. Our study demonstrated no significant effects of MCF treatments across both years on total starch content despite numerical increases compared to non-inoculated control tubers ($p = 0.665$ and 0.303, prospectively). These numerical increases might result from the MCF treatments because the bacterial strains coated on the synthetic fertilizers included those with phosphorus solubilization characteristics, leading to greater phosphorus availability by treated potato plants and contributing to the higher total starch content in tubers (Bertoft and Blennow 2016).

Based on Agriculture and Agri-Food Canada (2014), the Goldrush potato cultivar is among table cultivars which are excellent for boiling and baking as well as fresh marketing. Cultivars such as Russet Burbank and Shepody are classified as very suitable for French fries, while Kennebec and Superior are categorized as suitable for the chips industry. The starch content of potato tubers significantly influences the quality of potato products (Stark et al., 2020). Potato tubers with higher starch content are deemed suitable for food use, processing, or starch production (Raigond et al., 2020). Therefore, potato growers must select cultivars that suit the local environment for higher yield quantity and better biochemical composition (quality).

### 3.5. Conclusions

Following a two-year and multi-planting date study, it can safely be concluded that using both evaluated microbial consortia (EVL and EB) were efficient in growth promotion and increasing marketable-size and total tuber yield when compared to the non-treated control. Agronomic variables measured throughout the growing season were considerably increased in response to the MCF treatments in both years and growth stages but not always with statistical significance.

In terms of the doses of the coating microbial consortia, there were variations in overall growth promotion. The recommended doses (1x) of EVL and double dose (2x) of EB resulted in greater growth promotion in 2018. Results for EVL (1x and 2x) were close in 2019, but treatment with EB (1x) resulted in the highest overall increase in both in-season variables and final yield and yield components. Double doses of EVL and EB did not result in any additional plant growth and yield increases in both growing seasons, compared to the recommended doses. In-season growth promotion, especially in leaf area and an increase in nitrogen concentration at the flowering stage justify the increased yield, which is the most important economic gain for potato growers and producers.

This study introduces a new method to inoculate the beneficial microorganisms into agricultural soils. The significant results indicate that a microbial consortium can be coated on synthetic fertilizers and banded as starter fertilizers at seeding to promote vigorous plant growth and increased agricultural productivity.

In sum, factors such as soil nutrient availability, microbial community composition, and environmental stresses can significantly impact the activity and effectiveness of microbial consortia in potato production. Understanding the interactions between these factors and microbial consortia is crucial for optimizing their use in sustainable potato cultivation practices. The application of potentially beneficial microbial consortia as MCF can increase aspects of potato plant growth and enhance final potato tuber yield. Further research is needed to comprehensively assess the relationship between seeding dates, growing degree days, and the MCF treatments to understand their impacts on potato yield and develop strategies for maximizing potato production in the region.

## References

Agriculture and Agri-Food Canada (2014). Potato Market Information Review. Pages 1-57 *in* Agriculture and Agri-Food Canada, ed. Market Analysis and Information Section, Horticulture and Cross Sectoral Division.

Agriculture and Agri-Food Canada (2015). The Biology of Solanum tuberosum (L.) (Potatoes). the Guidance Document Repository (GDR). Section 4.3 Cultivation and use as a crop.

Aksoy, E., Demirel, U., Bakhsh, A., Zia, M., Naeem, M., Saeed, F., … & Çalışkan, M. E. (2021). Recent advances in potato (solanum tuberosum l.) breeding. Advances in Plant Breeding Strategies: Vegetable Crops, 409-487. https://doi.org/10.1007/978-3-030-66965-2_10

Ali, Ahmed M., et al. "Effect of potassium solubilizing bacteria (Bacillus cereus) on growth and yield of potato." *Journal of Plant Nutrition* 44.3 (2021): 411-420.

Antar, M., Gopal, P., Msimbira, L. A., Naamala, J., Nazari, M., Overbeek, W., … & Smith, D. L. (2020). Inter-organismal signaling in the rhizosphere. Rhizosphere Biology: Interactions Between Microbes and Plants, 255-293. https://doi.org/10.1007/978-981-15-6125-2_13

Antar, M., Lyu, D., Nazari, M., Shah, A., Zhou, X., & Smith, D. L. (2021). Biomass for a sustainable bioeconomy: an overview of world biomass production and utilization. Renewable and Sustainable Energy Reviews, 139, 110691. https://doi.org/10.1016/j.rser.2020.110691

Bai, Y., Zhou, Y., Yue, T., Huang, Y., He, C., Jiang, W., ... & Wang, J. (2023). Plant growth-promoting rhizobacteria Bacillus velezensis JB0319 promotes lettuce growth under salt stress by modulating plant physiology and changing the rhizosphere bacterial community. *Environmental and Experimental Botany*, *213*, 105451.

Balderas-Ruíz, K. A., Bustos, P., Santamaria, R. I., González, V., Cristiano-Fajardo, S. A., Barrera-Ortíz, S., ... & Serrano-Carreon, L. (2020). Bacillus velezensis 83 a bacterial strain from mango phyllosphere, useful for biological control and plant growth promotion. *Amb Express*, *10*, 1-19.

Batool, T., Ali, S., Seleiman, M. F., Naveed, N. H., Ali, A., Ahmed, K. B. S., … & Mubushar, M. (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-73489-z

Bertoft, E., & Blennow, A. (2016). Structure of potato starch. In *Advances in potato chemistry and technology* (pp. 57-73). Academic Press.

Biazin, B., Wondatir, S., Tilahun, G., Asaro, N., & Amede, T. (2021). Using aquacrop as a decision-support tool for small-scale irrigation systems was dictated by the institutional and market incentives in ethiopia. Frontiers in Water, 3. https://doi.org/10.3389/frwa.2021.6641277

Borriss, R. (2014). Towards a new generation of commercial microbial disease control and plant growth promotion products. In *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture* (pp. 329-337). Cham: Springer International Publishing.

Caradonia, F., Ronga, D., Tava, A., & Francia, E. (2022). Plant biostimulants in sustainable potato production: an overview. *Potato Research*, *65*(1), 83-104.

Cerozi, B. and Fitzsimmons, K. (2016). Use of bacillus spp. to enhance phosphorus availability and serve as a plant growth promoter in aquaponics systems. Scientia Horticulturae, 211, 277-282. https://doi.org/10.1016/j.scienta.2016.09.005

Chakrabarti, S. K., Sharma, S., & Shah, M. A. (2022). Potato pests and diseases: a global perspective. Sustainable Management of Potato Pests and Diseases, 1-23. https://doi.org/10.1007/978-981-16-7695-6_1

Choi, I., Chun, J., Choi, H. S., Park, J., Kim, N. G., Lee, S. K., ... & Cho, K. (2020). Starch characteristics, sugars and thermal properties of processing potato (Solanum tuberosum L.) cultivars developed in Korea. *American Journal of Potato Research*, *97*(3), 308-317.

Choudhary, D. K., & Johri, B. N. (2009). Interactions of Bacillus spp. and plants–with special reference to induced systemic resistance (ISR). *Microbiological research*, *164*(5), 493-513.

Chung, H. J., Li, X. Q., Kalinga, D., Lim, S. T., Yada, R., & Liu, Q. (2014). Physicochemical properties of dry matter and isolated starch from potatoes grown in different locations in Canada. *Food research international*, *57*, 89-94.

Conlon, M. and Bird, A. (2014). The impact of diet and lifestyle on gut microbiota and human health. Nutrients, 7(1), 17-44. https://doi.org/10.3390/nu7010017

Crosby, T. and Wang, Y. (2021). Effects of different irrigation management practices on potato (solanum tuberosum l.). Sustainability, 13(18), 10187. https://doi.org/10.3390/su131810187

Dalla Costa, L., Delle Vedove, G., Gianquinto, G., Giovanardi, R., & Peressotti, A. (1997). Yield, water use efficiency and nitrogen uptake in potato: influence of drought stress. *Potato research*, *40*, 19-34.

Ekin, Z. (2019). Integrated use of humic acid and plant growth promoting rhizobacteria to ensure higher potato productivity in sustainable agriculture. Sustainability, 11(12), 3417. https://doi.org/10.3390/su11123417

Elkhatib, H. A., Gabr, S. M., Mohamed, R. A., & Manaa, A. (2019). Impact of potassium fertilization rates and potassium solubilizing bacteria inoculation on the growth, yield and quality of potato (Solanum Tuberosum L.). *Alexandria Science Exchange Journal*, *40* (JANUARY-MARCH), 127-142.

Elkholy, M. M., El-Tohamy, S. A., & Hafez, W. A. (2012). Retionalization of mineral potassium fertilizer by using biodesolving potassium and its effect on yield and quality potatos. *Journal of Soil Sciences and Agricultural Engineering*, *3*(12), 1275-1285.

Ghadamgahi, F., Tarighi, S., Taheri, P., Saripella, G., Anzalone, A., Kalyandurg, P., … & Vetukuri, R. (2022). Plant growth-promoting activity of pseudomonas aeruginosa fg106 and its ability to act as a biocontrol agent against potato, tomato and taro pathogens. Biology, 11(1), 140. https://doi.org/10.3390/biology11010140

Guo, J., Muhammad, H., Lv, X., Wei, T., Ren, X., Jia, H., … & Li, H. (2020). Prospects and applications of plant growth promoting rhizobacteria to mitigate soil metal contamination: a review. Chemosphere, 246, 125823. https://doi.org/10.1016/j.chemosphere.2020.125823

Guzmán-Guzmán, P., & Santoyo, G. (2022). Action mechanisms, biodiversity, and omics approaches in biocontrol and plant growth-promoting Pseudomonas: an updated review. *Biocontrol Science and Technology*, *32*(5), 527-550.

Hafez, E., Omara, A., & Ahmed, A. (2019). The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. Agronomy, 9(9), 524. https://doi.org/10.3390/agronomy9090524

Hamed, Hoda A, Yomna A Moustafa, and Shadia M Abdel-Aziz. 2011. 'In vivo efficacy of lactic acid bacteria in biological control against Fusarium oxysporum for protection of tomato plant', Life Science Journal, 8: 462-68

Hartz, T.K., Moore, F.D. Prediction of potato yield using temperature and insolation data. *American Potato Journal* 55, 431–436 (1978). https://doi.org/10.1007/BF02852146

Hossain, A., Hong, X., Ibrahim, E., Li, B., Sun, G., Meng, Y., … & An, Q. (2019). Green synthesis of silver nanoparticles with culture supernatant of a bacterium pseudomonas rhodesiae and their antibacterial activity against soft rot pathogen dickeya dadantii. Molecules, 24(12), 2303. https://doi.org/10.3390/molecules24122303

Jabnoun-Khiareddine, H., Aydi-Ben-Abdallah, R., & Daami-Remadi, M. (2023). Multi-species endophytic Bacillus for improved control of potato soilborne and tuber-borne diseases in Tunisia: from laboratory to field conditions. *Egyptian Journal of Biological Pest Control*, *33*(1), 109.

Jampílek, J., Kos, J., & Kráľová, K. (2019). Potential of nanomaterial applications in dietary supplements and foods for special medical purposes. Nanomaterials, 9(2), 296. https://doi.org/10.3390/nano9020296

Jun, Z., Zhan, X., Jiang, Y., & Xu, J. (2018). Variations in climatic suitability and planting regionalization for potato in northern china under climate change. Plos One, 13(9), e0203538. https://doi.org/10.1371/journal.pone.02035388

Kalozoumis, P., Savvas, D., Ntatsi, G., Marakis, G., Simou, E., Tampakaki, A., … & Karapanos, I. (2021). Impact of plant growth-promoting rhizobacteria inoculation and grafting on tolerance of tomato to combined water and nutrient stress assessed via metabolomics analysis. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.670236

Khan, S. T. (2022). Consortia-based microbial inoculants for sustaining agricultural activities. Applied Soil Ecology, 176, 104503. https://doi.org/10.1016/j.apsoil.2022.104503

Kloepper, J. W., Schroth, M. N., & Miller, T. D. (1980). Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology*, *70*(11), 1078-1082.

Koch, M., Naumann, M., Pawelzik, E., Gransee, A., & Thiel, H. (2019). The importance of nutrient management for potato production part i: plant nutrition and yield. Potato Research, 63(1), 97-119. https://doi.org/10.1007/s11540-019-09431-2

Kooman, P. L., Fahem, M., Tegera, P., & Haverkort, A. J. (1996). Effects of climate on different potato genotypes 2. Dry matter allocation and duration of the growth cycle. *European Journal of Agronomy*, *5*(3-4), 207-217.

Krishnan, H., Kang, B., Krishnan, A., Kim, K., & Kim, Y. (2007). rhizobium etliusda9032 engineered to produce a phenazine antibiotic inhibits the growth of fungal pathogens but is impaired in symbiotic performance. Applied and Environmental Microbiology, 73(1), 327-330. https://doi.org/10.1128/aem.02027-06

Lim, S. and Zainual, N. (2019). Isolation of potential fluorescent pseudomonads from kuini (mangifera odorata) planted soil and their potential as biofertilizer. Microbiology Research, 10(1). https://doi.org/10.4081/mr.2019.7844

Lin, L., Li, C., Ren, Z., Qin, Y., Wang, R., Wang, J., ... & Xiong, X. (2023). Transcriptome profiling of genes regulated by phosphate-solubilizing bacteria Bacillus megaterium P68 in potato (Solanum tuberosum L.). *Frontiers in Microbiology*, *14*, 1140752.

Liu, J., Zhang, J., Zhu, M., Wan, H., Chen, Z., Yang, N., ... & Liu, F. (2022). Effects of plant growth promoting rhizobacteria (PGPR) strain Bacillus licheniformis with biochar amendment on potato growth and water use efficiency under reduced irrigation regime. *Agronomy*, *12*(5), 1031. https://doi.org/10.3390/agronomy12051031

Liu, Q., Tarn, R., Lynch, D., & Skjodt, N. M. (2007). Physicochemical properties of dry matter and starch from potatoes grown in Canada. *Food Chemistry*, *105*(3), 897-907.

Lu, Z. H., Yada, R. Y., Liu, Q., Bizimungu, B., Murphy, A., De Koeyer, D., ... & Pinhero, R. G. (2011). Correlation of physicochemical and nutritional properties of dry matter and starch in potatoes grown in different locations. *Food Chemistry*, *126*(3), 1246-1253.

Lutz, Matthias Peter, Vincent Michel, Chloé Martinez, and Cédric Camps. 2012. 'Lactic acid bacteria as biocontrol agents of soil-borne pathogens', Biological Control of Fungal and Bacterial Plant Pathogens, 78: 285-88.

Lyu, D., Msimbira, L. A., Nazari, M., Antar, M., Pagé, A., Shah, A., … & Smith, D. L. (2021). The coevolution of plants and microbes underpins sustainable agriculture. Microorganisms, 9(5), 1036. https://doi.org/10.3390/microorganisms9051036

Lyu, D., Zajonc, J., Pagé, A., Tanney, C. A. S., Shah, A., Monjezi, N., … & Smith, D. L. (2021). Plant holobiont theory: the phytomicrobiome plays a central role in evolution and success. Microorganisms, 9(4), 675. https://doi.org/10.3390/microorganisms9040675

Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, H. W., & Makaju, S. (2021). Rhizosphere microbiome manipulation for sustainable crop production. *Current Plant Biology*, *27*, 100210.

Mamun, A. A., Neumann, G., Moradtalab, N., Ahmed, A., Dupuis, B., Darbon, G., ... & Weinmann, M. (2024). Microbial Consortia Versus Single-Strain Inoculants as Drought Stress Protectants in Potato Affected by the Form of N Supply. *Horticulturae*, *10*(1), 102.

Mccleary, B. V., Gibson, T. S., & Mugford, D. C. (1997). Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of AOAC International*, *80*(3), 571-579.

Mendis, H. C., Thomas, V. P., Schwientek, P., Salamzade, R., Chien, J. T., Waidyarathne, P., ... & De La Fuente, L. (2018). Strain-specific quantification of root colonization by plant growth promoting rhizobacteria Bacillus firmus I-1582 and Bacillus amyloliquefaciens QST713 in non-sterile soil and field conditions. *PLoS One*, *13*(2), e0193119.

Miransari, M., & Smith, D. L. (2014). Plant hormones and seed germination. *Environmental and experimental botany*, *99*, 110-121.

Mondal, S., Halder, S. K., Yadav, A. N., & Mondal, K. C. (2020). Microbial consortium with multifunctional plant growth-promoting attributes: future perspective in agriculture. Advances in Plant Microbiome and Sustainable Agriculture, 219-258. https://doi.org/10.1007/978-981-15-3204-7_10

Msimbira, L. A., Naamala, J., Antar, M., Subramanian, S., & Smith, D. L. (2022). Effect of microbial cell-free supernatants extracted from a range of ph levels on corn (zea mays l.) and tomato (solanum lycopersicum l.) seed germination and seedling growth. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.789335

Msimbira, Levini A., Judith Naamala, Sowmyalakshmi Subramanian, and Donald L. Smith. (2023). "Cell-Free Supernatant (CFS) from *Bacillus subtilis* EB2004S and *Lactobacillus helveticus* EL2006H Cultured at a Range of pH Levels Modulates Potato Plant Growth under Greenhouse Conditions" *International Journal of Molecular Sciences* 24, no. 7: 6620. https://doi.org/10.3390/ijms24076620

Naamala, J., Msimbira, L. A., Antar, M., Subramanian, S., & Smith, D. L. (2022). Cell-free supernatant obtained from a salt tolerant bacillus amyloliquefaciens strain enhances germination and radicle length under nacl stressed and optimal conditions. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.788939

Naamala, J., Msimbira, L. A., Subramanian, S., & Smith, D. L. (2023). Lactobacillus helveticus el2006h cell-free supernatant enhances growth variables in zea mays (maize), glycine max l. merill (soybean) and solanum tuberosum (potato) exposed to nacl stress. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1075633

Naqqash, T., Hameed, S., Imran, A., Hanif, M., Majeed, A., & Elsas, J. (2016). Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.00144

Nyiraneza, J., Cambouris, A. N., Nelson, A., Khakbazan, M., Mesbah, M., Perron, I., … & Lafond, J. (2021). Potato yield, net revenue and specific gravity responses to nitrogen fertilizer under different canadian agroecozones. Agronomy, 11(7), 1392. https://doi.org/10.3390/agronomy11071392

O'Callaghan, M., Ballard, R. A., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use and Management*, *38*(3), 1340-1369.

Ojeda, J. J., Rezaei, E. E., Kamali, B., McPhee, J., Meinke, H., Siebert, S., ... & Ewert, F. (2021). Impact of crop management and environment on the spatiotemporal variance of potato yield at regional scale. Field Crops Research, 270, 108213.

Ojeda, J. J., Rezaei, E. E., Remenyi, T. A., Webb, M. A., Webber, H. A., Kamali, B., ... & Meinke, H. (2020). Effects of soil-and climate data aggregation on simulated potato yield and irrigation water requirement. *Science of the Total Environment*, *710*, 135589.

Ojeda, J. J., Rezaei, E. E., Remenyi, T. A., Webber, H. A., Siebert, S., Meinke, H., ... & Ewert, F. (2021). Implications of data aggregation method on crop model outputs–The case of irrigated potato systems in Tasmania, Australia. *European Journal of Agronomy*, *126*, 126276.

Oswald, A., & Calvo, P. (2009). Using rhizobacteria to improve productivity of potato. *15th Triennial International Society for Tropical Root Crops (ISTRC), Lima*, *2*(6).

Overbeek, W., Jeanne, T., Hogue, R., & Smith, D. L. (2021). Effects of microbial consortia, applied as fertilizer coating, on soil and rhizosphere microbial communities and potato yield. Frontiers in Agronomy, 3. https://doi.org/10.3389/fagro.2021.714700

Raigond, P., Atkinson, F. S., Lal, M. K., Thakur, N., Singh, B., & Mishra, T. (2020). Potato carbohydrates. *Potato: Nutrition and Food Security*, 13-36.

Riaz, M., Akhtar, N., Msimbira, L. A., Antar, M., Ashraf, S., Khan, S. N., … & Smith, D. L. (2022). Neocosmospora rubicola, a stem rot disease in potato: characterization, distribution and management. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.953097

Riaz, M., Mahmood, R., Antar, M., Akhtar, N., Khan, S. N., Anjum, M. A., … & Smith, D. L. (2022). A bacterial consortium and synthetic fertilizer based biocontrol approach against potato rot disease "neocosmospora rubicola". Frontiers in Agronomy, 4. https://doi.org/10.3389/fagro.2022.828070

Riseh, R. S., Vazvani, M. G., & Kennedy, J. F. (2023). The application of chitosan as a carrier for fertilizer: A review. International Journal of Biological Macromolecules, 126483.

Romano, I., Ventorino, V., & Pepe, O. (2020). Effectiveness of plant beneficial microbes: overview of the methodological approaches for the assessment of root colonization and persistence. *Frontiers in plant Science*, *11*, 6.

Roquigny, R., Arseneault, T., Gadkar, V., Novinscak, A., Joly, D., & Filion, M. (2015). Complete genome sequence of biocontrol strain pseudomonas fluorescens lbum223. Genome Announcements, 3(3). https://doi.org/10.1128/genomea.00443-15

Rosyidah, A., & Handoko, R. N. S. (2020, March). Response of Potato (Solanum tuberosum) in Medium Plains to Antagonistic Microbes and Potassium Fertilizers. In *5th International Conference on Food, Agriculture and Natural Resources (FANRes 2019)* (pp. 107-113). Atlantis Press.

Saini, I., Kaushik, P., Al-Huqail, A. A., Khan, F., & Siddiqui, M. H. (2021). Effect of the diverse combinations of useful microbes and chemical fertilizers on important traits of potato. *Saudi Journal of Biological Sciences*, *28*(5), 2641-2648.

Saravanakumar, D., Thomas, A., & Banwarie, N. (2019). Antagonistic potential of lipopeptide producing Bacillus amyloliquefaciens against major vegetable pathogens. *European Journal of Plant Pathology*, *154*, 319-335.

Schippers, B., Bakker, A. W., & Bakker, P. A. (1987). Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual review of Phytopathology*, *25*(1), 339-358.

Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., … & Smith, D. L. (2021). Pgpr in agriculture: a sustainable approach to increasing climate change resilience. Frontiers in Sustainable Food Systems, 5. https://doi.org/10.3389/fsufs.2021.667546

Singh, D. (2020). Analysis of yield and technological gaps of potato production in bihar. Economic Affairs, 65(1). https://doi.org/10.30954/0424-2513.1.2020.7

SkZ, A., Vardharajula, S., & Vurukonda, S. S. K. P. (2018). Transcriptomic profiling of maize (zea mays l.) seedlings in response to pseudomonas putida stain fbkv2 inoculation under drought stress. Annals of Microbiology, 68(6), 331-349. https://doi.org/10.1007/s13213-018-1341-3

Smith, D. L., Praslickova, D., & Ilangumaran, G. (2015). Inter-organismal signaling and management of the phytomicrobiome. *Frontiers in plant science*, *6*, 722.

Tahir, Muhammad, et al. "Regulation of antioxidant production, ion uptake and productivity in potato (Solanum tuberosum L.) plant inoculated with growth promoting salt tolerant Bacillus strains." *Ecotoxicology and environmental safety* 178 (2019): 33-42.

Uysa, Aysel, and Faik Kantar. "Effect of Bacillus subtilis and Bacillus amyloliquefaciens culture on the growth and yield of off-season potato (Solanum tuberosum L.)." *Acta Agronómica* 69.1 (2020): 26-31.

Van Oort, P. A. J., Timmermans, B. G. H., Meinke, H., & Van Ittersum, M. K. (2012). Key weather extremes affecting potato production in The Netherlands. *European Journal of Agronomy*, *37*(1), 11-22.

Wang, Y., Ji, L., Zhang, C., Wang, L., Gao, W., & Jiang, J. (2020). Bacillus megaterium wl-3 lipopeptides collaborate against phytophthora infestans to control potato late blight and promote potato plant growth. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.01602

Wang, Z., Li, Y., Zhuang, L., Yu, Y., Liu, J., Zhang, L., … & Wang, Q. (2019). A rhizosphere-derived consortium of bacillus subtilis and trichoderma harzianum suppresses common scab of potato and increases yield. Computational and Structural Biotechnology Journal, 17, 645-653. https://doi.org/10.1016/j.csbj.2019.05.003

Wang, Zhenshuo, et al. "A rhizosphere-derived consortium of Bacillus subtilis and Trichoderma harzianum suppresses common scab of potato and increases yield." *Computational and structural biotechnology journal* 17 (2019): 645-653.

Weger, L., Vlugt, C., Wijfjes, A., Bakker, P., Schippers, B., & Lugtenberg, B. (1987). Flagella of a plant-growth-stimulating pseudomonas fluorescens strain are required for colonization of potato roots. Journal of Bacteriology, 169(6), 2769-2773. https://doi.org/10.1128/jb.169.6.2769-2773.1987

Western Potato Council (2003). Guide to commercial potato production on the Canadian prairies. Western Potato Council, Portage la Prairie, Manitoba.

Yaghoubian, I., Antar, M., Ghassemi, S., Modarres-Sanavy, S. A. M., & Smith, D. L. (2022). The effects of hydro-priming and colonization with piriformospora indica and azotobacter chroococcum on physio-biochemical traits, flavonolignans and fatty acids composition of milk thistle (silybum marianum) under saline conditions. Plants, 11(10), 1281. https://doi.org/10.3390/plants11101281

Yasmin, F., Othman, R., Sijam, K., & Saad, M. S. (2007). Effect of PGPR inoculation on growth and yield of sweet potato. *J. Biol. Sci*, *7*(2), 421-424.

Yin, C., Hagerty, C. H., & Paulitz, T. C. (2022). Synthetic microbial consortia derived from rhizosphere soil protect wheat against a soilborne fungal pathogen. *Frontiers in Microbiology*, *13*, 908981.

Yousef, A. F., Ali, A. M., Azab, M. A., Lamlom, S. F., & Al-Sayed, H. M. (2023). Improved plant yield of potato through exogenously applied potassium fertilizer sources and biofertilizer. *AMB Express*, *13*(1), 124.

Zamioudis, C., Mastranesti, P., Dhonukshe, P., Blilou, I., & Pieterse, C. (2013). Unraveling root developmental programs initiated by beneficialpseudomonasspp. bacteria. Plant Physiology, 162(1), 304-318. https://doi.org/10.1104/pp.112.212597

Zarzyńska, K., Trawczynski, C., & Pietraszko, M. (2023). Environmental and agronomical factors limiting differences in potato yielding between organic and conventional production system. Agriculture, 13(4), 901. https://doi.org/10.3390/agriculture13040901

## Connecting statement between chapters 3 and 4

Chapter 4 addresses the third objective of this study, which is to evaluate the response of potato plants, grown under controlled environment (greenhouse) conditions, to microbe-coated fertilizers (MCF) and various levels of water-deficit stress. The controlled environment experiments were similar to field experiments in terms of MCF treatments, plant growth stages and most of the studied variables. The purpose of this was to validate the efficiency of the MCF, which had already been investigated under open-field conditions, and develop a sense of the role of abiotic stress in the effectiveness of the microbes delivered as MCF. The experiments produced plants in fully irrigated and water-stressed conditions using only one dose (1x) of MCF. Water was withheld to introduce water-deficit stress treatments once the plant population was established. Throughout the experiments, data on growth variables at two phenological stages (vegetative and flowering), physiological variables (e.g. photosynthetic rate, stomatal conductance, plant greenness-SPAD), plant tissue nitrogen content, harvested potato tuber yield and total starch content were measured to determine the role of MCF treatments in improving potato plant tolerance to mild and severe water-deficit stress conditions, compared to fully irrigated and non-inoculated control plants.

Mohammed Antar conducted experiments - the experimental design, MCF preparation and application, seeding, data collection and statistical analysis, interpretation of results, and writing the initial draft of this chapter, which was then reviewed and edited by Professors Donald Smith and Philippe Seguin. The data on agronomic variables, yield and plant greenness-SPAD were used as a case study in the Experimental Design course, and the statistical analysis was a result of these considerations and statistics supervisory input from Professor Pierre Dutilleul from the Department of Plant Science.

# Chapter 4 : Evaluation of two microbe-coated fertilizers and assessment of potential interactions with water on growth of potato under greenhouse conditions

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**Abstract**

Microbe-coated fertilizer is an efficient and sustainable approach to reducing the excessive use of synthetic fertilizers, improving potato plant growth and development under water-deficit stress. This study is unique and is the first to report a novel technology for the application of microbial inoculations under greenhouse conditions. This study assessed the performance of two novel microbial consortia coated on NPK fertilizer granules, which also showed significant effects on potato plant growth and yield under field conditions. The overall goal was to evaluate the role of synthetic fertilizer (NPK) and microbe-coated fertilizer containing *Bacillus, Lactobacillus* and *Pseudomonas* strains in reducing the potential negative impacts of mild and severe water-deficit stress on potato plants. We designed the experiments following a Completely Randomized Design (CRD) with three microbial treatments: control (Starter NPK fertilizer), EVL Coating® (NPK + a consortium of *Bacillus*, *Lactobacillus*, and *Pseudomonas* strains), Era Boost microbes (NPK + a consortium of five *Bacillus* strains), and three levels of water-deficit stress treatment (fully irrigated, 25% water withhold, and 50% water withhold). At two phenological growth stages (vegetative and flowering), we collected data on agronomic variables including plant height, leaf area, shoot fresh and dry weight, root length, and root fresh and dry weight. We also collected

agro-physiological data on leaf greenness with SPAD, photosynthetic rate and stomatal conductance using a portable LiCor instrument.

The MCF showed positive effects on most of the studied above and below-ground agronomic variables (plant height, leaf area and shoot fresh weight, root length and root dry weight), leaf greenness (SPAD), and most importantly total tuber yield, in at least one experiment. However, variables such as shoot dry weight, root fresh weight, and total nitrogen in plant tissues responded inconsistently to the MCFs. Physiological variables, including photosynthetic rate, stomatal conductance and total starch content, did not respond to the MCF treatments in both experiments. Visual observations of numerical values in most of the studied variables show a better performance of NPK+EVL than NPK+EB under the greenhouse conditions used, particularly for leaf area, shoot fresh weight, photosynthetic rate, nitrogen content, tuber yield and starch content. In conclusion, this study evaluated the responses of all the variables to the MCF under mild and severe water-deficit stress conditions without investigating the mechanisms of responses. Therefore, further research is essential to understand the effect of MCFs and the findings reported in this study to clarify the mechanism behind the growth-promotion and enhanced stress tolerance in potato plants. Looking at the microbial community in the potato plant rhizosphere and analyzing root exudates in stressed plants could increase understanding and help fill the knowledge gap.

### 4.1. Introduction

Millions of people consume potato tubers for carbohydrates and nutritional composition such as fiber, vitamins, and minerals. Besides being a valuable food source, the potato crop is an important part of the global agro-economy, agricultural producers, and agricultural industries, providing income and employment opportunities. For example, growers across Canada produced approximately 5.7 million (out of the globe’s 376 million) tonnes of potatoes in 2021. Canada’s potato production is geographically widespread, spanning from the west coast of the country to the east, making it genuinely national in scope. The distribution of production regionally is 36% in Atlantic Canada, 22% in Central Canada, and 42% in the Prairie Provinces and British Columbia. According to the Potato Market Information Review, as reported by the Crop and Horticulture Division of Agriculture and Agri-Food Canada in December 2022, the provinces that produced the most by volume were Prince Edward Island (23%), Manitoba (20%), Alberta (20%), New Brunswick (15%), Quebec (13%), and Ontario (7%).

The balance between global population projection and stable food supply requires, over the longer-term, the development of more sustainable agriculture. However, yield gaps and reductions because of poor agricultural practices, soil disturbance and degradations, excessive use of chemical inputs, and various abiotic and biotic stresses, including those linked to climate change, continuously influence agricultural output (Antar et al., 2021; Shah et al., 2021). One of the primary aims of contemporary agriculture is to minimize the environmental footprint while achieving consistent and superior crop yields with improved quality. In order to maintain crop performance while reducing the reliance on external inputs, it is crucial to enhance nutrient availability and nutrient use efficiency in sustainable plant production (Petrushin et al., 2024). Therefore, evaluating the potential for sustainable agricultural practices for the globe's fourth-largest crop is essential.

As a hot spot for plant and microbe interactions, the crop rhizosphere has a high microbial activity, in part because of root exudates attracting beneficial microorganisms and establishing orchestrated microbial communities, favouring nitrogen fixation, phytohormone production, microbe-to-plant signal production and nutrient solubilization for plant uptake (Dashti et al., 2000; Zhang and Smith, 2002; Gray et al., 2006; Miransari and Smith, 2014). The PGPR represent a large subgroup of microorganisms that colonize rhizospheres and promote plant development through direct and indirect mechanisms. The utilization of rhizospheric bacterial strains as growth-promotion agents is a promising approach to growing food crops sustainably, considering the worldwide demand for agricultural products and the negative consequences of the uncontrolled application of agrochemicals in modern agriculture (Ahluwalia et al., 2021; Antar et al., 2021).

Potato plants require significant water and nutrients, including nitrogen, phosphorous, and potassium, for healthy growth and to manage biotic and abiotic stresses. The shallow root system of the potato plant makes it highly susceptible to water stress at all stages of development, from emergence to tuber bulking (Koch et al., 2019; Naumann and Pawelzik, 2023; Demirel, 2023). The plant cannot always absorb enough water from the soil to support its needs, which leads to stomatal closure, reduced photosynthesis, and impaired leaf development (Akinci and Lösel, 2012; Bhattacharya, 2021). Ultimately, water stress decreases overall above-ground plant growth, reducing tuber yield below-ground (Al-Mahmud, 2014; Wagg et al., 2021).

PGPR-based microbial consortia are promising to mitigate water stress by establishing a symbiotic relationship with the plants. A previous study found that inoculating potato plants with a *Bacillus subtilis* strain increases tuber yield and soluble sugar by 15 and 32% under moderate water stress, and 17 and 25% under severe water stress (Batool et al., 2020). The increased yield and quality might result from the microbial nutrient solubilization and biosynthesis of phytohormones and/or signals not only for root growth stimulation but also for photosynthesis regulation through the opening and closure of stomata. Another study reported that some PGPR can also regulate water use efficiency in plants under water stress by regulating stomatal conductance. For example, *Bacillus subtilis* reduced stomatal conductance by 30% and transpiration rates by 34%, which decreases water loss and improves plant water use efficiency under water stress (de Lima et al., 2019). This is probably because some PGPR can produce abscisic acid, a stress hormone that regulates stomatal closure and water use efficiency (Cohen et al., 2015), and because they produce microbe-to-plant signals that enhance crop plant stress tolerance (Mabood et al., 2014; Smith et al., 2017). Evidence has shown that the PGPR *Pseudomonas* spp. produces indole-3-acetic acid, which increases lateral root primordium by 66% and root hair length by 150% in the model plant *Arabidopsis* (Zamioudis et al., 2013). This improvement might allow more water uptake and improve the plant's ability to tolerate water stress. It has been reported that *Lactobacillus* has proven efficient as a biocontrol agent, demonstrating efficacy in controlling several fungal and bacterial phytopathogens. *Lactobacillus* strains, functioning as biostimulants, have shown the ability to enhance plant growth and seed germination and to mitigate the effects of abiotic stresses (Lamont et al., 2017; Raman et al., 2022). Therefore, PGPR have the potential to help plants cope with water-deficit stress through a range of mechanisms.

This study evaluated the effectiveness of two commercially available PGPR consortia, namely EVL Coating® (a consortium of *Bacillus*, *Lactobacillus* and *Pseudomonas* strains) and Era Boost microbes (a consortium of five *Bacillus* strains) on potato plants exposed to mild and severe water-deficit stresses. We hypothesized that (i) microbe-coated fertilizers (MCF) reduce water-deficit stress (WS) effects on potato grown under mild and severe water-deficit stress and (ii) microbe-coated fertilizers increase crop yield.

## 4.2. Materials and Methods

### 4.2.1. Site description

The experiments were conducted in the research greenhouse of the Macdonald Campus of McGill University (45°24’30”N 73°56’23”W) in Sainte-Anne-de-Bellevue, Quebec, Canada (Appendix 4-1).

Potato (*Solanum tuberosum* cv Goldrush) was grown under controlled environmental conditions in the greenhouse from April to August 2019 and from February to June 2021. We planned the second trial for April 2020, but the COVID-19 pandemic caused a delay.

Potato plants were exposed to a photosynthetic irradiance of 500–600 μE $m^{-2}$ $s^{-1}$, 65-70% relative humidity, and a 16/8 h (light/dark) photoperiod at 22 ± 1 °C (day)/18 ± 1 °C (night) while being grown in 24 L pots filled with G10 Agro Mix® (Fafard Inc., Quebec, Canada) soilless media.

*Table 4-1: Greenhouse potato growing medium G10 Agro Mix® specification.*

| *Medium* | *% N* | *% P2O5* | *% K2O* | *% Ca* | *% Mg* | *% S* | *% OM* | *% Moisture* |
|---|---|---|---|---|---|---|---|---|
| ***G10 Agro Mix®*** | 0.4 | 0.03 | 0.07 | 0.7 | 0.03 | 0.02 | 55 | 31 |

### 4.2.2. Plant Materials: Microbial Consortia Specifications

Potato (*Solanum tuberosum* L., Quality Seeds SEQ Marketing, Quebec City, Canada) Goldrush variety was chosen to evaluate two microbe-coated fertilizers under controlled environment (greenhouse) conditions. This variety has smooth, uniform tubers with minimal defects and excellent resistance to hollow heart, good resistance to common scab and moderate resistant to *Verticillium* wilt. It has good storability and a medium dormancy period. It is ideal for baking, boiling, French frying, and home and restaurant use because of its white flesh, texture, and flavour (Canadian Food Inspection Agency, 2013).

### 4.2.3. Experimental Materials: Synthetic Starter Fertilizer, EVL Coating® and Era Boost Consortia Specifications

EVL Coating ® (EVL) and Era Boost ® (EB) microbial consortia were used as treatments to prepare microbe-coated fertilizers. EVL Coating® is a microbial consortium commercialized by EVL Inc. (Saint-Hyacinthe, Quebec, Canada), which contains four bacterial strains (two *Bacillus*, one *Lactobacillus*, one *Pseudomonas*) and a fungal strain (*Saccharomyces*). EB is a microbial consortium commercialized by Ulysse Biotech (Trois-Rivières, Quebec, Canada), which contains five different *Bacillus* strains (Table 4-2). Both consortia were provided as ready-to-use products containing supplier-provided bioactive fermentation products.

Synthetic NPK (10.9-15.2-16.3) fertilizer was provided by SynAgri (Saint-Hyacinthe, Quebec, Canada) as a control fertilizer and carrier of the EVL and EB microbial consortia. Both microbial consortia were superficially spray-coated onto fertilizer granules in an industrial mixer at the manufacturer's recommended rate (Table 4-2). Then, microbe-coated fertilizer was air-dried and applied to the pots within 24 h. Synthetic NPK fertilizer without microbial coating was the control. We applied all treatments simultaneously and banded them into the experimental pots as a starter fertilizer at seeding. Each experimental pot received 30 g of microbe-coated fertilizer (MCF) based on a conversion of the recommended rate of fertilizer (kg $ha^{-1}$) under field conditions.

*Table 4-2: Summary of the experimental material and fertilization rate of the investigated products*

| Treatments | Description | Active substance | Rate per pot |
|---|---|---|---|
| **Control** | NPK Fertilizer (no microbes) | N = 100 kg N $ha^{-1}$<br>$P_2O_5$ = 140 kg $P_2O_5$ $ha^{-1}$<br>$K_2O$ = 150 kg $K_2O$ $ha^{-1}$ | 30 g $pot^{-1}$ |
| **NPK+EVL** | EVL Coating® (Biostimulants) | *Bacillus amyloliquefaciens*<br>*Bacillus subtitles*<br>*Lactobacillus helveticus*<br>*Pseudomonas putida*<br>*Saccharomyces cerevisiae* | 2 L $t^{-1}$ $ha^{-1}$<br>($7 \times 10^8$ cfu $mL^{-1}$)<br>700 million viable cells $mL^{-1}$ |

| | | | |
|---|---|---|---|
| **NPK+EB** | Era Boost Pro (Ulysse Biotech biostimulants) | *Bacillus licheniformis* U35<br>*Bacillus megaterium* U48<br>*Bacillus megaterium* U49<br>*Bacillus velezensis* U47<br>*Bacillus velezensis* U50 | 3.5 L $t^{-1}$ $ha^{-1}$<br>($4 \times 10^8$ cfu $g^{-1}$)<br>400 million viable cells $g^{-1}$ |

### 4.2.4. Experimental Setup and Design

In 2019 and 2021, we organized the treatments in the greenhouse following a completely randomized design (CRD), with each treatment comprising five replicates for each growth stage (vegetative, flowering, and harvest (full tuberization)). Under greenhouse conditions, the efficiency of the two microbe-coated fertilizers was evaluated under two levels of water-deficit stress (WS). The mild and severe water-deficit stress levels were introduced 15 days after establishing the plant population (Table 4-3). The water-deficit stress treatments (water with hold) were monitored using a Soil Moisture Equipment Corp. gauge (Jet Fill Tensiometer, Model 2725, Santa Barbara, California, USA). The stimulation effects were determined based on data collected on plant growth, physiology, and yield. The collected data helped evaluate the plant water-deficit stress tolerance levels (i.e., no stress, mild stress, and severe stress) in response to the microbe-coated fertilizers (MCF).

*Table 4-3: Summary of the treatments and their times of application*

| Treatments | Description | Application |
|---|---|---|
| **Fertilizer MCF** | Control (NPK only) | *Applied at sowing potato seed tubers* |
| | NPK + EVL microbes | |
| | NPK + EB microbes | |
| **Water-deficit stress (WS)** | No stress (Full Irrigation) | *WS Applied once the population was established for nearly 15 days post-vegetative stage* |
| | Mild stress (25% water-hold) | |
| | Mild stress (50% water-hold) | |

### 4.2.5. Treatments, Sampling and Data Collection

In each experiment, pots were arranged in a completely randomized design (CRD) with two microbe-coated fertilizers, EVL, EB, and uncoated NPK fertilizer as the control. Treatments were replicated (n = 5) in five pots. Immediately before planting the potato, microbe-coated fertilizer was added as a starter fertilizer in the pot at a depth of 10 cm. The potato was planted by hand, 5 cm below the soil surface. All pots received 30 g of 100 kg N $ha^{-1}$, 140 kg $P_2O_5$ $ha^{-1}$ and 150 kg $K_2O$ $ha^{-1}$ as synthetic NPK starter fertilizer. In addition, pots receiving EVL had about $7\times10^8$ CFU $mL^{-1}$ of *Bacillus*, *Lactobacillus* and *Pseudomonas* strains, while the EB had approximately $4\times10^8$ CFU $g^{-1}$ of the five *Bacillus* strains.

Data on the number of emerging seed tubers was collected daily until the full stand was established. Five pots were sampled per treatment at two phenological stages: vegetative and flowering. Values were recorded for agronomic variables, including plant height, number of branches, leaf area (using an LI-300 area meter - LI-COR Inc, Lincoln, Nebraska, USA), shoot and root fresh and dry weights, and root length. Then, the plant shoots were oven-dried at 60 $^oC$ for 2-3 days for biomass determination, total nitrogen, and ash content analysis. Data were also collected on plant physiology variables using a LI-COR 6400 Portable Photosynthesis Meter for photosynthetic rate and stomatal conductance determinations on leaves and a SPAD meter for plant leaf greenness determination from an average of 5 leaves.

The potato tubers were harvested at the end of the season when all the plants reached maturity. At this stage, the plant loses its leaves, tuber growth ceases, and the shoots turn yellow and then die. Data was collected from five pots and assessed for yield components. All the tubers harvested from each plant were grouped into marketable size (3-6 cm diameter), below marketable size (< 3 cm diameter) and above marketable size (> 6 cm diameter) ranges. Data were recorded on the total number of tubers per plant, weight, and grading sizes. For total yield, data were collected on the weight of marketable size tubers, plus the weight of tubers greater and lower than the marketable size.

### 4.2.6. Potato Plant Tissues and Tuber Analysis

Total Nitrogen (N): Potato above-ground plant tissue was collected from five pots per treatment at two phenological stages, vegetative and flowering. Plant tissues were oven-dried at 50 °C for 48 h and then ground for 2-3 min using a 1-mm sieve grinder (Model 4 Wiley Mill, Thomas Scientific - New Jersey USA). After grinding, 60-70 mg of each sample were weighed and encapsulated in a tin container (Elemental Microanalysis, Isomass Scientific Inc. - Alberta, Canada) for the total N analysis using an elemental analyzer (FLASH 2000 Series Organic Elemental Analyzers, Thermo Scientific - Milan, Italy).

Estimating total starch in potato tubers: After harvesting potatoes, 3 tubers were randomly selected from each pot, washed, peeled, and cut into ~2-3 cm slices for a total of 10 g (Velásquez-Herrera et al., 2017). The prepared slices were immediately freeze-dried to be used to estimate the total starch. The total starch content in potato tubers was estimated using a total starch assay kit (K-TSTA, Megazyme, Wicklow, Ireland) following AOAC method 991.11 (2019). The estimated starch content was expressed as total starch percentage and calculated following the method described in Mccleary et al. (1997).

### 4.2.7. Statistical Analysis

The data collected from two experiments was subjected to a normal distribution determination before performing the analysis of variance (ANOVA) with PROC GLM using SAS statistical software, Version 9.4 of the SAS System for Windows (SAS Institute, Inc, Cary, NC, USA) to determine the main effects of the treatments. Through a two-way ANOVA, means of microbe-coated fertilizers (control NPK, NPK+EB and NPK+EVL) and water-deficit stress (no stress, mild stress and severe stress) treatments were calculated to determine statistical significances of both factors on response variables, above-ground (plant height, leaf area, shoot fresh and dry weights), below ground (root length, root fresh and dry weights), physiological variables (photosynthetic rate, stomatal conductance, leaf greenness-SPAD), plant tissue nitrogen content, tuber weight and total starch content.

We used the Tukey's Honest Significant Differences (HSD) method for multiple comparison tests to determine whether any differences between the control and other treatments were statistically significant at $P < 0.05$. The values in the results section are the means $\pm$ standard error of the studied treatments. We generated graphs of mean values and results tables using Microsoft Excel (Microsoft 365 for Enterprise Version 2401).

## 4.3. Results

Microbial inoculations can potentially contribute to plant growth and development by improving nutrient solubilization, phytohormone production, and signal compounds, leading to better nutrient availability for easy uptake, improved shoot and root biomass because of growth regulation with hormones and enhanced tolerance of unfavourable abiotic stresses, including water deficit.

The use of synthetic fertilizers at various rates has been widely studied to optimize potato plant production. However, concerns about their environmental impact and low nutrient use efficiency have led to research into alternative fertilization methods. Microbial fertilizers have shown promising results in increasing soil fertility, improving plant growth, and enhancing productivity.

In our previous study, under field conditions, we demonstrated the efficiency of the unique microbe-coated fertilizer technology in promoting potato growth and yield, with specific doses showing optimal results. Integrating microbial consortia and blended synthetic (NPK) fertilizers has shown potential for significantly enhancing potato productivity, especially under stressful conditions.

In this study, we present the results of two previously field-tested novel microbe-coated fertilizers evaluated for ability to enhance potato plant above-ground agronomic variables (plant height, leaf area, shoot fresh and dry weights), below-ground variables (root length, root fresh and dry weight), physiological variables (photosynthetic rate, stomatal conductance, leaf greenness-SPAD), plant tissue nitrogen content, tuber yield and total starch content in response to water-deficit stress.

Most of the seven growth variables, three physiological variables, nitrogen content, total starch and yield that were measured in this study responded to the WS treatment at least at one growth stage. One exception to this was root length, which did not respond consistently to either WS treatment across experimental repetitions. Statistically significant responses of variables to the applied treatments are summarized in Table 4-4.

While almost all variables responded to the WS, few responded consistently to the fertilizer treatment. Only leaf area, root length, and leaf greenness (SPAD) consistently responded to the MCF treatments at the vegetative stage (VS) of experiment one and one of the growth stages in experiment 2. These are the only variables for which our first hypothesis was confirmed. Overall, far fewer variables responded significantly to the MCF treatments in the second experiment/year, showing an inconsistency in the experiment between the two years. This was the main reason for performing statistical analysis separately for each experiment/year.

Statistically significant interactions in the two-way ANOVA models were observed only for plant height (PH) in the first experiment, leaf area (LA) in the second experiment, root length (RL) in the vegetative stages of both years, and leaf greenness (SPAD readings) across both experiments and growth stages, except at the vegetative stage (VS) in experiment two. For statistical analysis of the data generated in these experiments, we used the Tukey test to determine if significant main effects or interactions, through the ANOVA model, were detected based on data from the specific treatments.

*Table 4-4: Summary of statistically significant effects of treatments on measured variables as determined by ANOVA. An **NS** indicates **p > 0.05**, ***** indicates **p < 0.05**, ****** indicates **p < 0.01**, ******* indicates **p < 0.001**. **PH** = plant height; **LA** = leaf area; **SFW** = shoot fresh weight; **SDW** = shoot dry weight; **RL** = root length; **RFW** = root fresh weight; **RDW** = root dry weight; **SPAD** = leaf greenness; **PR** = photosynthetic rate; **SC** = stomatal conductance; **%N** = plant tissue nitrogen content; **TY** = tuber yield; **%S** = total starch in potato tubers; **VS** = Vegetative Stage; **FS** = Flowering Stage*

| *Year* | *Factors* | *PH* | *LA* | *SFW* | *SDW* | *RL* | *RFW* | *RDW* | *SPAD* | *PR* | *SC* | *%N* | *TY* | *%S* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Experiment 1 - VS* | WS | *** | *** | *** | *** | ns | *** | *** | *** | *** | *** | *** | | |
| | MCF | ns | ** | * | *** | *** | ns | *** | *** | ns | ns | * | | |
| | Interaction WS x MCF | * | ns | ns | ns | ** | ns | ns | *** | ns | ns | ns | | |
| *Experiment 1 – FS and Yield* | WS | *** | *** | *** | *** | ns | *** | *** | *** | *** | *** | *** | *** | *** |
| | MCF | * | ** | ** | ns | *** | ns | ns | *** | ns | ns | ns | *** | ns |
| | Interaction WS x MCF | ns | ns | ns | ns | ns | ns | ns | *** | ns | ns | ns | ns | ns |
| *Experiment 2 - VS* | WS | ** | *** | ** | *** | ns | ns | *** | ns | *** | *** | *** | | |
| | MCF | ** | ns | ns | ns | ** | ns | ns | * | ns | ns | ns | | |
| | Interaction WS x MCF | ns | ** | ns | ns | * | ns | ns | ns | ns | ns | ns | | |
| *Experiment 2 - FS and Yield* | WS | *** | *** | * | *** | * | ** | ns | *** | *** | *** | *** | *** | *** |
| | MCF | ns | * | ns | ns | ns | * | ns | ns | ns | ns | ns | *** | ns |
| | Interaction WS x MCF | ns | *** | ns | ns | ns | ns | ns | *** | ns | ns | ns | ns | ns |

### 4.3.1. Effect of Microbe-coated fertilizers on above-ground variables: Plant Height, Leaf Area, Shoot Fresh Weight and Dry Weight

During the two experiments, in each of two years, data on plant height (PH), leaf area (LA), shoot fresh (SFW) and shoot dry (SDW) weights were collected at the vegetative (VS) and flowering stages (FS) to evaluate the capacity of two the studied microbe-coated fertilizers (MCF) with regard to their effectiveness in improving these variables under mild and severe water-deficit stress (WS). As we predicted in our first hypothesis, there was a clear effect and increases in growth variables at the vegetative (VS) and flowering (FS) stages in both experiments and growth stages, but not always with statistically significant differences between treatments (Table 4-5, Appendix 4-2 and 4-3).

In experiment 1, WS had a significant effect on all above-ground variables, while the MCF treatments showed the same tendency for all variables except for the PH at VS and SDW at the FS. In Experiment 2, WS treatment resulted in the same trend and significantly affected all the studied variables, whereas the MCF treatments were only significant for the PH at the VS and LA at the FS (Table 5). In terms of the interaction effects of WS and MCF treatments, PH at the VS in experiment 1, LA at the VS, and FS in experiment 2 showed significance (Table 4-5).

#### 4.3.1.1. Effect of MCF on Plant Height (PH)

Plant height is one of the critical variables for potato plant growth and development. In this study, the response of potato PH to the MCF treatments varied. In experiment 1, plants were almost the same height at the VS, leading to non-significant differences between treatments. At the FS, there were significant differences between MCF treatments in that plants inoculated with MCF treatments (NPK+EB: 97 cm and NPK+EVL: 95 cm) were taller than control plants (91 cm) grown under only NPK treatment (Table 4-3). In experiment 2, the trend was different in that plants at the VS were taller when the had received MCF treatments (NPK+EB: 68 cm and NPK+EVL: 66 cm) than the control treatment, which was 62 cm (Table 4-5, Figure 4-1).

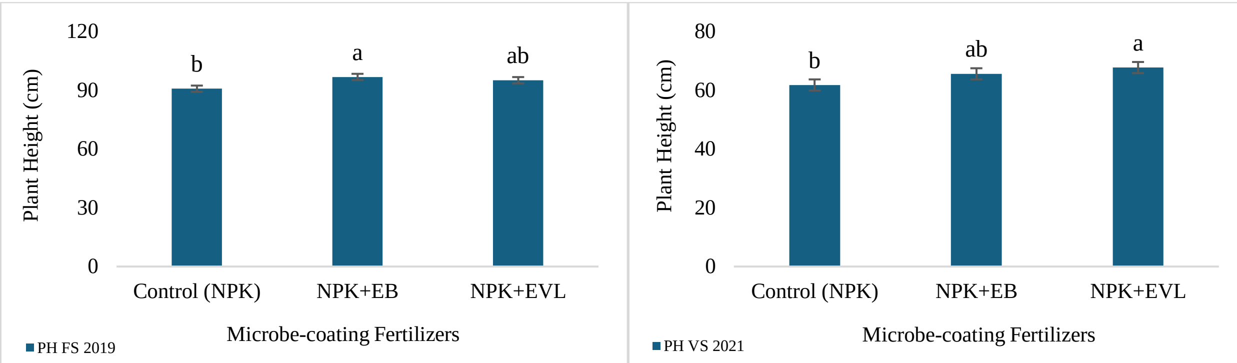



*Figure 4-1: Response of potato plant height (PH) to the microbe-coated fertilizer treatments at the flowering stage (FS) in experiment 1 (2019) and at the vegetative stage (VS) in experiment 2 (2021).*

Data analysis in this study showed a significant interaction effect of MCF treatments and WS ($p = 0.0219$) on potato PH at the VS (Table 4-5, Figure 4-2). The significant interaction effect reduced the PH in mild and severe water-stressed plants (56 and 53 cm, respectively) compared to non-stressed plants (63 cm) (Table 4-5). Non-stressed control plants were taller than those of other treatments within the same group or under mild and severe WS with or without MCF treatments. These results indicate that MCF treatments did not diminish the adverse effects of WS on the PH during the earlier stages of potato plant growth.

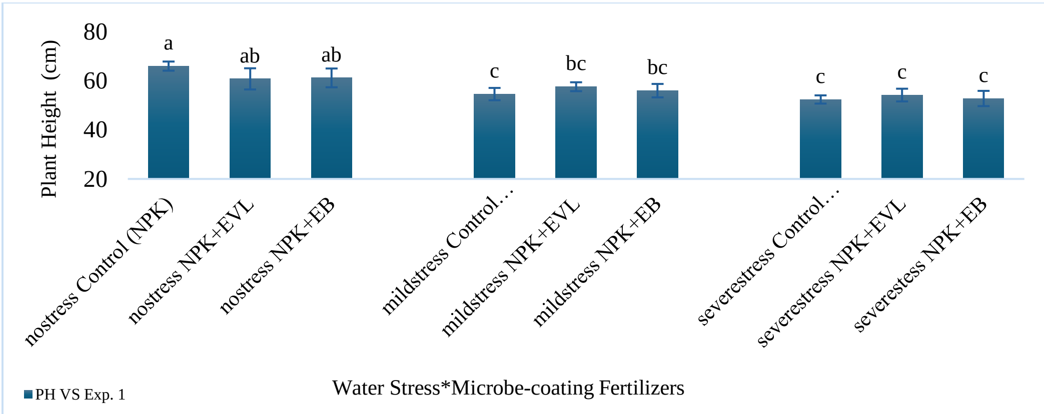



*Figure 4-2: The interaction effects of water-deficit stress and microbe-coated fertilizer treatments on potato plant height at the vegetative stage in experiment 1. Bars are the mean values (cm) of each treatment, and error bars are the standard deviation of the means. Dissimilar letters indicate significant differences (p = <0.05, Tukey HSD).*

#### 4.3.1.2. Effect of MCF on Leaf Area (LA)

Plant leaf area is an important variable for plant growth, determining the capacity of plants to intercept sunlight and balance water and gas exchange for vigorous plant development. This variable is related to total photosynthesis, along with stomatal conductance and even plant greenness, which are presented in subsection (3.3.3).

In this study, the response of potato LA to the MCF treatments was positive at the VS ($p$ = 0.035) and the FS ($p$ = 0.003) despite the significant effects of water-deficit stress at both stages ($p < 0.0001$). The increase in response to the MCF treatments was higher at the FS than at VS in the first experiment. At both stages, both MCF-treated plants had greater LA than the control, but NPK+EVL was the best with 5798 $cm^2$ at the VS and 6859 $cm^2$ at the FS, while the control plants were 5490 and 6345 $cm^2$, respectively (Table 4-5).

In experiment 2, LA significantly responded to the MCF treatments in the FS, in which NPK+EB and NPK+EVL were 12557 $cm^2$ at the VS and 11824 $cm^2$ at FS, while the LA for control plants was 11307 $cm^2$ (Table 4-5, Figure 4-3).

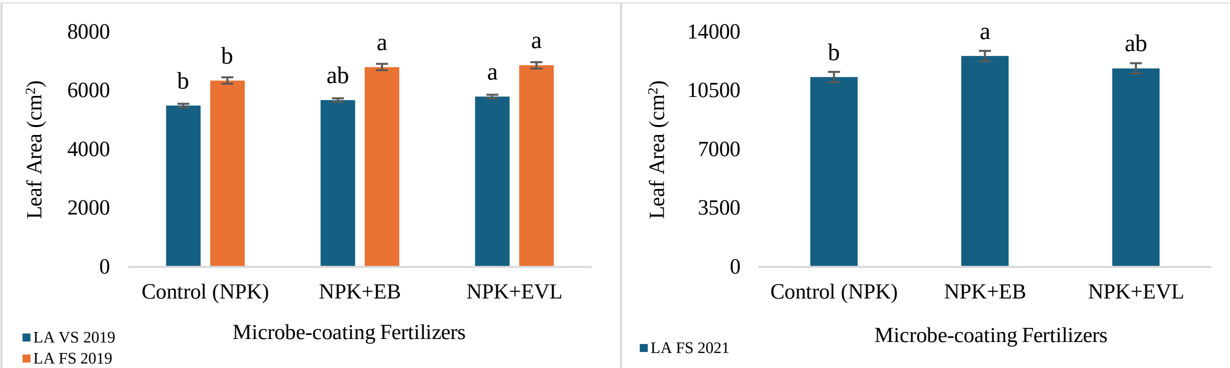



*Figure 4-3: Response of leaf area (LA) to the microbe-coated fertilizer treatments at the vegetative (VS) and flowering (FS) stages in experiment 1 (2019) and at the flowering stage (FS) in experiment 2 (2021).*

Data analysis in this study showed significant interactive effects of MCF treatments and WS at the VS ($p$ = 0.0139) and FS ($p$ = 0.0008) on potato LA only in the second experiment (Table 4-4). While the two-way ANOVA model showed a significant interaction between WS and MCF factors for the LA, the only significant difference across both treatments was the lower LA under mild stress for plants treated with NPK+EB in the VS. Other than this, the different MCF treatments at the same WS treatment level showed no statistically significant differences between themselves and the control (Figure 4-4).

At the FS, the trend is the same, and the different MCF treatments at the same WS treatment level showed no statistically significant differences. However, mild stresses and NPK+EB-treated plants differed significantly from the non-stressed and severely stressed plants (Figure 4-4). This showed the efficiency of MCF treatments, particularly NPK+EB, in enhancing plant growth and mitigating the mild WS effects on potato LA. However, we reject the hypothesis that the MCF treatments reduce the effects of WS for this variable because of the absence of statistical differences between the MCF treatments and the control within the same WS level.

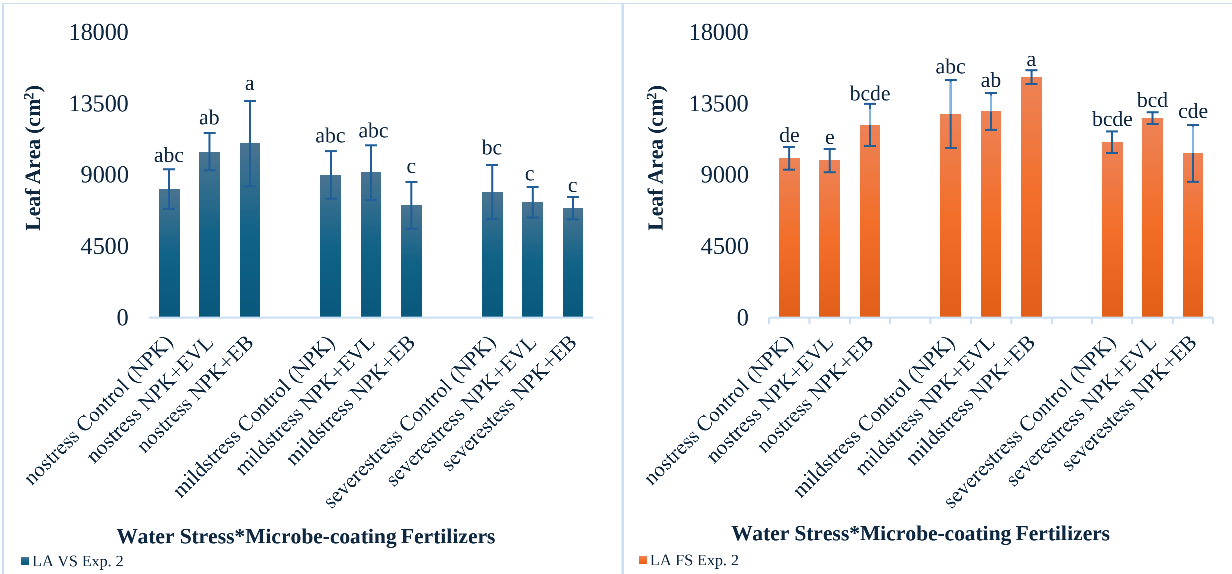



*Figure 4-4: The interaction effects of water-deficit stress and microbe-coated fertilizer treatments on potato leaf area at the vegetative and flowering stages in experiment 2. Bars are the mean values (cm) of each treatment, and error bars are the standard deviation of the means. Dissimilar letters indicate significant differences (p = <0.05, Tukey HSD).*

4.3.1.3. Effect of MCF on Shoot Fresh and Dry Weights

Healthy and vigorous plant shoot growth are crucial aspects of plant development and productivity. Several factors contribute to healthy shoot growth in plants, including environmental conditions. Key determinants of healthy shoot growth are light, temperature, water accessibility and the availability of essential nutrients, which significantly impact shoot growth.

In this study, we collected shoot growth data for each factor at two phenological stages in two experiments by measuring SFW before oven-drying shoots to determine dry biomass as SDW. An increase in SFW and SDW shows healthy growth and plant development in response to the MCF treatments.

In experiment 1, MCF-treated plants showed significant increases in SWF and SDW at both VS and FS ($p$ = 0.024 and $p$ = 0.0021, respectively) despite the significant water-deficit stress effects ($p$ = < 0.0001). The plants treated with NPK+EB and NPK+EVL recorded greater SFW at the VS (381and 382 g, respectively) and the FS (778 and 790 g, respectively) compared to the control plants, which weighed 355 g at the VS and 747 g at the FS. The pattern was similar for the SDW at the VS, in which SDW was significantly higher for MCF-treated plants (Table 4-5, Figure 4-5).

In experiment 2, there was no significant difference between any of the MCF treatments or significant interactions regarding SFW and SDW, despite the significant effects of water-deficit stress on both variables. However, there was a numerical increase in SFW ($p$ = 0.5871) and SDW ($p$ = 0.215) at the VS without statistical significance (Table 4-5).

Data analysis for the two phenological stages in both experiments showed no interactive effects of the MCF treatments and water-deficit stress on potato SFW and SDW (Table 4-5).

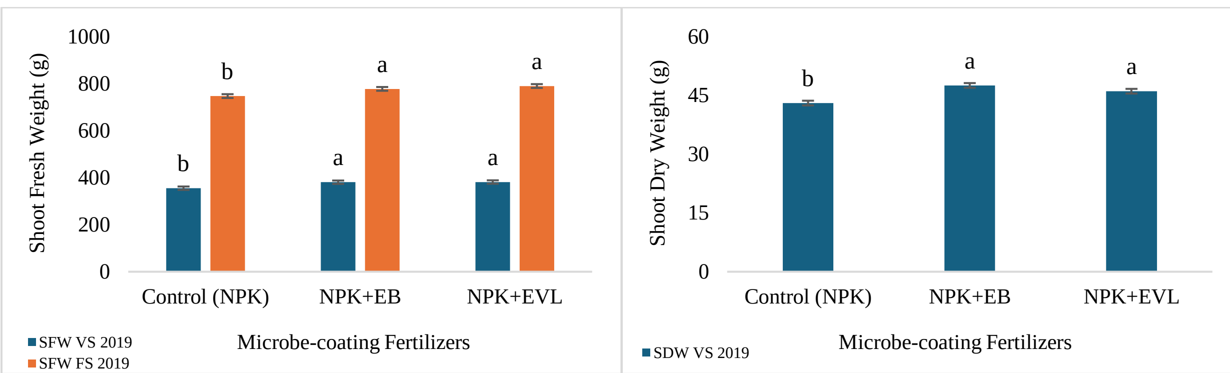



*Figure 4-5: Response of above-ground variables to the microbe-coated fertilizer treatments. SFW at the vegetative (VS) and flowering (FS) stages in experiment 1 (2019), and SDW at the vegetative stage (VS) in experiment 2 (2021).*

*Table 4-5: A summary of the analysis of variance (ANOVA) representing mean and p-values of above-ground agronomic variables at the vegetative (VS) and flowering (FS) stages studied in the first and second experiments. Columns indicate means (±SE). Means in the same column with the same letter are not significantly different according to the Tukey HSD test at p<0.05.* ***Bold*** *values are significant p-values.*

| | ***Variable →*** | ***Plant Height (cm)*** | | ***Leaf Area (cm²)*** | | ***Shoot Fresh Weight (g)*** | | ***Shoot Dry Weight (g)*** | |
|---|---|---|---|---|---|---|---|---|---|
| | ***Growth Stage →*** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** |
| ***Experiment 1*** | **Factors ↓** | | | | ***Water-deficit Stress*** | | | | |
| | **No Stress** | 63±1 a | 110±2 a | 6665±60 a | 8738±108 a | 445±7 a | 927±8 a | 53±1 a | 105±2 a |
| | **Mild Stress** | 56±1 b | 89±2 b | 5254±60 b | 5824±108 b | 355±7 b | 721±8 b | 44±1 b | 90±2 b |
| | **Severe Stress** | 53±1 c | 84±2 b | 5045±60 c | 5444±108 c | 317±7 c | 667±8 c | 39±1 c | 80±2 c |
| | | | | | ***Microbe-coated fertilizers*** | | | | |
| | **Control (NPK)** | 58±1 a | 91±2 b | 5490±60 b | 6345±108 b | 355±7 b | 747±8 b | 43±1 b | 92±2 a |
| | **NPK+ EB** | 57±1 a | 97±2 a | 5677±60 ab | 6803±108 a | 381±7 a | 778±8 a | 48±1 a | 90±2 a |
| | **NPK+ EVL** | 58±1 a | 95±2 ab | 5798±60 a | 6859±108 a | 382±7 a | 790±8 a | 46±1 a | 93±2 a |
| | | | | | ***p-value*** | | | | |
| | **Stress (S)** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| | **Fertilizer (F)** | 0.5853 | **0.0323** | **0.0035** | **0.003** | **0.024** | **0.0021** | **<0.0001** | 0.6365 |
| | **Interaction S*F** | **0.0219** | 0.1528 | 0.1758 | 0.6909 | 0.8104 | 0.8009 | 0.2681 | 0.7371 |
| ***Experiment 2*** | | | | | ***Water-deficit Stress*** | | | | |
| | **No Stress** | 68±2 a | 101±2 a | 9841±402 a | 10696±307 b | 629±26.7 a | 851±29 ab | 103±1.8 a | 125±2 b |
| | **Mild Stress** | 65±2 ab | 95±2 a | 8403±402 b | 13662±307 a | 607±26.7 ab | 940±29 a | 91±1.8 b | 132±2 a |
| | **Severe Stress** | 62±2 b | 87±2 a | 7358±402 b | 11329±307 b | 532±26.7 b | 822±29 b | 88±1.8 b | 108±2 c |
| | | | | | ***Microbe-coated fertilizers*** | | | | |
| | **Control (NPK)** | 62±2 b | 94±2 a | 8334±402 a | 11307±307 b | 573±26.7 a | 895±29 a | 91±1.8 a | 123±2 a |
| | **NPK+ EB** | 66±2 ab | 95±2 a | 8311±402 a | 12557±307 a | 584±26.7 a | 858±29 a | 95±1.8 a | 119±2 a |
| | **NPK+ EVL** | 68±2 a | 94±2 a | 8957±402 a | 11824±307 ab | 611±26.7 a | 860±29 a | 96±1.8 a | 124±2 a |
| | | | | | ***p-value*** | | | | |
| | **Stress (S)** | **0.0068** | **<0.0001** | **0.0005** | **<0.0001** | **0.0378** | **0.0189** | **<0.0001** | **<0.0001** |
| | **Fertilizer (F)** | **0.0041** | 0.9122 | 0.4445 | **0.0234** | 0.5871 | 0.6147 | 0.2105 | 0.3047 |
| | **Interaction S*F** | 0.4466 | 0.8319 | **0.0139** | **0.0008** | 0.2314 | 0.0626 | 0.1276 | 0.0664 |

### 4.3.2. Effect of Microbe-coated fertilizers on below-ground variables: Root Length, Root Fresh Weight and Dry Weight

Vigorous plant root growth is an essential indicator of overall plant development and productivity. Applying microbial fertilizers has shown significant benefits for plant root growth by colonizing the root zone and promoting root development, contributing to improved nutrient uptake, stress tolerance, and enhanced plant productivity.

In this two-year study, RL, RFW and SDW were collected at the VS and FSs to assess the response of these variables to the microbe-coated fertilizers (MCF) under mild and severe water-deficit stress. There was an apparent effect and increase in RL at the two phenological stages in experiment 1 and only at the VS in experiment 2. Despite numerical increases in these variables, the impact of the MCF treatments was only significant for RDW at the VS in experiment 1 and RFW at the FS in experiment 2 (Table 4-6).

The impact of water-deficit stress on RFW and RDW at both phenological stages was significant in experiment 1 but not in the RL. In experiment 2 (Table 4-6 and Appendix 4-2), stress significantly impacted RL and RFW at the FS and RDW at the VS. Regarding the interaction effects of water-deficit stress and MCF treatments, only RL at the VS in both experiments showed significant effects.

#### 4.3.2.1. Effect of MCF on Root Length

Root length is an important variable for plant growth, determining the capacity of a plant to extend its roots to uptake water and nutrients. Increasing RL means expanding root surface area, number of root tips, and overall root volume, contributing to improved plant growth, particularly under WS conditions. These changes due to microbial inoculations were reported, causing us to focus on the efficiency of MCF treatments in root growth and biomass.

In this study, the response of potato RL to the MCF treatments was positive at the VS ($p$ = 0.0008) and the FS ($p$ = 0.0008) when the WS effects were absent. In experiment 1, both MCF-treated plants resulted in greater RL, but NPK+EB was the best, with 75 cm at the VS and 77 cm at the FS, compared to control plants (66 and 67 cm, respectively) (Table 4-6).

In experiment 2, the MCF treatments did not increase the RL at the VS, in which control plants had longer roots than MCF-treated plants. At the FS, the trend remained similar in spite of a numerical increase in RL in MCF-treated plants (Table 4-6).

In this study, the ANOVA procedure showed a significant interactive effect of MCF treatments and water-deficit stress at the VS of both experiments ($p$ = 0.0055 and $p$ = 0.0521) on potato RL (Table 4-6). The Tukey test results show that the only significant differences occurred under mild WS in both experiments/years. The WS effects were insignificant in the experiment, but the non-stressed plant performance was greater than the mild and severe-stressed plants. However, the MCF treatments reduced the negative effect of the WS stress and enhanced the RL considerably in plants at the VS under mild WS, in which both NPK+EB and NPK+EVL had a root length of 75.6 cm compared to 56 cm for control plants (Table 4-6 and Figure 4-6). This shows the efficiency of MCF treatments in improving stress tolerance and lowering the adverse effects of mild WS, specifically earlier in the growing season when plants are in the establishment phase.

In experiment 2, the NPK+EB treatment had significantly lower RL under mild stress, while the NPK+EVL treatment was not significantly different from the control. The contradictory result was noted for the plants grown under severe WS in which NPK+EVL had lower RL than NPK+EB, leading to insignificant effects on RL, compared to the control plants (Figure 4-6). As these results are contradictory between years/experiments, we reject our first hypothesis that the MCF treatments reduce the WS effects for this variable.

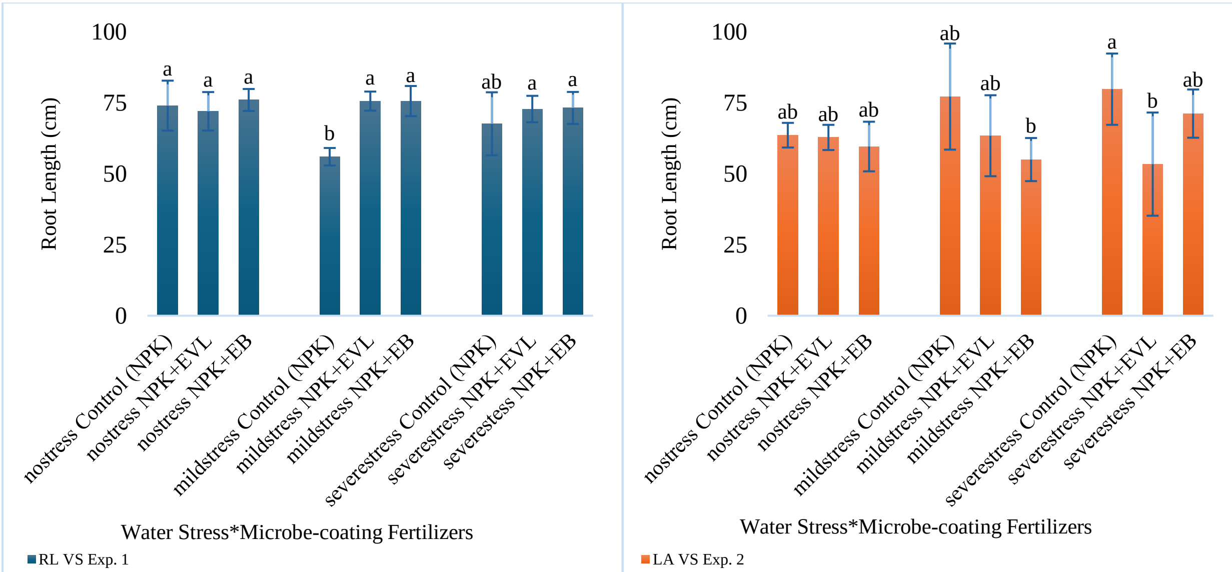



*Figure 4-6: The interaction effects of water-deficit stress and microbe-coated fertilizer treatments on potato root length at the vegetative stages in experiments 1 and 2. Bars are the mean values (cm) of each treatment, and error bars are the standard deviation of the means. Dissimilar letters indicate significancy (p = < 0.05, Tukey HSD).*

#### 4.3.2.2. Effect of MCF on Root Fresh and Dry Weights

Root biomass is an additional important variable determining plant ability to establish and grow, especially under stressful conditions. Microbes have the potential to develop an association with plant roots, resulting in more significant root proliferation because of the direct mechanisms of microbes in terms of nutrient solubilization, microbe-to-plant signaling and phytohormone compound production. Therefore, determining how potato plant roots were altered in response to the MCF under water-deficit stress and unstressed conditions is important in understanding MCF effects and potential benefits in agricultural systems.

In two experiments, we collected root biomass data at the VS and FS by measuring RFW before oven-drying the roots to determine RDW. In experiment 1, we did not observe any significant differences between treatments for RFW, whereas the MCF treatments significantly affected RDW ($p < 0.0001$) at the VS and showed a tendency toward statistical significance ($p = 0.0589$) at the FS (Table 4-6). The effect of the MCF was similar, but NPK+EB showed greater biomass levels: RFW (24 g) and RDW (3 g), compared to the control plants at the VS. In the FS, the MCF treatments increased RDW, and the performance of NPK+EVL was slightly greater. Data analysis showed significant effects of WS on RWF and RDW at both growth stages (Table 4-6, Figure 4-7, Appendix 4-2).

In experiment 2, the MCF treatments only caused significant effects on RFW ($p$ = 0.0382) at the FS in which NPK+EVL significantly increased RFW (28 g) compared to the control plants in which RFW resulted in 24 g (Table 4-6 and Figure 4-7). However, there was a numerical increase in RFW and RDW at the VS, but without statistical differences (Table 4-6). We observed significant WS effects at the FS for RFW and the VS for RDW (Appendix 4-3).

There were no significant interaction effects between the MCF treatments and water-deficit stress at either growth stage for both experiments.

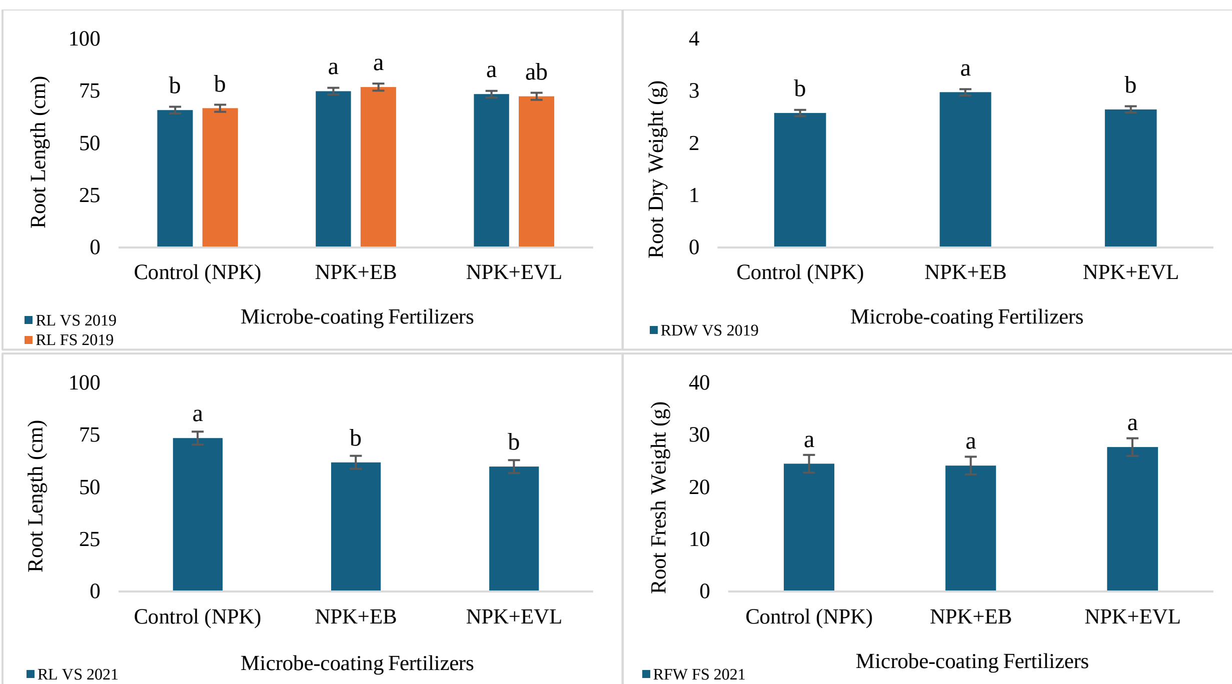



*Figure 4-7: Response of below-ground variables to the microbe-coated fertilizer treatments. RL at the vegetative (VS) and flowering (FS) stages in experiment 1 (2019), RDW at the vegetative stage (VS) in experiment 1 (2019), RL at the vegetative stage (VS) in experiment 2 (2021), and RFW at the flowering stage (FS) in experiment 2 (2021).*

*Table 4-6: A summary of the analysis of variance (ANOVA) representing mean and p-values of below-ground agronomic variables at the vegetative (VS) and flowering (FS) stages studied in the first and second experiments. Columns indicate means (±SE). Means in the same column with the same letter are not significantly different according to the Tukey HSD test at p<0.05.* ***Bold*** *values are significant p-values.*

| | ***Variables →*** | ***Root length (cm)*** | | ***Root Fresh Weight (g)*** | | ***Root Dry Weight (g)*** | |
|---|---|---|---|---|---|---|---|
| | ***Growth Stages →*** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** |
| ***Experiment 1*** | **Factors ↓** | ***Water-deficit Stress*** | | | | | |
| | **No Stress** | 74±2 a | 72±2 a | 30±1 a | 31±1 a | 3.5±0.1 a | 10 ±0.2 a |
| | **Mild Stress** | 69±2 a | 72±2 a | 21±1 b | 25±1 b | 2.4±0.1 b | 9±0.2 b |
| | **Severe Stress** | 71±2 a | 73±2 a | 19±1 b | 24±1 b | 2.2±0.1 c | 8 ±0.2 c |
| | | ***Microbe-coated fertilizers*** | | | | | |
| | **Control (NPK)** | 66±2 b | 67±2 b | 23±1 a | 26±1 a | 2.6±0.1 a | 8.7±0.2 a |
| | **NPK+ EB** | 75±2 a | 77±2 a | 24±1 a | 27±1 a | 3±0.1 b | 9.2±0.2 a |
| | **NPK+ EVL** | 73±2 a | 72±2 ab | 23±1 a | 27±1 a | 2.6±0.1 a | 9.3±0.2 a |
| | | ***p-value*** | | | | | |
| | **Stress (S)** | 0.1181 | 0.9183 | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| | **Fertilizer (F)** | **0.0008** | **0.0008** | 0.75 | 0.5934 | **<0.0001** | 0.0589 |
| | **Interaction S*F** | **0.0055** | 0.9436 | 0.9221 | 0.9705 | 0.1132 | 0.8024 |
| ***Experiment 2*** | | ***Water-deficit Stress*** | | | | | |
| | **No Stress** | 62±3.1 a | 70±2 a | 18±2 a | 28±1 a | 7.1±0.2 a | 8.7±0.3 a |
| | **Mild Stress** | 65±3.1 a | 63±2 b | 16±2 a | 25±1 ab | 6±0.2 b | 8.7±0.3 a |
| | **Severe Stress** | 68±3.1 a | 63±2 ab | 17±2 a | 24±1 b | 5.8±0.2 b | 8±0.3 a |
| | | ***Microbe-coated fertilizers*** | | | | | |
| | **Control (NPK)** | 74±3.1 a | 65±2 a | 15±2 a | 24±1 a | 6±0.2 a | 9±0.3 a |
| | **NPK+ EB** | 62±3.1 b | 65±2 a | 19±2 a | 24±1 a | 6.5±0.2 a | 8.1±0.3 a |
| | **NPK+ EVL** | 60±3.1 b | 66±2 a | 17±2 a | 28±1 a | 6.3±0.2 a | 8.3±0.3 a |
| | | ***p-value*** | | | | | |
| | **Stress (S)** | 0.382 | **0.0247** | 0.6556 | **0.0097** | **<0.0001** | 0.0889 |
| | **Fertilizer (F)** | **0.0071** | 0.8065 | 0.3346 | **0.0382** | 0.1505 | 0.0806 |
| | **Interaction S*F** | **0.0521** | 0.2387 | 0.8917 | 0.4889 | 0.5007 | 0.2507 |

#### 4.3.3. Physiological variables: Leaf Greenness (SPAD), Photosynthetic Rate, Stomatal Conductance and Plant Tissue Total Nitrogen

Water-deficit stress significantly affects plant physiology and nutrient balance in tissues, impacting their growth, development, and ability to cope with environmental challenges. Gaining insight into the physiological responses of plants to water stress is important for devising methods to improve their ability to withstand water-deficit stress and grow vigorously under conditions of restricted water availability.

The Soil Plant Analysis Development (SPAD) chlorophyll meter is a widely used tool for estimating leaf chlorophyll content, which indirectly indicates plant nitrogen content. It measures leaf greenness by acquiring absorbance of leaves in red and near-infrared regions at specific light wavelengths, based on the principle that leaf nitrogen content is reflected in leaf greenness. The SPAD meter has been used in various studies to estimate chlorophyll content in specific plant species, including potato, to assess nitrogen status, photosynthetic pigments, and plant health under a range of environmental conditions and across a number of crop types. The use of SPAD meter readings has been correlated with chlorophyll concentration, gas exchange, chlorophyll fluorescence, and specific leaf weight in different growth stages of plants. However, the accuracy of SPAD meter readings can be affected by factors such as cultivar, leaf anatomical characteristics and position, which may cause variation and inaccuracy (Chaimala et al., 2021; Mehrabi and Sepaskhah, 2021; Wasaya et al., 2021).

In this study, the MCF treatments and WS stress had significant effects on potato plant leaf greenness (SPAD measurement) at both growth stages. However, the responses were not consistent in the two experiments.

In experiment 1, the results of the two-way ANOVA show significant effects of both MCF treatments ($p$ < 0.0001) on SPAD measurements, in which NPK+EB were significantly higher

(45.3 units) at the VS and (43.7 units) at the FS compared to the control plants (41.7 and 38.5 units, respectively). The SPAD readings increased despite the significant effects of mild and severe WS at both growth stages (Table 4-7). There was an interactive effect of MCF and WS on SPAD readings at both growth stages.

In experiment 2, MCF treatments ($p = 0.0514$) showed a tendency toward a significant effect at the VS, where NPK+EVL treatment resulted in a higher SPAD reading (48.5 units) than the control plants, in the absence of WS effects. During the FS, no MCF treatment effects were observed; however, WS and its interaction effects with MCF were significant for SPAD readings. All MCF treatments and WS levels caused greater greenness at the VS than the FS (Table 4-7).

The two-way ANOVA model showed a significant interaction between MCF and WS factors (Table 4-7 and Figure 4-8). In the VS of experiment 1, the NPK+EB treatment caused significantly higher leaf greenness (47.9 units) than the control in the non-stressed (41.8 units) and severe stress (37.4 units) treatments. The NPK+EVL treatment differed significantly (44.3 units) from the control (41.8) in non-stressed plants (Table 4-7). The same treatment caused significantly lower SPAD unit levels than the control, under mild WS, than the control. These interactions do not follow the pattern we expected in our hypothesis: the effect of the MCF treatments would increase with increasing WS. NPK+EB's impact on the non-stressed and severely stressed plants but not the mildly stressed plants does not fit this pattern.

The two FSs of experiments 1 and 2 presented in Figure 4-8, manifested a similar pattern. The NPK+EB treatment had significantly higher leaf greenness (SPAD units) than the control in the non-stressed and severely stressed plants in experiment 1. In contrast, in experiment 2, it was significantly lower than the control under no stress and significantly higher under severe WS.

NPK+EVL was not significantly different from the control under any WS treatment except for the mild stress in experiment 2, with significantly lower greenness.

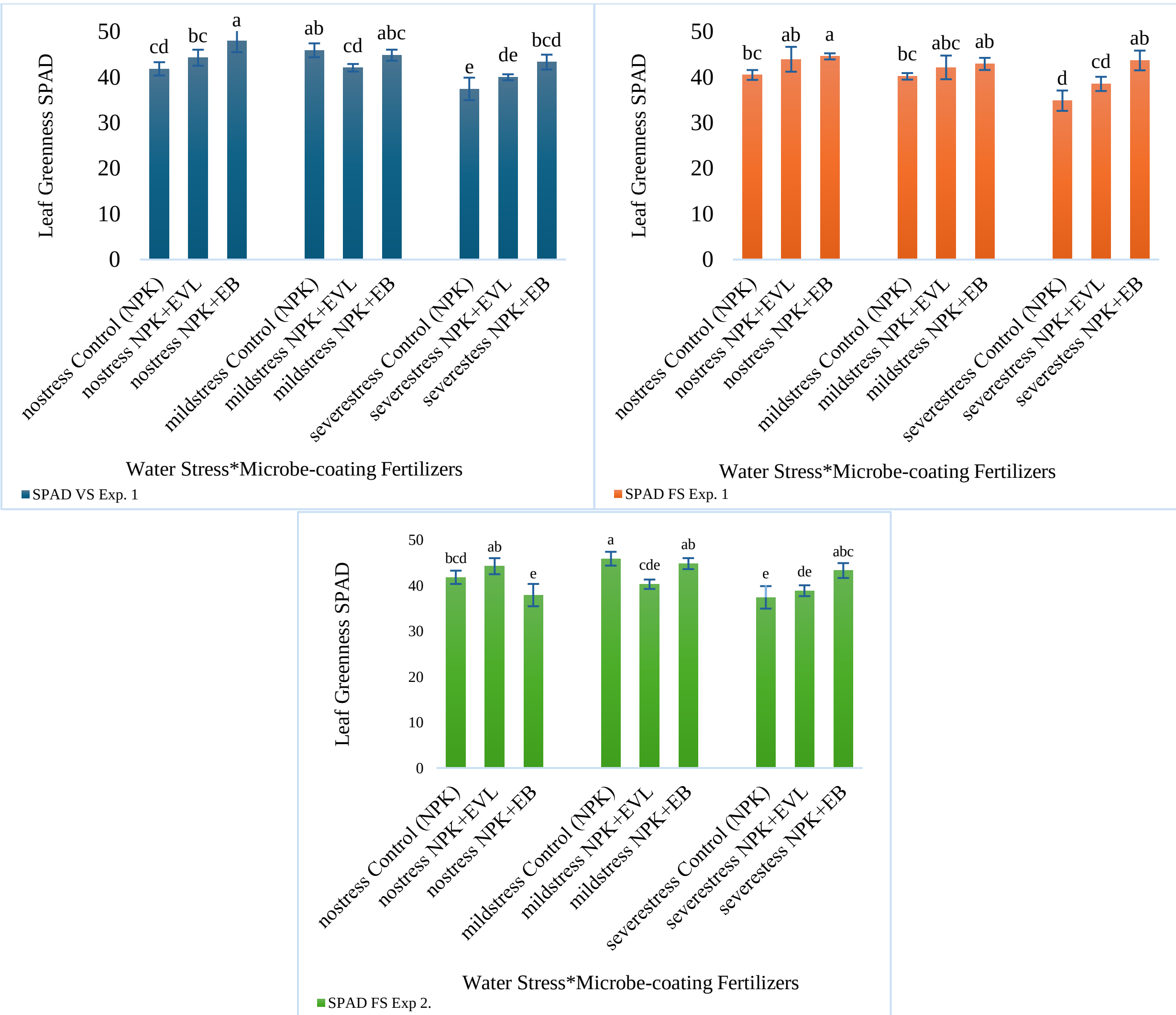



*Figure 4-8: The interaction effects of water-deficit stress and microbe-coated fertilizer treatments on potato leaf greenness (SPAD) at the vegetative and flowering stages in experiment 1 and the flowering stage in experiment 2. Bars are the mean values (cm) of each treatment, and error bars are the standard deviation of the means. Dissimilar letters indicate significant differences (p = < 0.05, Tukey HSD).*

The SPAD results indicate NPK+EVL did not affect potato leaf greenness under non-stressed or severely stressed conditions, but it may reduce greenness under mild WS. Meanwhile, NPK+EB may increase plant greenness under severe stress while having variably positive or negative effects in non-stressed potato plants and no significant effect on mildly stressed plants. Considering that the effect of NPK+EB was greatest at the highest level of WS, we consider this to be a genuine interaction, and we accept our first hypothesis, i.e., that the EB-coated fertilizer reduces the effects of WS as measured by leaf greenness.

Leaf greenness is a valuable indicator of physiological performance, reflecting the impact of stress-related factors on plant health and photosynthetic capacity. Leaf area and greenness contribute greatly to photosynthesis but can be negatively influenced by water-deficit stress, impacting plant ability to open and close stomata to control transpiration and other gas exchanges.

The results of this study show no significant effects of the MCF treatments on photosynthetic rate and stomatal conductance despite numerical increases in these two critical physiological variables, particularly at the VS of both experiments (Table 4-7). The positive effects of the MCF treatments were notable in reducing the stress effects but without statistical significance.

The WS effects were significant ($p < 0.001$) across all growth stages, except for the FS ($p = 0.001$) in the second experiment. No MCF treatments and WS interaction effects were observed at either stage or in either experiment (Table 4-7).

Plant/leaf greenness is positively correlated with nitrogen concentration in plant tissue. The MCF treatments significantly affected leaf greenness (SPAD readings) and prolonged potato shoot growth at the VS and FS in both experiments. However, the collected data and two-way ANOVA results show a significant difference in the MCF treatment ($p = 0.0452$) on plant tissue %N at the VS of the first experiment. NPK+EB and NPK+EVL resulted in higher %N levels: 2.6 and 2.7%, respectively, compared to 2.5% in the control plant tissues. This increase was considerable, while there was a significant reduction in the %N in mildly and severely stressed plants compared to the unstressed control plant tissues (Table 4-7).

*Table 4-7: The analysis of variance (ANOVA) showing mean and p-values of physiological variables and nitrogen content at the vegetative (VS) and flowering (FS) stages studied in the first and second experiments. Columns indicate means (±SE). Means in the same column with the same letter are not significantly different according to the Tukey HSD test at p<0.05.* ***Bold*** *values are significant p-values.*

| | ***Variables →*** | ***Leaf Greenness SPAD*** | | ***Photosynthetic Rate $\mu mol\ m^{-2}\ sec^{-1}$*** | | ***Stomatal Conductance mmol $m^{-2}\ s^{-1}$*** | | ***Total Nitrogen (%N)*** | |
|---|---|---|---|---|---|---|---|---|---|
| | ***Growth Stages →*** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** | **Vegetative Stage** | **Flowering Stage** |
| ***Experiment 1*** | **Factors ↓** | | | ***Water-deficit Stress*** | | | | | |
| | **No Stress** | 44.7±0.4 a | 43±1 a | 14.5±0.4 a | 12.9±0.4 a | 0.17±0.01 a | 0.15±0.01 a | 3.5±0.1 a | 3.3±0.04 a |
| | **Mild Stress** | 44.3±0.4 a | 42±1 a | 10.5±0.4 b | 11.2±0.4 b | 0.07±0.01 b | 0.07±0.01 b | 2.6±0.1 b | 2.6±0.04 b |
| | **Severe Stress** | 40.2±0.4 b | 39±1 b | 8.4±0.4 c | 9.6±0.4 c | 0.06±0.01 b | 0.06±0.01 b | 1.7±0.1 c | 1.6±0.04 c |
| | | | | ***Microbe-coated fertilizers*** | | | | | |
| | **Control (NPK)** | 41.7±0.4 b | 38.5±1 c | 10.9±0.4 a | 11.7±0.4 a | 0.09±0.01 a | 0.09±0.01 a | 2.5±0.1 b | 2.4±0.04 a |
| | **NPK+ EB** | 45.3±0.4 a | 43.7±1 a | 11.1±0.4 a | 11.1±0.4 a | 0.1±0.01 a | 0.09±0.01 a | 2.6±0.1 ab | 2.5±0.04 a |
| | **NPK+ EVL** | 42.1±0.4 b | 41.5±1 b | 11.4±0.4 a | 11.5±0.4 a | 0.1±0.01 a | 0.09±0.01 a | 2.7±0.1 a | 2.5±0.04 a |
| | | | | ***p-value*** | | | | | |
| | **Stress (S)** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| | **Fertilizer (F)** | **<0.0001** | **<0.0001** | 0.5975 | 0.7356 | 0.8579 | 0.9849 | **0.0452** | 0.2960 |
| | **Interaction S*F** | **<0.0001** | **0.0053** | 0.9916 | 0.9842 | 0.9949 | 0.9951 | 0.8011 | 0.8431 |
| ***Experiment 2*** | | | | ***Water-deficit Stress*** | | | | | |
| | **No Stress** | 46.2±1 b | 41.3±0.4 b | 14.3±0.4 a | 10.9±0.2 a | 0.17±0.02 a | 0.12±0.01 a | 3.7±0.1 a | 3.1±0.03 a |
| | **Mild Stress** | 47.8±1 a | 43.7±0.4 a | 10.8±0.4 b | 10.4±0.2 a | 0.11±0.02 ab | 0.09±0.01 b | 2.8±0.1 b | 1.4±0.03 b |
| | **Severe Stress** | 47.2±1 b | 39.9±0.4 b | 9±0.4 c | 9.6±0.2 b | 0.08±0.02 b | 0.07±0.01 b | 1.8±0.1 c | 1.1±0.03 c |
| | | | | ***Microbe-coated fertilizers*** | | | | | |
| | **Control (NPK)** | 46.4±1 a | 41.7±0.4 a | 11.2±0.4 a | 10±0.2 a | 0.11±0.02 a | 0.09±0.01 a | 2.7±0.1 a | 1.88±0.03 a |
| | **NPK+ EB** | 46.4±1 a | 42 ±0.4 a | 11.4±0.4 a | 10.3±0.2 a | 0.12±0.02 a | 0.09±0.01 a | 2.7±0.1 a | 1.88±0.03 a |
| | **NPK+ EVL** | 48.5±1 a | 41.1±0.4 a | 11.5±0.4 a | 10.6±0.2 a | 0.12±0.02 a | 0.1±0.01 a | 2.8±0.1 a | 1.89±0.03 a |
| | | | | ***p-value*** | | | | | |
| | **Stress (S)** | 0.2403 | **<0.0001** | **<0.0001** | **0.001** | **0.0071** | **<0.0001** | **<0.0001** | **<0.0001** |
| | **Fertilizer (F)** | **0.0514** | 0.3778 | 0.8797 | 0.2721 | 0.9385 | 0.5167 | 0.2444 | 0.9669 |
| | **Interaction S*F** | 0.3116 | **<0.0001** | 0.9971 | 0.7835 | 0.9978 | 0.9988 | 0.9981 | 0.9991 |

### 4.3.4. Tuber Yield and Total Strach Content in Potato Tubers

Total yield was significantly impacted by both the stress level ($p < 0.0001$) and the MCF treatments ($p < 0.0001$). The results for the TY of both years are presented in Table 4-8 and Figures 4-10 and 4-11. As postulated by our second hypothesis, treatment of the MCF led to a higher TY. MCF-treated plants had significantly more TY than the control across both experiments. However, the significance under WS was evident in both experiments when non-stressed plants yielded more tubers than mild and severely stressed plants.

In experiment 1, NPK+ EVL resulted in 786.4 g of TY, compared to NPK+EB at 780.5 g and control plants at 683.8 g. In experiment 2, the TY result followed the same pattern, in which NPK+EVL treated plants produced significantly more potato yield (793 g) than NPK+EB (773.9 g) and control plants (585.8 g). These results confirm the efficiency of both MCF treatments in reducing the negative impact of WS (Table 4-8, Figures 4-9 and 4-10).

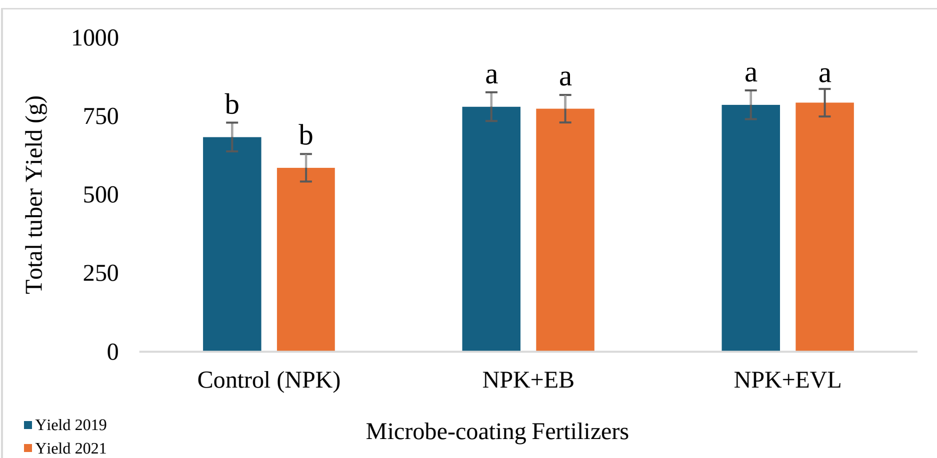



*Figure 4-9: Yield response to the microbe-coated fertilizer treatments in experiments 1 (2019) and 2 (2021). Dissimilar letters indicate significant differences (p = 0.05, Tukey HSD).*

The two-way ANOVA results revealed no significant interaction between the WS and the MCF factors. While the EB and EVL microbe-coated fertilizers improve yield, they do not do so at higher levels of WS tolerance (Figure 4-11). If this were the case, we would have expected to see an interaction, with the effect on TY increasing under stressed conditions.

After harvesting tubers, total starch (%S) analysis was performed on stressed and stress-free tubers. The results indicated no significant effects of the MCF treatments on %S in either experiment. However, there was a slight numerical increase in %S in tubers harvested in mild WS conditions in both experiments (68.8 and 69.2%, respectively), compared to the non-stressed tubers (68.1 and 68.4%, respectively) (Table 4-8).

*Table 4-8: A summary of the analysis of variance (ANOVA) representing mean and p-values of potato tuber yield and total (%) starch in the first and second experiments. Columns indicate means (±SE). Means in the same column with the same letter are not significantly different according to the Tukey HSD test at p<0.05.* ***Bold*** *values are significant p-values.*

| ***Variables →*** | ***Yield (g/plant)*** | | ***Total (%) Starch*** | |
|---|---|---|---|---|
| ***Experiments →*** | **Experiment 1** | **Experiment 2** | **Experiment 1** | **Experiment 2** |
| ***Factors ↓*** | ***Water-deficit Stress*** | | | |
| ***No Stress*** | 974±46 a | 829±44 a | 68.1±0.2 a | 68.4±0.2 a |
| ***Mild Stress*** | 679±46 b | 654±44 b | 68.8±0.2 b | 69.2±0.2 b |
| ***Severe Stress*** | 598±46 c | 670±44 b | 66.8±0.2 c | 67.6±0.2 c |
| | ***Microbe-coated fertilizers*** | | | |
| ***Control (NPK)*** | 684±46 b | 585.8±44 a | 67.8±0.2 a | 68.3±0.2 a |
| ***NPK+ EB*** | 781±46 a | 774±44 b | 67.9±0.2 a | 68.3±0.2 a |
| ***NPK+ EVL*** | 786±46 a | 793±44 b | 68±0.2 a | 68.6±0.2 a |
| | ***p-value*** | | | |
| ***Stress (S)*** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| ***Fertilizer (F)*** | **<0.0001** | **<0.0001** | 0.5473 | 0.4371 |
| ***Interaction S*F*** | 0.1194 | 0.0818 | 0.9352 | 0.6485 |

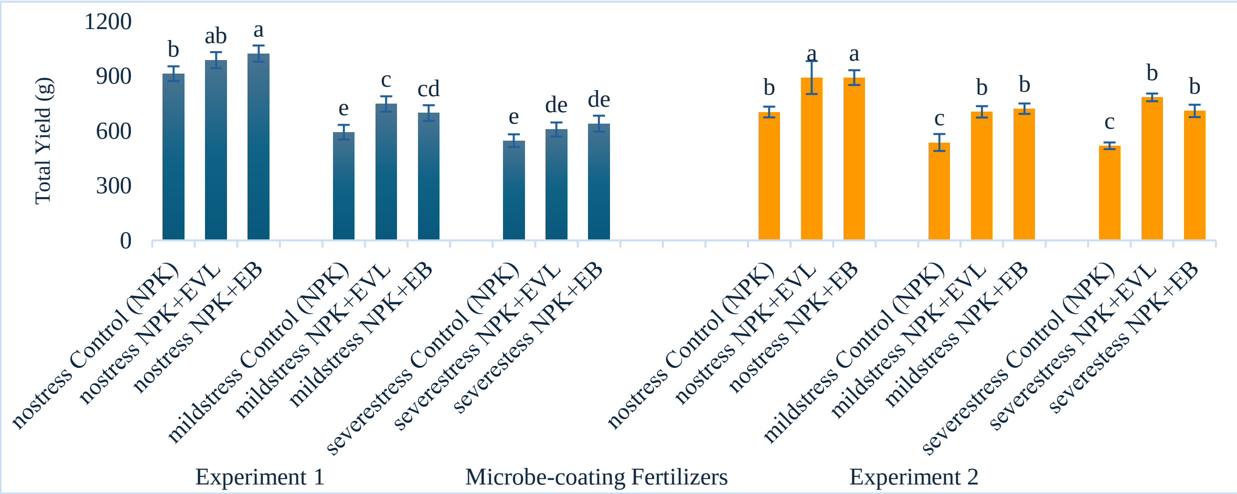



*Figure 4-10: The interaction effects of water-deficit stress and microbe-coated fertilizer treatments on potato yield in experiments 1 and 2. Bars are the mean values (cm) of each treatment, and error bars are the standard deviation of the means. Dissimilar letters indicate significant differences (p = < 0.05, Tukey HSD).*

## 4.4. Discussion

Global human population growth and the associated need for a reliable and sufficient food supply in the long term necessitate sustainable agriculture. Yield gaps and declines occur due to poor farming practices, soil disturbance from excessive use of chemical inputs, and numerous abiotic (Antar et al., 2021; Shah et al., 2021) and biotic stresses (Riaz et al., 2022a,b), which continuously influence agricultural output. To achieve plant productivity and food security goals without compromising yield quality, agricultural producers can consider sustainable and environmentally friendly approaches such as PGPR (Lyu et al., 2021 a, b). Drought, or water deficiency stress, are worldwide issues that negatively influence plant growth and productivity, with far-reaching global consequences. Various methods enhance drought tolerance, including agronomic practices, traditional breeding, and genetic engineering. These methods are typically time-consuming and not environmentally sustainable (Bouremani et al., 2023; Singh et al., 2024). Microbial inoculant technologies and their cell-free supernatants and signaling compounds have been reported to enhance plant growth under water deficit conditions. However, plant growth promotion and stimulation technologies need further exploration to understand the mechanisms contributing to the process (Msimbira et al., 2022; Naamala et al., 2022; Naamala et al., 2023; Shah et al., 2022; Nazari and Smith, 2023). The beneficial impacts of PGPR on plants are promising in controlled studies but tend to be less reliable in field situations due to a range of factors affecting their effectiveness and compatibility (Bacon et al., 2014; O'Callaghan et al., 2022).

In this study, we attempted to evaluate microbe-coated fertilizers (MCF), a novel technology that was previously tested under field conditions and exhibited promising results under diverse environmental conditions (dry and wet years), particularly for yield enhancement. It is possible for MCFs to enhance plant growth because the microbes coated on synthetic NPK fertilizer provide additional nutrient availability due to major nutrient solubilization (P and K), phytohormone production (IAA, CK and GA), siderophore prosecution for chelated iron, and to exude microbe-to-plant signals such as lipochitooligosaccharides (LCO) and thuricin17 possessing phyto-stimulation properties (Antar et al., 2021).

Because it is a novel technology for delivering microbes to soil, no studies have been available on MCF to compare to. However, cell-free supernatants derived from individual bacterial strains (EVL microbes) studied in this project were investigated recently for their ability to mitigate abiotic stresses, namely pH and salinity (Msimbira et al., 2022; Naamala et al., 2023). In addition, studies have been conducted on the use of *Bacillus* (Gerayeli et al., 2017; Lagzian et al., 2023; Marković et al., 2023), *Pseudomonas* (Vrieze et al., 2018; 2020; Riaz et al., 2022) and *Lactobacillus* (Omafuvbe et al., 2011; Steglińska et al., 2022) strains as single (Mamun et al., 2024) or consortium inoculants (Santoyo et al., 2021) on various crops in response to biotic and abiotic stresses. Many uncertainties regarding the optimal utilization of PGPR or microbial products to achieve maximum crop production efficiency have led to additional questions emerging from these uncertainties. Microbial inoculants have not been extensively used to promote potato growth. The effectiveness of live microorganisms in stimulating plant growth has been limited in laboratory and controlled environments (Velivelli et al., 2014). The application of MCF as a new microbial inoculation approach will acquire more interest when additional research validates its efficiency and compatibility. A specific focus of this study was to assess the potential of two microbe-coated fertilizers on potato plants subjected to mild and severe water deficit stresses.

#### 4.4.1. Effect of the MCF and WS on the agronomic variables and yield

Growth variables such as PH, LA, SFW and SDW are correlated with shoot system development and biomass, all of which contribute to determining healthy growth and development, leading to enhanced plant productivity. Vigorous plant shoot growth is a crucial aspect of plant development and productivity. Several factors contribute to plant growth, including genetics, environmental conditions, and agricultural practices (e.g. fertilization). One of the key determinants of vigorous shoot growth is the availability of essential nutrients. Macronutrients such as nitrogen, phosphorus, and potassium are vital in promoting shoot growth. Nitrogen promotes vegetative green growth, phosphorus aids in root development, and potassium enhances plant health, stem strength, and disease resistance, contributing to overall plant health. However, drought or water stress triggers several responses in potato, including changes in growth rates, alteration of physiological variables, and variation in production.

The vulnerability of potato growth stages, tuber initiation and bulking to water stress has been highlighted by Shin et al. (2010). Research has also shown that WS delays emergence, slows plant development, reduces plant biomass, and dramatically decreases tuber number, size, and yield (Nasir and Tóth, 2022). The results of this study show that microbe-coated fertilizers (MCF) are efficient in promoting potato plant growth, particularly under water-deficit stress (WS) conditions.

Plant height, one of the important variables, clearly responded to the MCF, particularly at the FS of experiment 1 and the VS stage of experiment 2 (Table 4-5). The PH growth stimulation positively affects the number of leaves on the stem, contributing to plant biomass (SFW and SDW). The root system is a growth-determining factor due largely to its roles in water and nutrient uptake. Research shows that PGPR can enhance root length and overall root system functioning, particularly in water and salinity-stressed environments. PGPR can colonize the root system, stimulate plant growth, and increase root length and number, promoting faster plant growth and increased production. It enhances tolerance against abiotic stresses by improving nutrient absorption. PGPR also increase seedling root length, surface area, number of root tips, and root volume, potentially improving plant growth and stress tolerance (Vacheron et al., 2013; Verbon and Liberman, 2016; Vargas et al., 2019; Zafar-ul-Hye et al., 2019).

In this study, the response of the root system to MCF treatments under WS was varied. However, the MCF significantly affected RL in both experiments and only for RFW at the FS of experiment two. The variation may be due to the negative impact of WS on the root system and the efficiency of the MCF. Overall, the response of most of the variables to the MCF was significant despite the water-stressed environment. The composition of the microbial consortia differed in the EVL microbial consortium, comprised of *Bacillus*, *Lactobacillus* and *Pseudomonas* strains. The Era Boost consortium includes a group of five *Bacillus* strains. Diversity in consortium-based microbial inoculations has been reported to benefit plants by improving nutrient uptake and overall plant performance (Mamun et al., 2024; Petrushin et al., 2024), and this diversity may have been a factor in the effects of these consortia in the current experimentation.

In a recent study conducted on the cell-free supernatants (CFS) derived from *Bacillus subtilis* and *lactobacillus helveticus* strains of EVL® Coating/product, which was used as fertilizer coatings in our study, showed significant effects on potato plant height, stem diameter, and shoot and root fresh weights grown under various levels of acidity stress (Msimbira et al., 2023). The above-mentioned strains were the composition of the. In addition, the same strains demonstrated significant effects on seed germination rates for both corn and tomato (Msimbira et al., 2022). In another study on *Lactobacillus helveticus*, the authors reported the CFS derived from this strain increased shoot fresh weight, leaf greenness and photosynthetic rate when potato plants grow under salinity stress (Naamala et al., 2023). In addition, *Bacillus amyloliquefaciens* cell-free supernatant shows biostimulation properties and could enhance seed germination and radicle length in corn and soybean grown under salt stress (Naamala et al., 2022). The positive results of these studies on the application of compounds derived from a single strain or a combination of two strains indicate the efficiency of EVL microbial inoculants in enhancing plant tolerance to abiotic stresses and promoting plant growth and development.

The diverse composition of the Era Boost Pro microbes from the Ulysse Biotechnology® product suggests the advantage of using consortium-based microbial inoculants rather than single inoculum. The EB strains were tested and selected based on their ability to solubilize nutrients. For example, *Bacillus velezensis* U47, *Bacillus megaterium* U48, and *Bacillus megaterium* U49 are able to solubilize organic phosphorus and calcium; *Bacillus velezensis* U50 has been shown to produce enzymes involved in the solubilization of organic phosphorus; *Bacillus velezensis* U47 produces a siderophore to chelate iron; and *Bacillus megaterium* U48 produces phytohormone compounds, specifically IAA (Personal communication with an industrial partner – Unshared data due to confidentiality).

Based on these studies and other research conducted on both microbial consortia, the growth promotion could be related to phytohormone production (auxin and cytokinin) by the MCF, which results in a relatively higher root and root biomass. In addition, the nutrient (P and K) solubilizing characteristics and siderophore production by the studied strains contribute to nutrient availability for plant growth and development under abiotic stress conditions (Gururani et al., 2012; Antar et al., 2020; Batool et al., 2020; Shah et al. 2021; Lyu et al., 2021), also a factor potentially contributing to plant growth enhancement.

Related to effects on plant growth and development variables, potato yield is susceptible to water stress. To achieve high yields, the soil water content in the root zone should not be below 50% of the maximum accessible water (Wilkinson et al., 2019). Water stress significantly affects tuber yield, influenced by dry matter allocation to tubers and also tuber water content, which constitutes 75-80% of tuber mass. Plant reaction to WS varies substantially depending on the cultivar, growth stages, and the duration of the stress (Dietz et al., 2021; Ierna and Mauromicale, 2022). It is crucial to consider factors such as root development, shoot growth and nutrient availability to enhance potato yield under stressed conditions because their disturbance negatively affects yield.

In this study, we demonstrated enhanced yield across both experiments/years (Table 4-8), achieved by improved shoot and root growth in both experiments (Tables 4-5 and 4-6). The MCF treatments increased above and below-ground variables, leading to TY increases compared to the control plants within the same water-deficit stress level. The growth promotion and enhanced tolerance to water-deficit stress in response to the MCF treatments are generally thought to be through microbial inoculant ability to solubilize essential nutrients and production of phytohormone compounds and their analogous or effectors for overall plant growth improvement (Ullah et al., 2018; Etesami and Adl, 2020; Fattahi et al., 2021; Yadav et al., 2021; EL Sabagh et al., 2022; Bouremani et al., 2023).

Complementing the growth stimulation evidence provided here, regarding the EVL and EB microbial consortia, a number of other studies have reported PGPR-produced phytohormone compounds, ACC deaminase and nutrient acquisition as involved in water stress alleviation. Plant hormones such as auxin, cytokinin, abscisic acid, and gibberellin are produced in response to water stress to manage processes related to drought tolerance mechanisms (Zafar-ul-Hye et al., 2019; Zia et al., 2021; Antar et al., 2021). These hormones trigger physiological processes related to plant growth and development in general and in response to water stress. Changing the levels of these phytohormones allows plants to perceive a stressful circumstance and to regulate gene expression in response (Salvi et al., 2021; Khan et al., 2022).

Drought stress significantly reduces auxin accumulation in plant tissues, with indole-3-acetic acid (IAA) being the best-known auxin phytohormone. Plant growth-promoting rhizobacteria produced IAA, which improves root architecture, water and nutrient uptake, and cellular defense against water stress. A decrease in IAA under stress conditions can increase ABA levels, causing auxin growth modulation (Barnawal et al., 2017; Raheem et al., 2018). Water stress influences abscisic acid accumulation, regulating plant-water balance and cellular tolerance to dehydration. It is rapidly produced in plant chloroplasts and roots, triggering stomatal closure and reducing water loss (Porcel et al., 2014).

Cytokinin promotes stomatal opening, decreases root growth, and stimulates shoot growth. Water stress significantly reduces plant cytokinin levels, facilitating adaptive plant responses and increasing plant survival under water-scarce conditions (Liu et al., 2013; Zaheer et al., 2019). Gibberellin is a primary growth regulator and protects plants against stress by regulating aspects of metabolism that scavenge reactive oxygen species (ROS) and maintain the photochemical efficiency of photosystem II (Cohen et al., 2009; Kang et al., 2019).

PGPR uses the enzyme ACC deaminase to mitigate ethylene stress, which reduces endogenous ethylene levels and negatively affects plants. Plant drought tolerance is linked to bacterial upregulation of the ACC deaminase gene. Some bacterial strains in the genera *Bacillus* and *Pseudomonas* increase plant water stress resistance by breaking down and lowering ethylene concentration in plant tissues to less toxic levels (Bal et al., 2013; Amna et al., 2019; Gamalero et al., 202).

The production of phytohormones by PGPR is a multifaceted process that contributes to enhanced plant growth, improved root system functioning, and mitigated abiotic stress conditions. These findings underscore the potential of microbe-coated fertilizers (MCF) as a sustainable and effective strategy for promoting plant growth and increasing crop productivity, particularly under conditions of water-deficit stress.

### 4.4.2. Effect of the MCF and WS on the physiological variables, plant tissue nitrogen and tuber starch content

Water-deficit stress dramatically affects several physiological processes in plants, including leaf greenness/chlorophyll content, photosynthetic rate and stomatal conductance. The changes in photosynthesis rate and nitrogen content with leaf age are closely correlated. Stomatal conductance declines more rapidly with decreasing water potential than photosynthetic activity in water-stressed potato plants (Romero et al., 2017). Measurement of photosynthesis capacity is a technique often used to assess potato plant responses to water stress. It is associated with decreasing stomatal conductance when leaf water potential values drop, affecting the capacity of plants to photosynthesize. In greenhouse conditions and under natural light, photosynthetic rates ranging from 1 to 3.6 $\mu CO_2\ m^{-2}\ s^{-1}$ indicate water stress (Vasquez-Robinet et al., 2008).

The findings of this study show that water-deficit stress significantly affected the physiological variables studied in both experiments (Table 4-7). It has been reported that severe water stress considerably decreases leaf greenness, photosynthetic rate and stomatal conductance, compared to mild or non-stressed potato plants; other studies have reported adverse effects of water stress on photosynthetic capacity due to disruption in gas exchange and stomatal closure (Jacques et al., 2020). In this work, it was observed that the photosynthetic rate was higher at the vegetative stage than at the flowering stage, which was expected across both experiments. These differences occurred because the water stress was applied to the potato plants when the plants were established, and data was collected after only 15 days of stress introduction. Other studies have reported similar findings on variations in photosynthetic rate and stomatal conductance as plants advance through growth and development (Dwelle et al., 1981; Saeidi and Abdoli, 2015)

In this study, the effects of the microbe-coated fertilizers on photosynthetic rate and stomatal conductance were not statistically significant (Table 4-7). Recent studies showed increased photosynthetic rate and stomatal conductance in potato plants grown under low pH stress, at pH 5, in response to the cell-free supernatants from *Bacillus* and *Lactobacillus*. However, no significance was observed when potato plants were exposed to pH 7 and pH 8, indicating variation in response to stress levels (Msimbira et al., 2023). In a similar study, cell-free supernatants from a *Lactobacillus* strain significantly increased potato plant photosynthetic rate and leaf greenness under 100 mM salt stress but not at higher levels of salt stress (Naamala et al., 2023).

Several factors influence plant photosynthetic rate and stomatal conductance, including the leaf chlorophyll content, leaf position, growth stage, leaf age, leaf area and timing of these measurements. Environmental factors such as water availability, light intensity, $CO_2$ concentration, humidity and temperature can also influence the photosynthetic rate and stomatal conductance (Stutte et al., 1996; Fleisher et al., 2006; Timlin et al., 2006; Tanios et al., 2018; Msimbira et al., 2022). Considering the abovementioned factors and the sensitivity of photosynthesis and stomatal conductance, as measured using a portable photosynthesis system (Li-Cor 6400, Lincoln, NE, USA), care should be taken in making broad conclusions.

SPAD is an important tool that indirectly and rapidly estimates leaf greenness through chlorophyll concentration associated with the nitrogen content of potato leaves (Deblonde and Ledent, 2001; Rudack et al., 2017). However, SPAD accuracy can be affected by factors such as chloroplast movement and leaf morphology characteristics, resulting in variations in readings among plant species (Croft and Chen, 2019).

With regard to leaf greenness and nitrogen content in plant tissue, our findings show consistently significant effects of water-deficit stress on these variables (Table 4-7). Previous studies show similar results regarding the negative impact of water stress conditions on SPAD readings estimating leaf greenness or chlorophyll content (Torres Netto et al., 2005; Genc et al., 2013). However, this was not the case for studies of mild water stress, which indicate decreases in plant leaf area, and increasing chlorophyll concentration, which is correlated with higher SPAD readings (Romero et al., 2017; Teixeira and Pereira, 2007). Studies have shown that plant resilience to water stress can be associated with decreasing cell density of shoot tissues, reducing shoot biomass, and increasing concentration of nitrogen compounds and chlorophyll. This can be monitored, at least in part, through leaf greenness and chlorophyll concentration (Saravia et al., 2016; El-Mageed et al., 2017).

In this study, we highlighted that the microbe-coated fertilizer effect was significant for leaf greenness across both experiments and only on nitrogen content in the first experiment (Table 4-7). This significance in SPAD measurements may have occurred due to the MCF treatment effects increasing potato plant ability to acquire sufficient nitrogen from the growing medium for healthy and prolonged shoot growth under water-deficit stress. Our findings are supported by Msimbira et al. (2023) and Naamala et al. (2023), who reported significant increases in leaf greenness when potato plants were grown under abiotic (acidity and salinity) stresses and treated with cell-free supernatants from the bacterial strains studied in the current research project.

The application of SPAD readings to diverse plant species and environmental conditions underscores its importance in plant research and agricultural management (Kalaji et al., 2016). However, many studies illustrate inconsistency in results form SPAD meters used as a method to estimate leaf greenness/chlorophyll and nitrogen content. Therefore, employing more accurate technologies might help better understand these physiological variables and how they respond to water stress (Ramírez et al., 2014; Rolando et al., 2015).

One of the variables considered in this study to assess MCF and water-deficit stress effects on potato, was total starch content in tubers. Our results showed no significant effects of MCF treatments on total starch in either experiment. However, water-deficit stress significantly lowered the starch content in severely stressed plants. Surprisingly, mildly stressed plants had higher starch contents than the control plants, but statistical significance was absent in both years. Our findings are consistent with Li et al. (2021), which demonstrated increased starch content in tubers of potato plants grown under mild water-deficit stress. Other studies showed a contrary result in which water stress did not significantly affect starch content, indicating that decreases in starch yield were because of reductions in tuber yield (Rudack et al., 2017). Water stress may induce a response in potato plants that affect starch concentration. Researchers have found that water stress promotes sucrose production and hinders starch production in developing potato tubers, suggesting a change in starch metabolism during water stress (Dahal et al., 2019).

Potato starch amylose concentration fluctuates because of environmental conditions, such as water availability and stress. Water stress can indirectly affect the functional properties of potato starch by influencing the amylase content. Additional research is required to understand the precise mechanisms influencing the amount and quality of starch produced by potato plants.

### 4.5. Conclusions

This study is unique and the first to report a novel technology for the application of microbial inoculations, as micobe-coated fertilizers (MCF), under greenhouse conditions. The study aimed to evaluate two ready-to-use microbial consortia (EB and EVL), coated on synthetic NPK fertilizers, under water-deficit stress conditions when applied to an economically valued crop, potato. The MCF treatments were delivered at the sowing of seed potatoes as a one-time application. The findings of this study were quite interesting, as treatment with the MCF resulted in significant positive effects on most of the studied above and below-ground agronomic variables (plant height, leaf area and shoot fresh weight, root length and root dry weight), leaf greenness (SPAD), and most importantly total tuber yield), in at least one of the two experiments. However, variables such as shoot dry weight, root fresh weight, and total nitrogen concentration in plant tissues responded inconsistently to the MCFs. Physiological variables, including photosynthetic rate, stomatal conductance and total starch content, did not respond to the MCF treatments in either experiment. The numerical values in most of the studied variables suggest a better performance of NPK+EVL than NPK+EB, although the two performed about the same under field conditions, particularly for leaf area, shoot fresh weight, photosynthetic rate, nitrogen concentration, tuber yield and starch content. In conclusion, this study evaluated the responses of all the variables to the MCF under mild and severe water-deficit deficit stress conditions without investigating the mechanisms of responses. Therefore, further research is essential to understand the effect of MCFs, and the findings reported in this study, as well as to clarify the mechanism behind the growth promotion and enhanced stress tolerance in potato plants. Examining the microbial community in the potato plant rhizosphere and analyzing root exudates in stressed plants could increase understanding and fill an important knowledge gap.

**References**


Ahluwalia, O., Singh, P. C., & Bhatia, R. (2021). A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resources, Environment and Sustainability*, *5*, 100032.

Akinci, Ş. and Lösel, D. M. (2012). Plant water-stress response mechanisms. Water Stress. https://doi.org/10.5772/29578

Al-Mahmud, A. (2014). Plant canopy, tuber yield and growth analysis of potato under moderate and severe drought condition. Journal of Plant Sciences (Science Publishing Group), 2(5), 201. https://doi.org/10.11648/j.jps.20140205.18

Aloo, B. N., Mbega, E. R., & Makumba, B. A. (2020). Rhizobacteria-based technology for sustainable cropping of potato (Solanum tuberosum L.). *Potato Research*, *63*, 157-177.

Amna, D. B. U., Sarfraz, S., Xia, Y., Kamran, M. A., Javed, M. T., et al. (2019). Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC- deaminase producing Bacillus strains under induced salinity stress. Ecotoxicol. Environ. Safety 183, 109466. https://doi: 10.1016/j.ecoenv.2019.109466

Antar, M., Gopal, P., Msimbira, L. A., Naamala, J., Nazari, M., Overbeek, W., … & Smith, D. L. (2020). Inter-organismal signaling in the rhizosphere. Rhizosphere Biology: Interactions Between Microbes and Plants, 255-293. https://doi.org/10.1007/978-981-15-6125-2_13

Antar, M., Lyu, D., Nazari, M., Shah, A., Zhou, X., & Smith, D. L. (2021). Biomass for a sustainable bioeconomy: an overview of world biomass production and utilization. Renewable and Sustainable Energy Reviews, 139, 110691. https://doi.org/10.1016/j.rser.2020.110691

Bachmann, D., Roscher, C., & Buchmann, N. (2018). how do leaf trait values change spatially and temporally with light availability in a grassland diversity experiment?. Oikos, 127(7), 935-948. https://doi.org/10.1111/oik.04533

Bacon, C. W., Palencia, E. R., & Hinton, D. M. (2014). Abiotic and biotic plant stress-tolerant and beneficial secondary metabolites produced by endophytic bacillus species. Plant Microbes Symbiosis: Applied Facets, 163-177. https://doi.org/10.1007/978-81-322-2068-8_8

Bal, H. B., Nayak, L., Das, S., & Adhya, T. K. (2013). Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. *Plant and soil*, *366*, 93-105.

Barnawal, D., Bharti, N., Pandey, S. S., Pandey, A., Chanotiya, C. S., & Kalra, A. (2017). Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiologia plantarum*, *161*(4), 502-514.

Batool T, Ali S, Seleiman M F, et al. Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. Scientific Reports, 2020, 10: 16975.

Bhattacharya, A. (2021). Effect of soil water deficit on growth and development of plants: a review. Soil Water Deficit and Physiological Issues in Plants, 393-488. https://doi.org/10.1007/978-981-33-6276-5_5

Boguszewska-Mańkowska, D., Zarzyńska, K., & Wasilewska-Nascimento, B. (2022). Potato (Solanum tuberosum L.) Plant Shoot and Root Changes under Abiotic Stresses—Yield Response. *Plants*, *11*(24), 3568.

Bouremani, N., Cherif-Silini, H., Silini, A., Bouket, A. C., Luptakova, L., Alenezi, F. N., ... & Belbahri, L. (2023). Plant growth-promoting rhizobacteria (PGPR): a rampart against the adverse effects of drought stress. *Water*, *15*(3), 418.

Chaimala, A., Jogloy, S., Vorasoot, N., Holbrook, C. C., Kvien, C. K., & Laohasiriwong, S. (2021). The variation of relative water content, spad chlorophyll meter reading, stomatal conductance, leaf area, and specific leaf area of jerusalem artichoke genotypes under different durations of terminal drought in tropical region. Journal of Agronomy and Crop Science, 209(1), 12-26. https://doi.org/10.1111/jac.12561

Cohen A C, Bottini R, Piccoli P. Role of abscisic acid producing PGPR in sustainable agriculture. Bacterial metabolites in sustainable agroecosystem, 2015: 259-282.

Cohen, A. C., Travaglia, C. N., Bottini, R., & Piccoli, P. N. (2009). Participation of abscisic acid and gibberellins produced by endophytic Azospirillum in the alleviation of drought effects in maize. *Botany*, *87*(5), 455-462.

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669-678.

Croft, H. and Chen, J. (2018). Leaf pigment content. Comprehensive Remote Sensing, 117-142. https://doi.org/10.1016/b978-0-12-409548-9.10547-0

Dahal, K., Li, X., Tai, H. H., Creelman, A., & Bizimungu, B. (2019). Improving potato stress tolerance and tuber yield under a climate change scenario – a current overview. Frontiers in Plant Science, 10. https://doi.org/10.3389/fpls.2019.00563

Dashti, N., Prithiviraj, B., Hynes, R. K., & Smith, D. L. (2000). Root and rhizosphere colonization of soybean [glycine max (l.) merr.] by plant-growth-promoting rhizobacteria at low root zone temperatures and under short-season conditions. Journal of Agronomy and Crop Science, 185(1), 15-22. https://doi.org/10.1046/j.1439-037x.2000.00394.x

Dawwam, G. E., El-Beltagy, A. E., Emara, H., Abbas, I. H., & Hassan, M. M. (2013). Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Annals of Agricultural Sciences, 58(2), 195-201. https://doi.org/10.1016/j.aoas.2013.07.007

de Lima B C, Moro A L, Santos A C P, et al. Bacillus subtilis ameliorates water stress tolerance in maize and common bean. Journal of Plant Interactions, 2019, 14(1): 432-439.

Deblonde, P. and Ledent, J. (2001). Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. European Journal of Agronomy, 14(1), 31-41. https://doi.org/10.1016/s1161-0301(00)00081-2

Demirel, U. (2023). Environmental requirements of potato and abiotic stress factors. Potato Production Worldwide, 71-86. https://doi.org/10.1016/b978-0-12-822925-5.00011-6

Dietz, K., Zörb, C., & Geilfus, C. (2021). Drought and crop yield. Plant Biology, 23(6), 881-893. https://doi.org/10.1111/plb.13304

Dwelle, R. B., Kleinkopf, G. E., & Pavek, J. J. (1981). Stomatal conductance and gross photosynthesis of potato (Solanum tuberosum L.) as influenced by irradiance, temperature, and growth stage. *Potato Research*, *24*, 49-59.

EL Sabagh, A., Islam, M. S., Hossain, A., Iqbal, M. A., Mubeen, M., Waleed, M., ... & Abdelhamid, M. T. (2022). Phytohormones as growth regulators during abiotic stress tolerance in plants. *Frontiers in Agronomy*, *4*, 765068.

El-Mageed, H. N. A., Ibrahim, M., & Elbeltagi, A. (2017). The effect of water stress on nitrogen status as well as water use efficiency of potato crop under drip irrigation system. Misr Journal of Agricultural Engineering, 34(3), 1351-1374. https://doi.org/10.21608/mjae.2017.97473

Esfahani, M., Abbasi, H., Rabiei, B., & Kavousi, M. (2007). Improvement of nitrogen management in rice paddy fields using chlorophyll meter (spad). Paddy and Water Environment, 6(2), 181-188. https://doi.org/10.1007/s10333-007-0094-6

Etesami, H., & Adl, S. M. (2020). Plant growth-promoting rhizobacteria (PGPR) and their action mechanisms in availability of nutrients to plants. *Phyto-Microbiome in stress regulation*, 147-203.

Fattahi, M., Mohammadkhani, A., Shiran, B., Baninasab, B., Ravash, R., & Gogorcena, Y. (2021). Beneficial effect of mycorrhiza on nutritional uptake and oxidative balance in pistachio (Pistacia spp.) rootstocks submitted to drought and salinity stress. *Scientia Horticulturae*, *281*, 109937.

Fleisher, D. H., Timlin, D., & Reddy, V. R. (2006). Temperature influence on potato leaf and branch distribution and on canopy photosynthetic rate. Agronomy Journal, 98(6), 1442-1452. https://doi.org/10.2134/agronj2005.0322

Gamalero, E., Lingua, G., & Glick, B. R. (2023). Ethylene, ACC, and the plant growth-promoting enzyme ACC deaminase. *Biology*, *12*(8), 1043.

Genc, L., Inalpulat, M., Kizil, U., Mirick, M., Smith, S., and Mendes, M. (2013). Determination of water stress with spectral reflectance on sweet corn (Zea mays L.) using classification tree (CT) analysis. Zemdirbyste-Agric. 100, 81–90. https://doi: 10.13080/z-a.2013.100.011

Gerayeli, N., Ravari, S. B., & Tarighi, S. (2017). Evaluation of the antagonistic potential of bacillus strains against pectobacterium carotovorum subsp. carotovorum and their role in the induction of resistance to potato soft rot infection. European Journal of Plant Pathology, 150(4), 1049-1063. https://doi.org/10.1007/s10658-017-1344-0

Gray, E. J., Lee, K., Souleimanov, A., Falco, M. R. D., Zhou, X., Ly, A., … & Smith, D. L. (2006). A novel bacteriocin, thuricin 17, produced by plant growth promoting rhizobacteria strain bacillus thuringiensis neb17: isolation and classification. Journal of Applied Microbiology, 100(3), 545-554. https://doi.org/10.1111/j.1365-2672.2006.02822.x

Gururani, M. A., Upadhyaya, C. P., Venkidasamy, B., Venkatesh, J., Nookaraju, A., & Park, S. W. (2012). Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in solanum tuberosum through inducing changes in the expression of ros-scavenging enzymes and improved photosynthetic performance. Journal of Plant Growth Regulation, 32(2), 245-258. https://doi.org/10.1007/s00344-012-9292-6

Ierna, A., & Mauromicale, G. (2022). How irrigation water saving strategy can affect tuber growth and nutritional composition of potato. *Scientia Horticulturae*, *299*, 111034.

Iwama, K. (2008). Physiology of the potato: new insights into root system and repercussions for crop management. Potato Research, 51(3-4), 333-353. https://doi.org/10.1007/s11540-008-9120-3

Jacques, M. M., Gallichand, J., Celicourt, P., & Gumiere, T. (2020). Impacts of water stress severity and duration on potato photosynthetic activity and yields. Frontiers in Agronomy, 2. https://doi.org/10.3389/fagro.2020.590312

Kalaji, H. M., Dąbrowski, P., Cetner, M. D., Samborska, I. A., Lukasik, I., Brestič, M., … & Panchal, B. (2016). A comparison between different chlorophyll content meters under nutrient deficiency conditions. Journal of Plant Nutrition, 40(7), 1024-1034. https://doi.org/10.1080/01904167.2016.1263323

Kang, S. M., Radhakrishnan, R., Khan, A. L., Kim, M. J., Park, J. M., Kim, B. R., ... & Lee, I. J. (2014). Gibberellin secreting rhizobacterium, Pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiology and Biochemistry*, *84*, 115-124.

Khan, N., Bano, A., Ali, S., & Babar, A. (2020). Crosstalk amongst phytohormones from planta and pgpr under biotic and abiotic stresses. Plant Growth Regulation, 90(2), 189-203. https://doi.org/10.1007/s10725-020-00571-x

Koch, M., Naumann, M., Pawelzik, E., Gransee, A., & Thiel, H. (2019). The importance of nutrient management for potato production part i: plant nutrition and yield. Potato Research, 63(1), 97-119. https://doi.org/10.1007/s11540-019-09431-2

Kong, Z., & Liu, H. (2022). Modification of rhizosphere microbial communities: A possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. *Frontiers in Plant Science*, *13*, 920813.

Lagzian, A., Riseh, R. S., Sarikhan, S., Ghorbani, A., Khodaygan, P., Borriss, R., … & Veltri, P. (2023). Genome mining conformance to metabolite profile of bacillus strains to control potato pathogens. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-46672-1

Lamont, J. R., Wilkins, O., Bywater-Ekegärd, M., & Smith, D. L. (2017). From yogurt to yield: potential applications of lactic acid bacteria in plant production. Soil Biology and Biochemistry, 111, 1-9. https://doi.org/10.1016/j.soilbio.2017.03.015

Li, F., Deng, H., Wang, Y., Li, X., Chen, X., Liu, L., … & Zhang, H. (2021). Potato growth, photosynthesis, yield, and quality response to regulated deficit drip irrigation under film mulching in a cold and arid environment.. https://doi.org/10.21203/rs.3.rs-142714/v1

Liu, F., Xing, S., Ma, H., Du, Z., & Ma, B. (2013). Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in Platycladus orientalis container seedlings. *Applied microbiology and biotechnology*, *97*, 9155-9164.

Lyu, D., Msimbira, L. A., Nazari, M., Antar, M., Pagé, A., Shah, A., … & Smith, D. L. (2021). The coevolution of plants and microbes underpins sustainable agriculture. Microorganisms, 9(5), 1036. https://doi.org/10.3390/microorganisms9051036

Lyu, D., Zajonc, J., Pagé, A., Tanney, C. A. S., Shah, A., Monjezi, N., … & Smith, D. L. (2021). Plant holobiont theory: the phytomicrobiome plays a central role in evolution and success. Microorganisms, 9(4), 675. https://doi.org/10.3390/microorganisms9040675

Mabood, F., Zhou, X., & Smith, D. L. (2014). Microbial signaling and plant growth promotion. Canadian Journal of Plant Science, 94(6), 1051-1063. https://doi.org/10.4141/cjps2013-148

Mamun, A. A., Neumann, G., Moradtalab, N., Ahmed, A., Dupuis, B., Darbon, G., Nawaz, F., Declerck, S., Mai, K., Vogt, W., Ludewig, U., & Weinmann, M. (2024, January 20). Microbial Consortia Versus Single-Strain Inoculants as Drought Stress Protectants in Potato Affected by the Form of N Supply. Horticulturae, 10(1), 102. https://doi.org/10.3390/horticulturae10010102

Marković, S., Milovanović, T. P., Jelušić, A., Iličić, R., Medić, O., Berić, T., … & Stanković, S. (2023). Biological control of major pathogenic bacteria of potato by bacillus amyloliquefaciens strains ss-12.6 and ss-38.4. Biological Control, 182, 105238. https://doi.org/10.1016/j.biocontrol.2023.105238

Mehrabi, F. and Sepaskhah, A. R. (2021). Leaf nitrogen, based on spad chlorophyll reading can determine agronomic parameters of winter wheat. International Journal of Plant Production, 16(1), 77-91. https://doi.org/10.1007/s42106-021-00172-2

Miransari, M. and Smith, D. L. (2014). Plant hormones and seed germination. Environmental and Experimental Botany, 99, 110-121. https://doi.org/10.1016/j.envexpbot.2013.11.005

Msimbira, L. A., Naamala, J., Antar, M., Subramanian, S., & Smith, D. L. (2022). Effect of microbial cell-free supernatants extracted from a range of ph levels on corn (zea mays l.) and tomato (solanum lycopersicum l.) seed germination and seedling growth. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.789335

Msimbira, L. A., Subramanian, S., Naamala, J., Antar, M., & Smith, D. L. (2022). Secretome analysis of the plant biostimulant bacteria strains bacillus subtilis (eb2004s) and lactobacillus helveticus (el2006h) in response to ph changes. International Journal of Molecular Sciences, 23(23), 15144. https://doi.org/10.3390/ijms232315144

Naamala, J., Msimbira, L. A., Antar, M., Subramanian, S., & Smith, D. L. (2022). Cell-free supernatant obtained from a salt tolerant bacillus amyloliquefaciens strain enhances germination and radicle length under nacl stressed and optimal conditions. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.788939

Naamala, J., Msimbira, L. A., Subramanian, S., & Smith, D. L. (2023). Lactobacillus helveticus el2006h cell-free supernatant enhances growth variables in zea mays (maize), glycine max l. merill (soybean) and solanum tuberosum (potato) exposed to nacl stress. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1075633

Nasir, M. W. and Tóth, Z. (2022). Effect of drought stress on potato production: a review. Agronomy, 12(3), 635. https://doi.org/10.3390/agronomy12030635

Naumann, M. and Pawelzik, E. (2023). Nutrient management in potato. Potato Production Worldwide, 101-120. https://doi.org/10.1016/b978-0-12-822925-5.00018-9

O'Callaghan, M., Ballard, R., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: limitations and opportunities. Soil Use and Management, 38(3), 1340-1369. https://doi.org/10.1111/sum.12811

Olmedo, C.A., Thuar, A.M. (2002). Greenhouse Evaluation of Plant Growth Promoting Rhizobacteria (PGPR). In: Pedrosa, F.O., Hungria, M., Yates, G., Newton, W.E. (eds) Nitrogen Fixation: From Molecules to Crop Productivity. Current Plant Science and Biotechnology in Agriculture, vol 38. Springer, Dordrecht. https://doi.org/10.1007/0-306-47615-0_340

Omafuvbe, B. O., Feruke-Bello, Y. M., & Adeleke, E. O. (2011). Aerobic Mesophilic Bacteria Associated With Irish Potato (Solanum Tuberosum L.) Spoilage and Their Susceptibility Pattern to Lactic Acid Bacteria and Antibiotics. *Ife Journal of Science*, *13*(2), 347-353.

Petrushin, I. S., Filinova, N. V., & Gutnik, D. I. (2024). Potato Microbiome: Relationship with Environmental Factors and Approaches for Microbiome Modulation. *International Journal of Molecular Sciences*, *25*(2), 750.

Porcel, R., Zamarreño, Á. M., García-Mina, J. M., & Aroca, R. (2014). Involvement of plant endogenous aba in bacillus megaterium pgpr activity in tomato plants. BMC Plant Biology, 14(1), 36. https://doi.org/10.1186/1471-2229-14-36

Raheem, A., Shaposhnikov, A., Belimov, A. A., Dodd, I. C., & Ali, B. (2018). Auxin production by rhizobacteria was associated with improved yield of wheat (Triticum aestivum L.) under drought stress. *Archives of Agronomy and Soil Science*, *64*(4), 574-587.

Raman, J., Kim, J., Choi, K. R., Eun, H., Yang, D., Ko, Y., … & Kim, S. (2022). Application of lactic acid bacteria (lab) in sustainable agriculture: advantages and limitations. International Journal of Molecular Sciences, 23(14), 7784. https://doi.org/10.3390/ijms23147784

Ramírez, D. A., Yactayo, W., Gutiérrez, R., Mareš, V., Mendiburu, F. D., Posadas, A., … & Quiroz, R. (2014). Chlorophyll concentration in leaves is an indicator of potato tuber yield in water-shortage conditions. Scientia Horticulturae, 168, 202-209. https://doi.org/10.1016/j.scienta.2014.01.036

Riaz, M., Akhtar, N., Msimbira, L. A., Antar, M., Ashraf, S., Khan, S. N., … & Smith, D. L. (2022). Neocosmospora rubicola, a stem rot disease in potato: characterization, distribution and management. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.953097

Riaz, M., Mahmood, R., Antar, M., Akhtar, N., Khan, S. N., Anjum, M. A., … & Smith, D. L. (2022). A bacterial consortium and synthetic fertilizer based biocontrol approach against potato rot disease "neocosmospora rubicola". Frontiers in Agronomy, 4. https://doi.org/10.3389/fagro.2022.828070

Rolando, J. L., Ramírez, D. A., Yactayo, W., Monneveux, P., & Quiroz, R. (2015). Leaf greenness as a drought tolerance related trait in potato (solanum tuberosum l.). Environmental and Experimental Botany, 110, 27-35. https://doi.org/10.1016/j.envexpbot.2014.09.006

Romero, Á. P., Alarcón, A., Valbuena, R. I., & Galeano, C. H. (2017). Physiological assessment of water stress in potato using spectral information. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01608

Rudack, K., Seddig, S., Sprenger, H., Köhl, K., Uptmoor, R., & Ordon, F. (2017). Drought stress-induced changes in starch yield and physiological traits in potato. Journal of Agronomy and Crop Science, 203(6), 494-505. https://doi.org/10.1111/jac.12224

Saeidi, M., & Abdoli, M. (2015). Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. Journal of Agricultural Science and Technology, 17(4), 885-898.

Salvi, P., Manna, M., Kaur, H., Thakur, T., Gandass, N., Bhatt, D., … & Muthamilarasan, M. (2021). Phytohormone signaling and crosstalk in regulating drought stress response in plants. Plant Cell Reports, 40(8), 1305-1329. https://doi.org/10.1007/s00299-021-02683-8

Santoyo, G., Guzmán-Guzmán, P., Parra-Cota, F. I., Santos-Villalobos, S. d. l., Orozco-Mosqueda, M. d. C., & Glick, B. R. (2021). Plant growth stimulation by microbial consortia. Agronomy, 11(2), 219. https://doi.org/10.3390/agronomy11020219

Saravia, D., Farfán-Vignolo, E. R., Gutiérrez, R., Mendiburu, F. D., Schafleitner, R., Bonierbale, M., … & Khan, M. A. (2016). Yield and physiological response of potatoes indicate different strategies to cope with drought stress and nitrogen fertilization. American Journal of Potato Research, 93(3), 288-295. https://doi.org/10.1007/s12230-016-9505-9

Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., … & Smith, D. L. (2021). Pgpr in agriculture: a sustainable approach to increasing climate change resilience. Frontiers in Sustainable Food Systems, 5. https://doi.org/10.3389/fsufs.2021.667546

Singh, D., Thapa, S., Singh, J. P., Mahawar, H., Saxena, A. K., Singh, S. K., ... & Chakdar, H. (2024). Prospecting the Potential of Plant Growth-Promoting Microorganisms for Mitigating Drought Stress in Crop Plants. *Current Microbiology*, *81*(3), 1-17.

Smith, D. L., Gravel, V., & Yergeau, É. (2017). Editorial: signaling in the phytomicrobiome. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00611

Steglińska, A., Kołtuniak, A., Motyl, I., Berłowska, J., Czyżowska, A., Cieciura-Włoch, W., … & Gutarowska, B. (2022). Lactic acid bacteria as biocontrol agents against potato (solanum tuberosum l.) pathogens. Applied Sciences, 12(15), 7763. https://doi.org/10.3390/app12157763

Stewart, D. C., Costa, C., Dwyer, L. M., Smith, D. L., Hamilton, R. I., & L., B. (2003). Canopy structure, light interception, and photosynthesis in maize. Agronomy Journal, 95(6), 1465-1474. https://doi.org/10.2134/agronj2003.1465

Stutte, G. W., Yorio, N., & Wheeler, R. M. (1996). Interacting effects of photoperiod and photosynthetic photon flux on net carbon assimilation and starch accumulation in potato leaves. Journal of the American Society for Horticultural Science, 121(2), 264-268. https://doi.org/10.21273/jashs.121.2.264

Tanios, S., Eyles, A., Tegg, R. S., & Wilson, C. R. (2018). Potato tuber greening: a review of predisposing factors, management and future challenges. American Journal of Potato Research, 95(3), 248-257. https://doi.org/10.1007/s12230-018-9648-y

Teixeira, J., and Pereira, S. (2007). High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. Environ. Exp. Bot. 60, 121–126. https://doi: 10.1016/j.envexpbot.2006.09.003

Timlin, D., Rahman, S. A., Baker, J. T., Reddy, V. R., Fleisher, D. H., & Quebedeaux, B. (2006). Whole plant photosynthesis, development, and carbon partitioning in potato as a function of temperature. Agronomy Journal, 98(5), 1195-1203. https://doi.org/10.2134/agronj2005.0260

Torres Netto, A., Campostrini, E., Oliveira, J. G., and Bressan-Smith, R. E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Sci. Hort. 104, 199–209. https://doi: 10.1016/j.scienta.2004.08.013

Ullah, A., Manghwar, H., Shaban, M., Khan, A. H., Akbar, A., Ali, U., ... & Fahad, S. (2018). Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environmental Science and Pollution Research*, *25*, 33103-33118.

Vacheron, J., Desbrosses, G., Bouffaud, M., Touraine, B., Moënne-Loccoz, Y., Müller, D., … & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00356

Vargas, R., Kenney, A., & Bilinski, T. (2019). Variable influences of water availability and rhizobacteria on the growth of schizachyrium scoparium (little bluestem) at different ages. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.00860

Vasquez-Robinet, C., Mane, S. P., Ulanov, A. V., Watkinson, J. I., Stromberg, V. K., De Koeyer, D., et al. (2008). Physiological and molecular adaptations to drought in Andean potato genotypes. J. Exp. Bot. 59, 2109–2123. doi: 10.1093/jxb/ern073

Velivelli, S. L. S., Sessitsch, A., & Prestwich, B. D. (2014). The role of microbial inoculants in integrated crop management systems. Potato Research, 57(3-4), 291-309. https://doi.org/10.1007/s11540-014-9278-9

Verbon, E. and Liberman, L. (2016). Beneficial microbes affect endogenous mechanisms controlling root development. Trends in Plant Science, 21(3), 218-229. https://doi.org/10.1016/j.tplants.2016.01.013

Vrieze, M. D., Germanier, F., Vuille, N., & Weisskopf, L. (2018). Combining different potato-associated pseudomonas strains for improved biocontrol of phytophthora infestans. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.02573

Vrieze, M. D., Varadarajan, A. R., Schneeberger, K., Bailly, A., Rohr, R. P., Ahrens, C. H., … & Weisskopf, L. (2020). Linking comparative genomics of nine potato-associated pseudomonas isolates with their differing biocontrol potential against late blight. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.00857

Wagg, C., Hann, S., Kupriyanovich, Y., & Li, S. (2021). Timing of short period water stress determines potato plant growth, yield and tuber quality. Agricultural Water Management, 247, 106731. https://doi.org/10.1016/j.agwat.2020.106731

Wang, Z., Li, Y., Zhao, Y., Zhuang, L., Yu, Y., Wang, M., … & Wang, Q. (2021). A microbial consortium-based product promotes potato yield by recruiting rhizosphere bacteria involved in nitrogen and carbon metabolisms. Microbial Biotechnology, 14(5), 1961-1975. https://doi.org/10.1111/1751-7915.13876

Wasaya, A., Manzoor, S., Yasir, T. A., Sarwar, N., Mubeen, K., Ismail, I. A., … & Sabagh, A. E. (2021). Evaluation of fourteen bread wheat (triticum aestivum l.) genotypes by observing gas exchange parameters, relative water and chlorophyll content, and yield attributes under drought stress. Sustainability, 13(9), 4799. https://doi.org/10.3390/su13094799

Wilkinson, S., Weston, A. K., & Marks, D. J. (2019). Stabilising urea amine nitrogen increases potato tuber yield by increasing chlorophyll content, reducing shoot growth rate and increasing biomass partitioning to roots and tubers. Potato Research, 63(2), 217-239. https://doi.org/10.1007/s11540-019-09436-x

Yadav, B., Jogawat, A., Gnanasekaran, P., Kumari, P., Lakra, N., Lal, S. K., ... & Narayan, O. P. (2021). An overview of recent advancement in phytohormones-mediated stress management and drought tolerance in crop plants. *Plant Gene*, *25*, 100264.

Yaghoubian, I., Antar, M., Ghassemi, S., Modarres-Sanavy, S. A. M., & Smith, D. L. (2022). The effects of hydro-priming and colonization with piriformospora indica and azotobacter chroococcum on physio-biochemical traits, flavonolignans and fatty acids composition of milk thistle (silybum marianum) under saline conditions. Plants, 11(10), 1281. https://doi.org/10.3390/plants11101281

Yasmin, F., Othman, R., & Maziz, M. N. H. (2020). Yield and nutrient content of sweet potato in response of plant growth-promoting rhizobacteria (PGPR) inoculation and N fertilization. *Biological*, *13*(1), 1117.

Zafar-ul-Hye, M., Bhutta, T., Shaaban, M., Hussain, S., Qayyum, M., Aslam, U., … & Zahir, Z. (2019). Influence of plant growth promoting rhizobacterial inoculation on wheat productivity under soil salinity stress. Phyton, 88(2), 119-129. https://doi.org/10.32604/phyton.2019.06570

Zafar-ul-Hye, M., Danish, S., Abbas, M., Ahmad, M., & Munir, T. M. (2019). ACC deaminase producing PGPR Bacillus amyloliquefaciens and Agrobacterium fabrum along with biochar improve wheat productivity under drought stress. *Agronomy*, *9*(7), 343.

Zaheer, M. S., Raza, M. A. S., Saleem, M. F., Erinle, K. O., Iqbal, R., & Ahmad, S. (2019). Effect of rhizobacteria and cytokinins application on wheat growth and yield under normal vs drought conditions. *Communications in Soil Science and Plant Analysis*, *50*(20), 2521-2533.

Zamioudis C, Mastranesti P, Dhonukshe P, et al. Unraveling root developmental programs initiated by beneficial Pseudomonas spp. bacteria. Plant Physiology, 2013, 162(1): 304-318.

Zarzyńska, K., Boguszewska-Mańkowska, D., & Nosalewicz, A. (2017). Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress. Plant, Soil and Environment, 63(4), 159-164. https://doi.org/10.17221/4/2017-pse

Zhang, F. and Smith, D. L. (2002). Interorganismal signaling in suboptimum environments: the legume-rhizobia symbiosis. Advances in Agronomy, 125-161. https://doi.org/10.1016/s0065-2113(02)76004-5

Zia, R., Nawaz, M. S., Siddique, M., Hakim, S., & Imran, A. (2021). Plant survival under drought stress: implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiological Research, 242, 126626. https://doi.org/10.1016/j.micres.2020.126626

# Chapter 5 : General Discussion, Conclusions and Future Directions

## 5.1. General Discussion

Climate change and depletion of water and land resources are putting pressure on the global food supply, which must increase to feed an estimated 9.7 billion people worldwide by 2050 (Kaur and Chauhan, 2023). This situation is exacerbated by increased fertilizer prices and mismanagement of fertilizer allocation. Fertilizer prices have risen in many countries, and small-hold agricultural producers in many areas lack access to fertilizers (Michelson et al., 2023; Mihoub et al., 2023). Rising uncertainty and high fertilizer prices are affecting food production prospects and agricultural producer livelihoods. To address this situation, initiatives should encourage alternative plant mineral nutrient sources for more sustainable crop production, and food security in changing climates (Molina-Santiago and Matilla, 2019; Prasad et al., 2020). These strategies support crop and food production and, in some cases, can mitigate the negative impacts of biotic and abiotic stresses.

Microbe-coated fertilizers (MCFs) could be an alternative fertilizer source, showing potential in this study when applied to potato plants under field and greenhouse conditions. The MCF contains plant PGPR, which are known as beneficial microbes, improving plant growth and enhancing soil health and fertility (Gray and Smith 2005). Utilizing plant-microbe interactions as alternative crop growth enhancement methods can improve crop productivity and reduce the environmental impact of synthetic chemical fertilizers (Bala, 2022).

These biologicals can be applied with synthetic fertilizers at seeding or as microbial cell-derived compounds for seed treatment and foliar spray application. Regardless of their means of application, these technologies have the potential to make Canadian crop production systems more climate change resilient by helping plants deal with abiotic stresses, especially those associated with developing climate change conditions. Therefore, we designed this study to determine if microbial inoculants can contribute to plant growth promotion and improved yield when potato plants are grown in diverse environmental conditions in field experiments and controlled environment (greenhouse) conditions in the presence of water-deficit stress. We think that microbial treatments help potato plants by activating direct mechanisms, including nutrient availability and use efficiency. Microbial-consortia-based fertilizer coatings evaluated in this study contained *Bacillus*, *Pseudomonas*, and *Lactobacillus*; strains from these genera have shown potential as growth-promotion agents for sustainable agriculture.

*Bacillus* species have been shown to promote potato plant growth through various mechanisms that boost plant development and productivity. It has been reported that combining *Bacillus subtilis* and *Bacillus megatorium* with humic acid enhanced potato yield through mixed culture inoculation (Ruzzi et al., 2015; Kaymak et al., 2023; Saharan and Nehra, 2011). The efficacy of these strains was attributed to their capacity to perform nitrogen fixation, phosphate solubilization, and production of significant amounts of IAA, resulting in, among other effects, enhanced root elongation and lateral root growth. In a study conducted by Devi et al. (2016), the authors reported that biological products containing *Bacillus subtilis, Bacillus amyloliquefaciens* and *Bacillus pumilus* could greatly help control common scab disease in crops, including potato and lead to improved yield.

In addition, *Bacillus* strains have been discovered that possess general plant growth-promoting capacity, as demonstrated by Calvo et al. (2010), who documented the potential plant growth-promoting properties of *Bacillus* isolates from the potato rhizosphere in Andean soils. These traits include the capacity to make nitrogen available, dissolve phosphorus, and generate phytohormones, all enhancing plant growth. In addition, Liu et al. (2022). emphasized the potential of *Bacillus* spp. strains to increase potato plant growth and water use efficiency under reduced irrigation conditions. This underscores the diverse potential roles of *Bacillus* strains in enhancing potato plant growth. A study demonstrated the impact of inoculating the potato rhizosphere with a *Bacillus subtilis* strain altered the microbial community by influencing plant-microbe interactions, contributing to improved yield and quality of potato tubers (Song et al., 2021; Zhang et al., 2023).

*Pseudomonas* species promote potato plant output through various processes that improve plant development and productivity. Multiple studies have highlighted *Pseudomonas* strain interactions in the rhizosphere and their beneficial effects on plant growth and development through phosphorus solubilization, siderophore and phytohormone production (Howie and Echandi 1992). It was observed that the abundance of *Pseudomonads* added as inoculants increased towards the end of the growing season, suggesting a possible contribution to plant growth and productivity promotion (Kloepper et al., 1980; Lifshitz et al., 1987; Andreate et al., 2009; Diallo et al., 2011). In addition, compounds and active substances derived from *Pseudomonas rhodesiae* showed antimicrobial properties which can contribute to plant health and development (Sah et al., 2021; Oteino et al., 2023). Studies have shown the crucial role of fluorescent *Pseudomonas* in siderophore production, which is vital in iron-deficient soils. *Pseudomonas* species have been shown to have a potential role in suppressing plant pathogens, indicating their potential to improve crop vigour and productivity (O'Sullivan and O'Gara, 1992; Omidvari et al., 2010; Syed et al., 2023).

The potential of *Lactobacillus* species as plant growth promotion agents has yet to be studied in depth. Therefore, current understanding is very limited regarding their potential for use in plant growth enhancement and overall contribution to the productivity of agricultural systems. A recent study conducted by Panetto et al. (2023) evaluated the potential of *Lactobacillus acidophilus* to improve nitrogen fixation, phosphorus solubilization, phytohormone and siderophore production, as well as some specific enzyme activities. The results showed that *Lactobacillus acidophilus* could fix nitrogen, solubilize phosphorus, synthesize siderophores and produce IAA. In addition to these activities, authors have reported increases in some growth variables. In addition, increased germination rate, shoot branching, and shoot and root growth of tomato were reported in response to plant treatment with *Lactobacillus acidophilus*, *Lactobacillus plantarum,* and *Lactobacillus sp.*, strains isolated from dairy sources (Hamed et al., 2011; Lutz et al., 2012; Limanska et al., 2013; Blainski et al., 2018). Similar results were observed when cucumber plants were inoculated with *Lactobacillus casei*, *Lactobacillus lactis* and *Lactobacillus plantarum (*Rzhevskaya et al., 2015; Kang et al., 2015). These findings indicate that *Lactobacillus* species have the capacity to enhance plant growth and contribute to increased productivity.

*Bacillus* species promote potato plant growth through nitrogen fixation, phosphate solubilization, phytohormone production, disease suppression, and overall plant health and productivity improvement. *Pseudomonas* species stimulate growth by generating bioactive chemicals, increasing nutrient availability, inhibiting pathogens, and establishing stronger plant-microbe interactions. *Lactobacillus* species encourage growth by affecting immunological responses and root development and producing bioactive compounds, indicating their potential as valuable resources in sustainable agriculture systems.

Our study further describes the importance of PGPR in improved crop productivity and for further development of sustainable agricultural production systems. Microbial inoculations might not be a complete replacement for synthetic fertilizers, but introducing microbes to plants can decrease the need for chemical fertilizers; nitrogen fertilizer is produced using energy-intensive methods, while mineral fertilizers such as phosphorus and potassium are extracted from non-renewable sources. Sustainable agriculture approaches strive to reduce crop fertilizer needs by, for instance, utilizing nutrients naturally found in the soil and transforming them into accessible forms through beneficial soil microbes with the use of microbial inoculants, which was the case in this study.

## 5.2. Revisiting the Objectives

Objectives one and two of this study were focused on evaluating the field performance and efficacy of the (EVL and EB) microbial consortia in a low-nutrient containing sandy soil, as well as at alternate seeding dates (soil temperature affected by early and late seeding) during 2018 and 2019 growing seasons. The results showed that the MCF significantly increased leaf area (15.3 - 23.1%), dry biomass (11.6 - 21.9%), total nitrogen concentration (3.9 - 13.8%), marketable tuber size (9.1 - 21.9%) and total tuber yield (9 - 20.8%), compared to non-inoculated control plants. Other evaluated variables also increased considerably in response to the MCF treatments but without statistical significance ($p > 0.05$). These results indicate the efficiency of both microbial consortia, when coated onto synthetic fertilizers, introducing a new technology for microbe-based agriculture and sustainable plant production.

To address the third objective, the MCF was evaluated for greenhouse performance to determine efficiency and consistency in potato production and the effect of mild and severe water deficit stress on this. The findings of this study indicated that the MCF showed positive effects on most of the studied above and below-ground agronomic variables (plant height, leaf area and shoot fresh weight, root length and root dry weight), leaf greenness (SPAD), and most importantly total tuber yield, in at least one experiment. However, variables such as shoot dry weight, root fresh weight, and total nitrogen in plant tissues responded inconsistently to the MCFs. Physiological variables, including photosynthetic rate, stomatal conductance and total starch content, did not respond to the MCF treatments in both experiments. Numerical increases in specific agronomic and developmental variable values indicated a better performance of NPK+EVL than NPK+EB, although the two performed about the same under field conditions, particularly for leaf area, shoot fresh weight, photosynthetic rate, nitrogen concentration, tuber yield and starch content.

These studies evaluated the responses of all the variables to MCF under field and greenhouse conditions without investigating the mechanisms of responses. Therefore, further research is essential to understand the effect of MCFs, and the findings reported in this study to clarify the mechanism behind the growth promotion and enhanced stress tolerance in potato plants. This could increase our understanding of these effects and fill some of the current knowledge gaps.

## 5.3. General Conclusions

The growing global human population requires increased food production, but climate change and limited farmland make this challenging. Chemical applications and molecular techniques have been used to address this challenge. A more sustainable agricultural approach is PGPR, which establish mutualistic interactions with host plants, improving nutrient absorption, stress resistance to stress (including water-deficit stress), and plant development through a range of mechanisms. Roots provide a stable habitat for microbe growth. Plant growth-promoting rhizobacteria are an under-exploited mechanism to enhance yield and improve crop plant resilience. However, environmental conditions such as water availability, soil temperature, pH, and fertility affect PGPR efficiency, altering the ability of cultivated plants to produce biomass and food materials under climate change-related extremes.

Water stress significantly affects the physiology and biochemistry of potato plants, affecting their growth, development, and ability to withstand environmental challenges.

Understanding plant agronomic and physiologic responses to environmental stresses is crucial for developing strategies to enhance their resilience and productivity given increasingly water-limited conditions associated with climate change.

Using synthetic fertilizers in crop production to supply plants with nutrients and improve plant productivity has been extensively investigated, but this approach has a negative environmental impact. Therefore, microbe-coated fertilizers can be a significant resource for plant growth improvement by establishing a beneficial association between plants and their rhizosphere microbial community, the phytomicrobiome. This can lead to improved nutrient uptake and increased tolerance to harsh environmental stresses (e.g. water-deficit stress) that can negatively affect crop growth and development from crop seeding to harvesting.

In this study, we presented the potential of microbe-coated fertilizers (MCF) for enhancing agronomic variables and yield components of potato plants grown in the field and under controlled environment conditions and the potential interaction with water stress conditions. However, more work is needed to expand the utilization of this novel technology in a broader range of crops in various regions, with variable soils and climate regimes, to validate its efficacy.

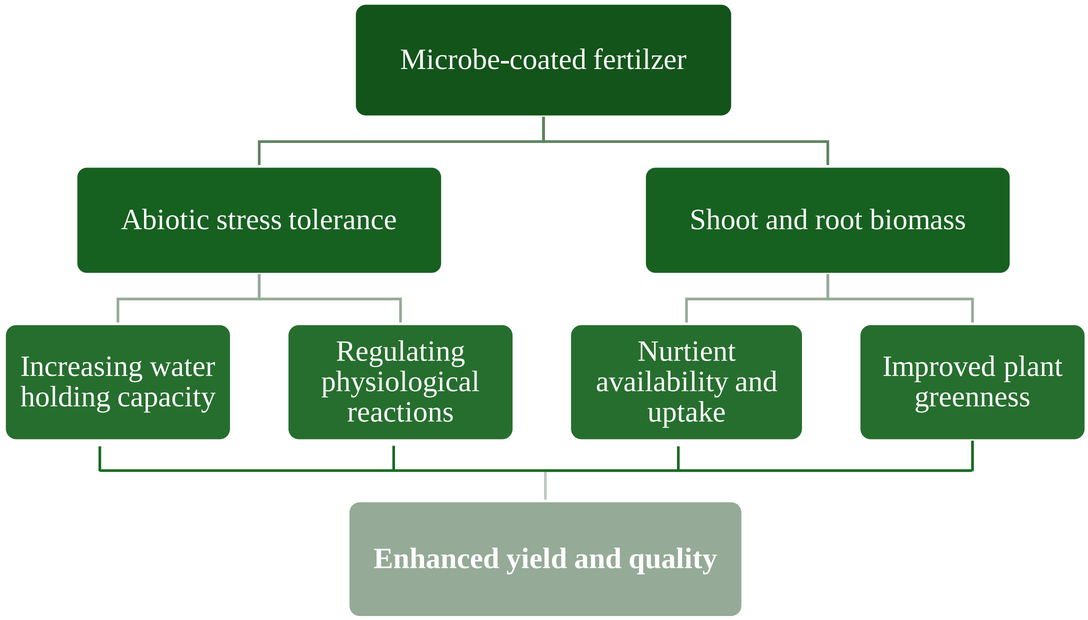



*Figure 5-1: Microbe-coated fertilizer and its potential role in this study*

### 5.4. Future Directions

This study evaluated the efficiency of a novel technology approach for microbial application: Microbe-coated fertilizer (MCF). We demonstrated positive responses by many crop agronomic variables in MCF-treated plants. However, the study did not involve understanding the mechanisms behind improved potato plant growth and development under field and greenhouse conditions. Therefore, we briefly provide a set of suggestions regarding knowledge gaps that remain to be investigated and filled:

- The MCF should be tested on various crops and soil types to validate its performance more widely. It would be interesting to determine its effects on plants subjected to other abiotic stresses (e.g. salinity, acidity, more extreme temperatures) to monitor the response of agronomic variables and overall plant growth, development and yield.

- Root-associated microbial communities: It is very important to investigate the viability of the MCF once they are applied. Tracking microbes after application will help to understand the viability of microbes after being coated on synthetic fertilizers.
- Proteomics studies will help us understand the protein profiles of plants grown in stressed and unstressed field conditions, and how these are affected by treatment with microbial consortia, and so will contribute to understanding how microbial communities (rather than single microbes) could contribute to plant stress responses and stress resilience.
- Metagenomics work would illustrate how microbial communities in plant tissues and the rhizosphere, are affected by phytomicrobiome (PGPR) inoculations and environmental conditions through providing insights into the complex relationships between plants and the diverse microbial communities that inhabit their surroundings.
- Transcriptomics is another potential tool in plant-microbe interaction studies, as it enables the comprehensive analysis of gene expression patterns in both plants and microbes during their interactions. This approach has the potential to facilitate the identification of specific genes involved in plant-microbe signaling, nutrient solubilization, enhanced abiotic stress tolerance and pest defence responses.
- Metabolomics: It would be interesting to investigate the metabolomic profile of the microbial consortia and consortium-inoculated plants to identify the compounds associated with growth promotion. This technique could identify metabolites involved in plant defence mechanisms and pathogen virulence and identify bioactive compounds produced by beneficial microbes that enhance plant stress resilience and overall health.

**Reference**

Adesemoye, A. O., & Kloepper, J. W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency. *Applied microbiology and biotechnology*, *85*, 1-12. https://doi.org/10.1007/s00253-009-2196-0

Adesemoye, A., Torbert, H., & Kloepper, J. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology*, 58(4), 921-929. https://doi.org/10.1007/s00248-009-9531-y

Adesemoye, A., Torbert, H., & Kloepper, J. (2010). Increased plant uptake of nitrogen from 15n-depleted fertilizer using plant growth-promoting rhizobacteria. *Applied Soil Ecology*, 46(1), 54-58. https://doi.org/10.1016/j.apsoil.2010.06.010

Adhikari, S., Srivastava, A., Sharma, M., & Shrestha, A. (2020). Response of potato clones to planting dates in pokhara, kaski, nepal. Journal of Agriculture and Natural Resources, 3(2), 175-183. https://doi.org/10.3126/janr.v3i2.32503

Ahmad, H. M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A. N., Gul, B., ... & Wang, X. (2022). Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: A review. *Frontiers in Plant Science, 13,* 875774. https://doi.org/10.3389/fpls.2022.875774

Aloo, B. (2021). *Characterization of rhizobacteria and their formulation into biofertilizers for potato (solanum tuberosum l.) growth promotion in Tanzania* (Doctoral dissertation, NM-AIST). https://doi.org/10.58694/20.500.12479/1329

Aloo, B. N., Mbega, E. R., & Makumba, B. A. (2020). Rhizobacteria-based technology for sustainable cropping of potato (Solanum tuberosum L.). *Potato Research*, *63*, 157-177.

Aloo, B. N., Mbega, E. R., Makumba, B. A., Hertel, R., & Daniel, R. (2021). Molecular identification and in vitro plant growth-promoting activities of culturable Potato (Solanum tuberosum L.) rhizobacteria in Tanzania. *Potato Research*, *64*, 67-95.

Ambrosini, A., de Souza, R., & Passaglia, L. M. (2016). Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. *Plant and Soil*, *400*, 193-207.

Amin, H. A., El Kammar, H. F., Saied, S. M., & Soliman, A. M. (2023). Effect of Bacillus subtilis on potato virus Y (PVY) disease resistance and growth promotion in potato plants. *European Journal of Plant Pathology*, *167*(4), 743-758.

Andreote, F. D., Araújo, W. L., Azevedo, J. L. d., Elsas, J. D. v., Rocha, U. N. d., & Overbeek, L. v. (2009). Endophytic colonization of potato (solanum tuberosum l.) by a novel competent bacterial endophyte, pseudomonas putida strain p9, and its effect on associated bacterial communities. Applied and Environmental Microbiology, 75(11), 3396-3406. https://doi.org/10.1128/aem.00491-09

Antar, M., Gopal, P., Msimbira, L. A., Naamala, J., Nazari, M., Overbeek, W., … & Smith, D. L. (2020). Inter-organismal signaling in the rhizosphere. Rhizosphere Biology: Interactions Between Microbes and Plants, 255-293. https://doi.org/10.1007/978-981-15-6125-2_13

Arora, S., & Jha, P. N. (2023). Drought-tolerant Enterobacter bugandensis WRS7 induces systemic tolerance in Triticum aestivum L.(Wheat) under drought conditions. *Journal of Plant Growth Regulation*, *42*(12), 7715-7730.

Babalola, O. and Fasusi, O. (2021). The multifaceted plant-beneficial rhizobacteria toward agricultural sustainability. Plant Protection Science, 57(2), 95-111. https://doi.org/10.17221/130/2020-pps

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in plant science*, *9*, 402666. https://doi.org/10.3389/fpls.2018.01473

Bala, K. (2022). Microbial fertilizer as an alternative to chemical fertilizer in modern agriculture. Beneficial Microorganisms in Agriculture, 111-130. https://doi.org/10.1007/978-981-19-0733-3_4

Baligar, V. C., Fageria, N. K., & He, Z. L. (2001). Nutrient use efficiency in plants. *Communications in soil science and plant analysis*, *32*(7-8), 921-950. https://doi.org/10.1081/css-100104098

Bamdad, H., Papari, S., Lazarovits, G., & Berruti, F. (2021). Soil amendments for sustainable agriculture: microbial organic fertilizers. Soil Use and Management, 38(1), 94-120. https://doi.org/10.1111/sum.12762

Barnawal, D., Bharti, N., Pandey, S., Pandey, A., Chanotiya, C., & Kalra, A. (2017). Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and tactr1/tadreb2 expression. Physiologia Plantarum, 161(4), 502-514. https://doi.org/10.1111/ppl.12614

Bastas, K. K. (2023). Bacterial diseases of potato and their control. In *Potato Production Worldwide* (pp. 179-197). Academic Press.

Batool, T., Ali, S., Seleiman, M., Naveed, N., Ali, A., Ahmed, K., … & Mubushar, M. (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. *Scientific Reports*, *10*(1), 16975. https://doi.org/10.1038/s41598-020-73489-z

Billah, M. and Bano, A. (2014). Role of plant growth promoting rhizobacteria in modulating the efficiency of poultry litter composting with rock phosphate and its effect on growth and yield of wheat. Waste Management & Research the Journal for a Sustainable Circular Economy, 33(1), 63-72. https://doi.org/10.1177/0734242x14559593

Blainski, J. M. L., Neto, A. C. d. R., Schimidt, E. C., Voltolini, J. A., Rossi, M. J., & Piero, R. M. D. (2018). Exopolysaccharides from lactobacillus plantarum induce biochemical and physiological alterations in tomato plant against bacterial spot. Applied Microbiology and Biotechnology, 102(11), 4741-4753. https://doi.org/10.1007/s00253-018-8946-0

Borriss, R., & Nord Reet, U. G. (2017). Plant growth promoting bacteria–early investigations, present state and future prospects. *International journal of plant research*, *30*, 211.

Bouhia, Y., Hafidi, M., Ouhdouch, Y., Zeroual, Y., & Lyamlouli, K. (2023). Organo-Mineral Fertilization Based on Olive Waste Sludge Compost and Various Phosphate Sources Improves Phosphorus Agronomic Efficiency, Zea mays Agro-Physiological Traits, and Water Availability. *Agronomy*, *13*(1), 249.

Bradshaw, J. E., & Bradshaw, J. E. (2021). Domestication to twenty-first-century potato cultivars. *Potato breeding: Theory and practice*, 3-51. https://doi.org/10.1007/978-3-030-64414-7_1

Buch, K., Saikanth, D. R. K., Singh, B. V., Mallick, B., Pandey, S. K., Prabhavathi, N., & Satapathy, S. N. (2023). Impact of Agrochemicals on Beneficial Microorganisms and Human Health. *International Journal of Environment and Climate Change*, *13*(10), 1135-1145.

Bukhat, S., Imran, A., Javaid, S., Shahid, M., Majeed, A., & Naqqash, T. (2020). Communication of plants with microbial world: exploring the regulatory networks for pgpr mediated defense signaling. Microbiological Research, 238, 126486. https://doi.org/10.1016/j.micres.2020.126486

Caliskan, M. E., Caliskan, S. E. V. G. I., & Arioglu, H. A. L. I. S. (2004, June). Effects of presprouting and planting date on growth and yield of potato crop in a Mediterranean type environment. In *Agronomy Section Meeting of the European Association for Potato Research* (pp. 23-27).

Calvo, P., Ormeño-Orrillo, E., Martínez-Romero, E., & Zúñiga, D. (2010). Characterization of bacillus isolates of potato rhizosphere from andean soils of peru and their potential pgpr characteristics. Brazilian Journal of Microbiology, 41(4), 899-906. https://doi.org/10.1590/s1517-83822010000400008

Cao, H., Ding, R., Kang, S., Du, T., Tong, L., Zhang, Y., ... & Shukla, M. K. (2023). Drought, salt, and combined stresses in plants: effects, tolerance mechanisms, and strategies. *Advances in Agronomy*, *178*, 107-163.

Cao, M., Narayanan, M., Shi, X., Chen, X., Li, Z., & Ma, Y. (2023). Optimistic contributions of plant growth-promoting bacteria for sustainable agriculture and climate stress alleviation. *Environmental Research*, *217*, 114924.

Caradonia, F., Buti, M., Flore, A., Gatti, R., Morcia, C., Terzi, V., … & Milc, J. (2022). Characterization of leaf transcriptome of grafted tomato seedlings after rhizospheric inoculation with azospirillum baldaniorum or paraburkholderia graminis. Agronomy, 12(10), 2537. https://doi.org/10.3390/agronomy12102537

Castro-Sowinski, S., Herschkovitz, Y., Okon, Y., & Jurkevitch, E. (2007). Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. *FEMS microbiology letters*, *276*(1), 1-11.

Castulo-Rubio, D., Alejandre-Ramírez, N., Orozco-Mosqueda, M., Santoyo, G., Macías-Rodríguez, L., & Valencia-Cantero, E. (2015). Volatile organic compounds produced by the rhizobacterium arthrobacter agilis umcv2 modulate sorghum bicolor (strategy ii plant) morphogenesis and sbfro1 transcription in vitro. Journal of Plant Growth Regulation, 34(3), 611-623. https://doi.org/10.1007/s00344-015-9495-8

Chandran, H., Meena, M., & Swapnil, P. (2021). Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture. Sustainability, 13(19), 10986. https://doi.org/10.3390/su131910986

Chaudhry, S., & Sidhu, G. P. S. (2022). Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Reports*, *41*(1), 1-31.

Chea, L., Pfeiffer, B., Schneider, D., Daniel, R., Pawelzik, E., & Naumann, M. (2021). Morphological and metabolite responses of potatoes under various phosphorus levels and their amelioration by plant growth-promoting rhizobacteria. International Journal of Molecular Sciences, 22(10), 5162. https://doi.org/10.3390/ijms22105162

Chizen, C. (2020). *The effects of nitrogen fertilizer rates and planting date on greenhouse gas emissions and potato production in Delta, British Columbia* (Doctoral dissertation, University of British Columbia).

Chouhan, G. K., Verma, J. P., Jaiswal, D. K., Mukherjee, A., Singh, S., Pereira, A. P. d. A., … & Singh, B. K. (2021). Phytomicrobiome for promoting sustainable agriculture and food security: opportunities, challenges, and solutions. Microbiological Research, 248, 126763. https://doi.org/10.1016/j.micres.2021.126763

Compant, S., Van Der Heijden, M. G., & Sessitsch, A. (2010). Climate change effects on beneficial plant–microorganism interactions. *FEMS microbiology ecology*, *73*(2), 197-214.

Contreras-Liza, S., Ramírez, R., & Olivas, D. (2022). Production of potato seed tubers under the effect of trichoderma sp. and rhizobacteria in greenhouse conditions. Revista De Ciências Agroveterinárias, 21(4), 419-427. https://doi.org/10.5965/223811712142022419

Crossay, T., Majorel, C., Redecker, D., Gensous, S., Medevielle, V., Durrieu, G., ... & Amir, H. (2019). Is a mixture of arbuscular mycorrhizal fungi better for plant growth than single-species inoculants?. *Mycorrhiza*, *29*, 325-339.

Cruz, S., Filho, A., Nascimento, A., & Vargas, P. (2016). Mineral nutrition and yield of sweet potato according to phosphorus doses. Comunicata Scientiae, 7(2), 183. https://doi.org/10.14295/cs.v7i2.958

Dahal, K., Li, X. Q., Tai, H., Creelman, A., & Bizimungu, B. (2019). Improving potato stress tolerance and tuber yield under a climate change scenario–a current overview. *Frontiers in plant science*, *10*, 416712. https://doi.org/10.3389/fpls.2019.00563

Darabi, A. (2013). Effect of planting date on total and marketable yield of potato cultivars in Khuzestan Province in Iran. *Seed and Plant Production Journal*, *29*(3), 369-386.

Daryanto, S., Wang, L., & Jacinthe, P. A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agricultural Water Management*, *179*, 18-33. https://doi.org/10.1016/j.agwat.2016.04.022

Das, P. P., Singh, K. R., Nagpure, G., Mansoori, A., Singh, R. P., Ghazi, I. A., … & Singh, J. (2022). Plant-soil-microbes: a tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. Environmental Research, 214, 113821. https://doi.org/10.1016/j.envres.2022.113821

Davies, K., & Whitbread, R. (1989). Factors affecting the colonisation of a root system by fluorescent Pseudomonads: The effects of water, temperature and soil microflora. *Plant and Soil*, *116*, 247-256.

Dawwam, G. E., Elbeltagy, A., Emara, H. M., Abbas, I. H., & Hassan, M. M. (2013). Beneficial effect of plant growth-promoting bacteria isolated from the roots of potato plant. *Annals of Agricultural Sciences*, *58*(2), 195-201.

Devi, A. R., Kotoky, R., Pandey, P., & Sharma, G. D. (2016). Application of Bacillus spp. for sustainable cultivation of potato (Solanum tuberosum L.) and the benefits. *Bacilli and agrobiotechnology*, 185-211.

Diallo, S., Crépin, A., Barbey, C., Orange, N., Burini, J., & Latour, X. (2011). Mechanisms and recent advances in biological control mediated through the potato rhizosphere. FEMS Microbiology Ecology, 75(3), 351-364. https://doi.org/10.1111/j.1574-6941.2010.01023.x

Dietz, K., Zörb, C., & Geilfus, C. (2021). Drought and crop yield. Plant Biology, 23(6), 881-893. https://doi.org/10.1111/plb.13304

Dimkpa, C. O., Fugice, J., Singh, U., & Lewis, T. D. (2020). Development of fertilizers for enhanced nitrogen use efficiency–Trends and perspectives. *Science of the Total Environment*, *731*, 139113. https://doi.org/10.1016/j.scitotenv.2020.139113

Dobosy, P., Endrédi, A., Sandil, S., Vetési, V., Rékási, M., Takács, T., & Záray, G. (2020). Biofortification of potato and carrot with iodine by applying different soils and irrigation with iodine-containing water. *Frontiers in Plant Science*, *11*, 593047.

Eid, M. A., Abdel-Salam, A. A., Salem, H. M., Mahrous, S. E., Seleiman, M. F., Alsadon, A. A., ... & Ibrahim, A. A. (2020). Interaction effects of nitrogen source and irrigation regime on tuber quality, yield, and water Use efficiency of Solanum tuberosum L. *Plants*, *9*(1), 110.

Ekin, Z. (2019). Integrated use of humic acid and plant growth promoting rhizobacteria to ensure higher potato productivity in sustainable agriculture. Sustainability, 11(12), 3417. https://doi.org/10.3390/su11123417

Elmaghraby, M. M., Abdellatif, A. A., Amer, M. N., & Sahu, P. K. (2023). Enhancing Productivity Through Multiple Microbial Inoculants. In *Metabolomics, Proteomes and Gene Editing Approaches in Biofertilizer Industry* (pp. 117-137). Singapore: Springer Nature Singapore.

El-Naqma, K. (2020). The Role of Humate Substances in Controlling Synergism and Antagonism of Nutrients Uptake by Potato Plants. *Environment, Biodiversity and Soil Security*, *4*(2020), 149-165.

El-Ramady, H. R., Alshaal, T. A., Amer, M., Domokos-Szabolcsy, É., Elhawat, N., Prokisch, J., & Fári, M. (2014). Soil quality and plant nutrition. *Sustainable Agriculture Reviews 14: Agroecology and Global Change*, 345-447. https://doi.org/10.1007/978-3-319-06016-3_11

Franco, J., Main, G., Navia, O., Ortuño, N., & Herbas, J. (2016). Improving productivity of traditional andean small farmers by bio-rational soil management: i. the potato case. Revista Latinoamericana De La Papa, 16(2), 270-290. https://doi.org/10.37066/ralap.v16i2.182

Frommel, M. I., Nowak, J., & Lazarovits, G. (1991). Growth enhancement and developmental modifications of in vitro grown potato (solanum tuberosum spp. tuberosum) as affected by a nonfluorescent pseudomonas sp. *Plant Physiology*, 96(3), 928-936. https://doi.org/10.1104/pp.96.3.928

Gelaye, Y., Ademe, D., & Alemayehu, M. (2021). Effect of Phosphorus and potassium fertilizer rates on growth and yield of potato (Solanum tuberosum L.), Northwestern Ethiopia. *Journal of Horticulture*, *8*(4), 1-8.

George, T. S., Taylor, M. A., Dodd, I. C., & White, P. J. (2017). Climate change and consequences for potato production: a review of tolerance to emerging abiotic stress. *Potato Research*, *60*, 239-268. https://doi.org/10.1007/s11540-018-9366-3

Gonzali, S., Kiferle, C., & Perata, P. (2017). Iodine biofortification of crops: agronomic biofortification, metabolic engineering and iodine bioavailability. *Current opinion in biotechnology*, *44*, 16-26.

Goswami, M., & Suresh, D. E. K. A. (2020). Plant growth-promoting rhizobacteria—alleviators of abiotic stresses in soil: a review. *Pedosphere*, *30*(1), 40-61. https://doi.org/10.1016/s1002-0160(19)60839-8

Gouda, S., Das, G., Paramithiotis, S., Shin, H., & Patra, J. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological Research, 206, 131-140. https://doi.org/10.1016/j.micres.2017.08.016

Gray, E. J. and Smith, D. L. (2005). Intracellular and extracellular pgpr: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biology and Biochemistry, 37(3), 395-412. https://doi.org/10.1016/j.soilbio.2004.08.030

Gupta, G., Parihar, S., Ahirwar, N., & Snehi, S. (2015). Plant growth promoting rhizobacteria (pgpr): current and future prospects for development of sustainable agriculture. Journal of Microbial & Biochemical Technology, 07(02). https://doi.org/10.4172/1948-5948.1000188

Gupta, S., Bhattacharyya, P., Kulkarni, M. G., & Doležal, K. (2023). Growth regulators and biostimulants: upcoming opportunities. *Frontiers in Plant Science*, *14*, 1209499.

Hafez, E., Omara, A., & Ahmed, A. (2019). The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. Agronomy, 9(9), 524. https://doi.org/10.3390/agronomy9090524

Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., ... & Imran, A. (2021). Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems*, *5*, 617157.

Hanif, M. K., Hameed, S., Imran, A., Naqqash, T., Shahid, M., & Elsas, J. D. v. (2015). Isolation and characterization of a î²-propeller gene containing phosphobacterium bacillus subtilis strain kps-11 for growth promotion of potato (solanum tuberosum l.). Frontiers in Microbiology, 06. https://doi.org/10.3389/fmicb.2015.00583

Harkhani, K., & Sharma, A. K. (2023). Alleviation of Drought Stress by Plant Growth-Promoting Rhizobacteria (PGPR) in Crop Plants: A Review. *Communications in Soil Science and Plant Analysis*, 1-24.

Hasanuzzaman, M., Raihan, M. R. H., Nowroz, F., & Fujita, M. (2022). Insight into the mechanism of salt-induced oxidative stress tolerance in soybean by the application of Bacillus subtilis: Coordinated actions of osmoregulation, ion homeostasis, antioxidant defense, and methylglyoxal detoxification. *Antioxidants*, *11*(10), 1856.

Henagamage, A. P. (2022). Characterization of plant growth promoting rhizobacteria isolated from potato (Solanum tuberosum L.) rhizosphere. *Journal of Science-FAS-SEUSL*, *3*(02), 01-10.

Hossain, M. E., Shahrukh, S., & Hossain, S. A. (2022). Chemical fertilizers and pesticides: impacts on soil degradation, groundwater, and human health in Bangladesh. In *Environmental degradation: challenges and strategies for mitigation* (pp. 63-92). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-95542-7_4

Howie, W. J. and Echandi, E. (1983). Rhizobacteria: influence of cultivar and soil type on plant growth and yield of potato. Soil Biology and Biochemistry, 15(2), 127-132. https://doi.org/10.1016/0038-0717(83)90091-3

Islam, M., Azad, O., Rana, M., Ryu, B., & Lim, Y. (2021). Led light pre-treatment improves pre-basic seed potato (solanum tuberosum l. cv. golden king) production in the aeroponic system. Agronomy, 11(8), 1627. https://doi.org/10.3390/agronomy11081627

Jain, A., Chakraborty, J., & Das, S. (2020). Underlying mechanism of plant–microbe crosstalk in shaping microbial ecology of the rhizosphere. Acta Physiologiae Plantarum, 42(1). https://doi.org/10.1007/s11738-019-3000-0

Jansa, J., Smith, F. A., & Smith, S. E. (2008). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi?. *New Phytologist*, *177*(3), 779-789.

Jiao, X., Takishita, Y., Zhou, G., & Smith, D. L. (2021). Plant associated rhizobacteria for biocontrol and plant growth enhancement. *Frontiers in plant science*, *12*, 634796. https://doi.org/10.3389/fpls.2021.634796

Jones, J. L. (1990). Effect of date of planting on contrasting potato varieties. *Dissertation Abstracts International. B, Sciences and Engineering*, *51*(1).

Jones, J. L., & Allen, E. J. (1983). Effects of date of planting on plant emergence, leaf growth, and yield in contrasting potato varieties. *The Journal of Agricultural Science*, *101*(1), 81-95.

Joshi, B., Chaudhary, A., Singh, H., & Kumar, P. A. (2020). Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (Oryza sativa L.). *Plant and Soil*, *457*, 225-240. https://doi.org/10.1007/s11104-020-04730-x

Jovovic, Z., Dolijanović, Ž., Spalevic, V., Dudic, B., Przulj, N., Velimirovic, A., … & Popovic, V. (2021). Effects of liming and nutrient management on yield and other parameters of potato productivity on acid soils in montenegro. Agronomy, 11(5), 980. https://doi.org/10.3390/agronomy11050980

Kabiraj, A., Majhi, K., Halder, U., Let, M., & Bandopadhyay, R. (2020). Role of Plant Growth-Promoting Rhizobacteria (PGPR) for crop stress management. *Sustainable agriculture in the era of climate change*, 367-389.

Kalatskaja, J. N., Baliuk, N. V., Rybinskaya, K. I., Herasimovich, K. M., Yalouskaya, N. A., Yarullina, L. G., & Tsvetkov, V. O. (2023). Increasing Potato Sustainability to PVY under Water Deficiency by Bacillus Bacteria with Salicylic Acid and Methyl Jasmonate. *International Journal of Plant Biology*, *14*(1), 312-328.

Kamran, S., Shahid, I., Baig, D., Rizwan, M., Malik, K., & Mehnaz, S. (2017). Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.02593

Kang, S. M., Khan, A. L., Waqas, M., You, Y. H., Kim, J. H., Kim, J. G., ... & Lee, I. J. (2014). Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus. *Journal of Plant Interactions*, *9*(1), 673-682.

Kang, S. M., Radhakrishnan, R., Khan, A. L., Park, J., Lee, S., … & Lee, I. (2014). Cucumber performance is improved by inoculation with plant growth-promoting microorganisms. Acta Agriculturae Scandinavica, Section B — Soil &Amp; Plant Science, 65(1), 36-44. https://doi.org/10.1080/09064710.2014.960889

Katoch, V., Shavnam, Sharma, S., & Negi, M. (2023). Utilization of Plant Growth-Promoting Rhizobacteria (PGPR) for Managing Recently Reported Potato Cyst Nematodes, Globodera spp. in North Himalayan Regions of India. *Potato Research*, 1-16.

Kaur, S., & Chauhan, B. S. (2023). Challenges and opportunities to sustainable crop production. *Plant Small RNA in Food Crops*, 25-43.

Kaymak, H., Ürüşan, A., Tiraşci, S., & Kaşka, M. (2023). Role of N2-Fixing Plant Growth-Promoting Rhizobacteria in Some Selected Vegetables. *Türk Tarım-Gıda Bilim ve Teknoloji dergisi*, *11*(6).

Keswani, C., Prakash, O., Bharti, N., Vílchez, J. I., Sansinenea, E., Lally, R. D., ... & Singh, H. B. (2019). Re-addressing the biosafety issues of plant growth promoting rhizobacteria. *Science of the Total Environment*, *690*, 841-852.

Khan, N., & Mehmood, A. (2023). Revisiting climate change impacts on plant growth and its mitigation with plant growth promoting rhizobacteria. *South African Journal of Botany*, *160*, 586-601.

Khan, S. T. (2022). Consortia-based microbial inoculants for sustaining agricultural activities. *Applied Soil Ecology*, *176*, 104503.

Khare, E., & Arora, N. K. (2014). Effects of soil environment on field efficacy of microbial inoculants. In *Plant microbes symbiosis: applied facets* (pp. 353-381). New Delhi: Springer India.

Kloepper, J. W., Schroth, M. N., & Miller, T. D. (1980). Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology, 70(11), 1078. https://doi.org/10.1094/phyto-70-1078

Knowles, N. and Knowles, L. (2006). Manipulating stem number, tuber set, and yield relationships for northern- and southern-grown potato seed lots. Crop Science, 46(1), 284-296. https://doi.org/10.2135/cropsci2005.05-0078

Koch, M., Naumann, M., Pawelzik, E., Gransee, A., & Thiel, H. (2020). The importance of nutrient management for potato production Part I: Plant nutrition and yield. *Potato research*, *63*, 97-119.

Kołodziejczyk, M. and Gwóźdź, K. (2022). Effect of plant growth regulators on potato tuber yield and quality. Plant Soil and Environment, 68(8), 375-381. https://doi.org/10.17221/215/2022-pse

Kour, D., Khan, S. S., Kaur, T., Kour, H., Singh, G., Yadav, A., & Yadav, A. N. (2022). Drought adaptive microbes as bioinoculants for the horticultural crops. *Heliyon*, *8*(5). https://doi.org/10.1016/j.heliyon.2022.e09493

Kumar, R., Sagar, V., Verma, V. C., Kumari, M., Gujjar, R. S., Goswami, S. K., ... & Prasad, P. V. (2023). Drought and Salinity Stress Induced Physio-Biochemical Changes in Sugarcane: An Overview of Tolerance Mechanism and Mitigating Approaches. *Frontiers in Plant Science*, *14*, 1225234.

Kumar, S., Biswas, S. K., Kumar, A., Rajput, V. D., Kumar, R., Kumar, S., ... & Kumar, R. (2023). Effect of Integrated Disease Management (IDM) Practices on Disease Severity and Incidence of Common Scab of Potato. *Potato Research*, 1-22.

Li, Y., Wang, J., Tang, J., Wang, E., Pan, Z., Pan, X., & Hu, Q. (2021). Optimum planting date and cultivar maturity to optimize potato yield and yield stability in North China. *Field Crops Research*, *269*, 108179.

Lifshitz, R., Kloepper, J. W., Kozlowski, M., Simonson, C., Carlson, J. E., Tipping, E. M., … & Zaleska, I. (1987). Growth promotion of canola (rapeseed) seedlings by a strain of pseudomonas putida under gnotobiotic conditions. Canadian Journal of Microbiology, 33(5), 390-395. https://doi.org/10.1139/m87-068

Limanska, Nataliya, Tetiana Ivanytsia, Olena Basiul, Kateryna Krylova, Vanessa Biscola, Jean Marc Chobert, Volodymyr Ivanytsia, and Thomas Haert. (2013). Effect of lactobacillus plantarum on germination and growth of tomato seedlings. Acta Physiologiae Plantarum, 35(5), 1587-1595. https://doi.org/10.1007/s11738-012-1200-y

Liu, J., Zhang, J., Zhu, M., Wan, H., Chen, Z., Yang, N., … & Liu, F. (2022). Effects of plant growth promoting rhizobacteria (pgpr) strain bacillus licheniformis with biochar amendment on potato growth and water use efficiency under reduced irrigation regime. Agronomy, 12(5), 1031. https://doi.org/10.3390/agronomy12051031

Liu, X. and Zhang, H. (2015). The effects of bacterial volatile emissions on plant abiotic stress tolerance. Frontiers in Plant Science, 6. https://doi.org/10.3389/fpls.2015.00774

Liu, X., Mei, S., & Salles, J. F. (2023). Inoculated microbial consortia perform better than single strains in living soil: A meta-analysis. *Applied Soil Ecology*, *190*, 105011.

Lyu, D., Msimbira, L. A., Nazari, M., Antar, M., Pagé, A., Shah, A., … & Smith, D. L. (2021). The coevolution of plants and microbes underpins sustainable agriculture. Microorganisms, 9(5), 1036. https://doi.org/10.3390/microorganisms9051036

Lyu, D., Zajonc, J., Pagé, A., Tanney, C. A. S., Shah, A., Monjezi, N., … & Smith, D. L. (2021). Plant holobiont theory: the phytomicrobiome plays a central role in evolution and success. Microorganisms, 9(4), 675. https://doi.org/10.3390/microorganisms9040675

Ma, S., Wang, Y., & Teng, W. (2023). Bacillus velezensis K-9 as a Potential Biocontrol Agent for Managing Potato Scab. *Plant Disease*, PDIS-12.

Mabood, F., Souleimanov, A., Khan, W. Z., & Smith, D. L. (2006). Jasmonates induce nod factor production by bradyrhizobium japonicum. Plant Physiology and Biochemistry, 44(11-12), 759-765. https://doi.org/10.1016/j.plaphy.2006.10.025

Mabood, F., Zhou, X., & Smith, D. L. (2014). Microbial signaling and plant growth promotion. *Canadian journal of plant science*, *94*(6), 1051-1063.

Magray, M. M. (2021). Plant Growth Regulators and Their Role in Horticultural Crop Production and Development. *Research Management in Horticultural Crops*, 31.

Mahalakshmi, S., Vijayapriya, M., & Pandeeswari, N. (2019). Studies on developing PGPR consortium with improved shelf life. *Journal of Pharmacognosy and Phytochemistry*, *8*(2S), 545-548.

Malboobi, M. A., Behbahani, M., Madani, H., Owlia, P., Deljou, A., Yakhchali, B., … & Hassanabadi, H. (2009). Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. World Journal of Microbiology and Biotechnology, 25(8), 1479-1484. https://doi.org/10.1007/s11274-009-0038-y

Marković, S., Milovanović, T. P., Jelušić, A., Iličić, R., Medić, O., Berić, T., & Stanković, S. (2023). Biological control of major pathogenic bacteria of potato by Bacillus amyloliquefaciens strains SS-12.6 and SS-38.4. *Biological Control*, *182*, 105238.

Marpaung, A. E., & Susilowati, D. N. (2021, August). Isolation and identification of phosphate solubilising bacteria from potato rhizosphere on andisol. In *IOP Conference Series: Earth and Environmental Science* (Vol. 810, No. 1, p. 012041). IOP Publishing.

Martini, M., Ferniah, R., Nurchayati, Y., & Jannah, S. (2022). Effects of plant growth-promoting rhizobacteria and trichoderma sp. on potato growth on medium plains. Kne Life Sciences. https://doi.org/10.18502/kls.v7i3.11157

Meena, R. K., Singh, R. K., Singh, N. P., Meena, S. K., & Meena, V. S. (2015). Isolation of low temperature surviving plant growth–promoting rhizobacteria (PGPR) from pea (Pisum sativum L.) and documentation of their plant growth promoting traits. *Biocatalysis and agricultural biotechnology*, *4*(4), 806-811.

Mellidou, I., & Karamanoli, K. (2022). Unlocking PGPR-mediated abiotic stress tolerance: What lies beneath. *Frontiers in Sustainable Food Systems*, *6*.

Michelson, H., Gourlay, S., Lybbert, T., & Wollburg, P. (2023). Purchased agricultural input quality and small farms. *Food Policy*, *116*, 102424.

Mihoub, A., Ahmad, I., & Radicetti, E. (2023). Editorial: alternative fertilizer harnessing plant-microbe interactions (afpmi) for improved soil and plantnutrient management. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1333927

Mix, K., Lopes, V.L. & Rast, W. Growing season expansion and related changes in monthly temperature and growing degree days in the Inter-Montane Desert of the San Luis Valley, Colorado. *Climatic Change* **114**, 723–744 (2012). https://doi.org/10.1007/s10584-012-0448-y

Molina-Santiago, C. and Matilla, M. A. (2019). Chemical fertilization: a short-term solution for plant productivity?. Microbial Biotechnology, 13(5), 1311-1313. https://doi.org/10.1111/1751-7915.13515

Mondal, S., Halder, S. K., Yadav, A. N., & Mondal, K. C. (2020). Microbial consortium with multifunctional plant growth-promoting attributes: future perspective in agriculture. *Advances in plant microbiome and sustainable agriculture: functional annotation and future challenges*, 219-258. https://doi.org/10.1007/978-981-15-3204-7_10

Monneveux, P., Ramírez, D. A., & Pino, M. T. (2013). Drought tolerance in potato (S. tuberosum L.): Can we learn from drought tolerance research in cereals?. *Plant Science*, *205*, 76-86. https://doi.org/10.1016/j.plantsci.2013.01.011

Morcillo, R. and Manzanera, M. (2021). The effects of plant-associated bacterial exopolysaccharides on plant abiotic stress tolerance. Metabolites, 11(6), 337. https://doi.org/10.3390/metabo11060337

Msimbira, L. A., & Smith, D. L. (2020). The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems*, *4*, 106.

Msimbira, L. A., Naamala, J., Antar, M., Subramanian, S., & Smith, D. L. (2022). Effect of microbial cell-free supernatants extracted from a range of ph levels on corn (zea mays l.) and tomato (solanum lycopersicum l.) seed germination and seedling growth. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.789335

Mushtaq, Z., Asghar, H. N., Zahir, Z. A., & Maqsood, M. (2021). Characterization of rhizobacteria for growth promoting traits and their potential to increase potato yield. *Pakistan Journal of Agricultural Sciences*, *58*(1).

Mystkowska, I. and Matyszczak, A. (2019). Content and uptake of selected microelements with potato tuber yield treated with biostimulators. Journal of Ecological Engineering, 20(8), 65-70. https://doi.org/10.12911/22998993/110815

Mystkowska, I., Zarzecka, K., Ginter, A., Gugała, M., & Sikorska, A. (2023). Total and protein nitrogen content in potato tubers under the influence of various care and nutrition methods with the use of biostimulants. Journal of Ecological Engineering, 24(2), 247-255. https://doi.org/10.12911/22998993/157019

Naamala, J., Msimbira, L. A., Antar, M., Subramanian, S., & Smith, D. L. (2022). Cell-free supernatant obtained from a salt tolerant bacillus amyloliquefaciens strain enhances germination and radicle length under nacl stressed and optimal conditions. Frontiers in Sustainable Food Systems, 6. https://doi.org/10.3389/fsufs.2022.788939

Naqqash, T., Hameed, S., Imran, A., Hanif, M. K., Majeed, A., & Elsas, J. D. v. (2016). Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.00144

Naqqash, T., Imran, A., Hameed, S., Shahid, M., Majeed, A., Iqbal, J., … & Malik, K. (2020). First report of diazotrophic brevundimonas spp. as growth enhancer and root colonizer of potato. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-69782-6

Nazari, M., & Smith, D. L. (2020). A PGPR-produced bacteriocin for sustainable agriculture: a review of thuricin 17 characteristics and applications. *Frontiers in plant science*, *11*, 916.

Obidiegwu, J. E., Bryan, G. J., Jones, H. G., & Prashar, A. (2015). Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Frontiers in plant science*, *6*, 151023. https://doi.org/10.3389/fpls.2015.00542

Omidvari, M., Sharifi, R. A., Ahmadzadeh, M., & Dahaji, P. A. (2010). Role of fluorescent pseudomonads siderophore to increase bean growth factors. Journal of Agricultural Science, 2(3). https://doi.org/10.5539/jas.v2n3p242

Orozco-Mosqueda, M. D. C., Kumar, A., Babalola, O. O., & Santoyo, G. (2023). Rhizobiome Transplantation: A Novel Strategy beyond Single-Strain/Consortium Inoculation for Crop Improvement. *Plants*, *12*(18), 3226.

O'Sullivan, D. and O'Gara, F. (1992). Traits of fluorescent pseudomonas spp. involved in suppression of plant root pathogens. Microbiological Reviews, 56(4), 662-676. https://doi.org/10.1128/mmbr.56.4.662-676.1992

Oswald, A., Velez, P., Dávila, D., & Pineda, J. (2010). Evaluating soil rhizobacteria for their ability to enhance plant growth and tuber yield in potato. Annals of Applied Biology, 157(2), 259-271. https://doi.org/10.1111/j.1744-7348.2010.00421.x

Oteino, N., Lally, R. D., Kiwanuka, S., Lloyd, A. T., Ryan, D., Germaine, K. J., … & Dowling, D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic pseudomonas isolates. Frontiers in Microbiology, 6. https://doi.org/10.3389/fmicb.2015.00745

Otieno, H. M., & Mageto, E. K. (2021). A review on yield response to nitrogen, potassium and manure applications in potato (Solanum tuberosum L.) production. *Archives of Agriculture and Environmental Science*, *6*(1), 80-86. https://doi.org/10.26832/24566632.2021.0601011

Pandey, P., Bisht, S., Sood, A., Aeron, A., Sharma, G. D., & Maheshwari, D. K. (2012). Consortium of plant-growth-promoting bacteria: future perspective in agriculture. *Bacteria in agrobiology: plant probiotics*, 185-200.

Pandey, V. V., Bhattacharya, A., & Pandey, A. (2023). Plant growth-promoting microbiomes: History and their role in agricultural crop improvement. *Plant-Microbe Interaction-Recent Advances in Molecular and Biochemical Approaches*, 1-44.https://doi.org/10.1016/b978-0-323-91875-6.00012-8

Panetto, L. D., Soares, J. D. R., Santos, C. H. B., Frezarin, E. T., Sales, L. R., Andrade, L. A. d., … & Rigobelo, E. C. (2023). Lactic bacteria with plant-growth-promoting properties in potato. Microbiology Research, 14(1), 279-288. https://doi.org/10.3390/microbiolres14010022

Papik, J., Folkmanova, M., Polivkova-Majorova, M., Šuman, J., & Uhlík, O. (2020). The invisible life inside plants: deciphering the riddles of endophytic bacterial diversity. Biotechnology Advances, 44, 107614. https://doi.org/10.1016/j.biotechadv.2020.107614

Pathak, D., Lone, R., Koul, K.K. (2017). Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR) Association in Potato (*Solanum tuberosum* L.): A Brief Review. In: Kumar, V., Kumar, M., Sharma, S., Prasad, R. (eds) Probiotics and Plant Health. Springer, Singapore. https://doi.org/10.1007/978-981-10-3473-2_18

Peralta, I. E., Clausen, A. M., Zorrilla, C., Ames, M., Digilio, A., & Rodriguez, F. (2021). Wild and cultivated potato species diversity, taxonomy, and conservation. *The Wild Solanums Genomes*, 51-94. https://doi.org/10.1007/978-3-030-30343-3_4

Petropoulos, S. A., Fernandes, Â., Polyzos, N., Antoniadis, V., Barros, L., & CFR Ferreira, I. (2020). The impact of fertilization regime on the crop performance and chemical composition of potato (Solanum tuberosum L.) cultivated in central Greece. *Agronomy*, *10*(4), 474.

Petrushin, I. S., Filinova, N. V., & Gutnik, D. I. (2024). Potato Microbiome: Relationship with Environmental Factors and Approaches for Microbiome Modulation. *International Journal of Molecular Sciences*, *25*(2), 750.

Pii, Y., Graf, H., Valentinuzzi, F., Cesco, S., & Mimmo, T. (2018). The effects of plant growth-promoting rhizobacteria (pgpr) on the growth and quality of strawberries. Acta Horticulturae, (1217), 231-238. https://doi.org/10.17660/actahortic.2018.1217.29

Prasad, S., Malav, L. C., Choudhary, J., Kannojiya, S., Kundu, M., Kumar, S., … & Yadav, A. N. (2020). Soil microbiomes for healthy nutrient recycling. Environmental and Microbial Biotechnology, 1-21. https://doi.org/10.1007/978-981-15-6949-4_1

Preeti, V., & Shahi, S. K. (2015). Isolation and characterization of bacterial isolates from potato rhizosphere as potent plant growth promoters. *International Journal of Current Microbiology and Applied Sciences*, *4*(3), 521-528.

Pyasi, R., Bhatt, D., & Singh, R. (2020). Response of potato crop to different combinations of inorganic fertilizers, organic manure and biofertilizers. *Journal of Pharmacognosy and Phytochemistry*, *9*(3), 1789-1792.

Rafedzi, E., Bakar, M., Bharudin, I., Kamarudin, S., Bakar, F., & Murad, A. (2022). Draft genome sequence of the drought-tolerant plant growth-promoting rhizobacterium bacillus altitudinis ukm rb11, isolated from upland paddy rhizosphere. Microbiology Resource Announcements, 11(10). https://doi.org/10.1128/mra.00617-22

Rahi, P. (2017). Phytomicrobiome: a reservoir for sustainable agriculture. Mining of Microbial Wealth and MetaGenomics, 117-132. https://doi.org/10.1007/978-981-10-5708-3_7

Rahman, H., Azad, O., Islam, M., Rana, S., Li, K., & Lim, Y. (2021). Production of potato (solanum tuberosum l.) seed tuber under artificial led light irradiation in plant factory. Plants, 10(2), 297. https://doi.org/10.3390/plants10020297

Rahman, M. M., Ali, E., Khan, A. A., Akanda, A. M., Uddin, M. K., Hashim, U., … & Hamid, S. B. A. (2012). Isolation, characterization, and identification of biological control agent for potato soft rot in bangladesh. The Scientific World Journal, 2012, 1-6. https://doi.org/10.1100/2012/723293

Ramírez-Cariño, H. F., Morales, I., Guadarrama-Mendoza, P. C., Tlilayatzi-Bello, A. A., & Valadez-Blanco, R. (2024). Beneficial traits of crop-associated rhizobacteria toward plant growth promotion, biofertilization, and nutrient enhancement of harvested agro-products. In *Bacterial Secondary Metabolites* (pp. 299-334). Elsevier.

Recep, K., Fikrettin, S., Erkol, D., & Cafer, E. (2009). Biological control of the potato dry rot caused by Fusarium species using PGPR strains. *Biological Control*, *50*(2), 194-198.

Reddy, C. A., & Saravanan, R. S. (2013). Polymicrobial multi-functional approach for enhancement of crop productivity. In *Advances in applied microbiology* (Vol. 82, pp. 53-113). Academic Press.

Redecker, D., Kodner, R., & Graham, L. E. (2000). Glomalean fungi from the Ordovician. Science, 289(5486), 1920-1921. https://doi.org/10.1126/science.289.5486.1920

Rehman, A., Sandhu, J., Alam, M., & Mehboob, S. (2021). A perspective on common scab (streptomyces scabiei) disease management strategies in potato crop. International Journal of Phytopathology, 10(3), 195-201. https://doi.org/10.33687/phytopath.010.03.3795

Rehman, F., Pervez, A., Khattak, B., & Ahmad, R. (2018). Plant growth promoting rhizobacteria impact ontypha latifoliaandphragmites australisgrowth and dissolved oxygen. Clean - Soil Air Water, 46(8), 1700353. https://doi.org/10.1002/clen.201700353

Ren, H., Huang, B., Calvo, L., Miesel, J., Yan, L., & Lv, C. (2020). Biochar and rhizobacteria amendments improve several soil properties and bacterial diversity. Microorganisms, 8(4), 502. https://doi.org/10.3390/microorganisms8040502

Riaz, M., Akhtar, N., Msimbira, L. A., Antar, M., Ashraf, S., Khan, S. N., … & Smith, D. L. (2022). Neocosmospora rubicola, a stem rot disease in potato: characterization, distribution and management. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.953097

Riaz, M., Mahmood, R., Antar, M., Akhtar, N., Khan, S. N., Anjum, M. A., … & Smith, D. L. (2022). A bacterial consortium and synthetic fertilizer based biocontrol approach against potato rot disease “neocosmospora rubicola”. Frontiers in Agronomy, 4. https://doi.org/10.3389/fagro.2022.828070

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M. L., ... & Daffonchio, D. (2015). Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental microbiology*, *17*(2), 316-331. https://doi.org/10.1111/1462-2920.12439

Ruzzi, M., & Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 124-134.

S, S. and Thomas, J. (2019). Screening growth promoting traits in maize using native rhizobacteria through multiple approaches. Journal of Pure and Applied Microbiology, 13(1), 355-360. https://doi.org/10.22207/jpam.13.1.39

Saghafi, D., Delangiz, N., Lajayer, B., & Ghorbanpour, M. (2019). An overview on improvement of crop productivity in saline soils by halotolerant and halophilic pgprs. 3 Biotech, 9(7). https://doi.org/10.1007/s13205-019-1799-0

Sah, S., Krishnani, S., & Singh, R. (2021). Pseudomonas mediated nutritional and growth promotional activities for sustainable food security. Current Research in Microbial Sciences, 2, 100084. https://doi.org/10.1016/j.crmicr.2021.100084

Saharan, B. S., & Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sci Med Res*, *21*(1), 30.

Saleem, M. H., Wang, X., Parveen, A., Perveen, S., Mehmood, S., Fiaz, S., ... & Ali, S. (2022). Alleviation of drought stress by root-applied thiourea is related to elevated photosynthetic pigments, osmoprotectants, antioxidant enzymes, and tubers yield and suppressed oxidative stress in potatoes cultivars. *PeerJ*, *10*, e13121.

Sandhu, A. S., Sharma, S. P., Bhutani, R. D., & Khurana, S. C. (2014). Effects of planting date and fertilizer dose on plant growth attributes and nutrient uptake of potato (Solanum tuberosum L.). *International Journal of Agricultural Sciences*, *4*(5), ii+-196.

Sati, D., Pande, V., Pandey, S. C., & Samant, M. (2023). Recent advances in PGPR and molecular mechanisms involved in drought stress resistance. *Journal of Soil Science and Plant Nutrition*, *23*(1), 106-124.

Sati, P., Dhakar, K., & Pandey, A. (2013). Microbial diversity in soil under potato cultivation from cold desert himalaya, india. ISRN Biodiversity, 2013, 1-9. https://doi.org/10.1155/2013/767453

Schwinghamer, T., Souleimanov, A., Dutilleul, P., & Smith, D. L. (2014). The plant growth regulator lipo-chitooligosaccharide (lco) enhances the germination of canola (brassica napus [l.]). Journal of Plant Growth Regulation, 34(1), 183-195. https://doi.org/10.1007/s00344-014-9456-7

Schwinghamer, T., Souleimanov, A., Dutilleul, P., & Smith, D. L. (2015). The response of canola cultivars to lipo-chitooligosaccharide (nod bj v [c18:1, mefuc]) and thuricin 17. Plant Growth Regulation, 78(3), 421-434. https://doi.org/10.1007/s10725-015-0104-4

Sebnie, W., Esubalew, T., & Mengesha, M. (2021). Response of potato (Solanum tuberosum L.) to nitrogen and phosphorus fertilizers at Sekota and Lasta districts of Eastern Amhara, Ethiopia. *Environmental Systems Research*, *10*, 1-8.

Seleim, M. A., Bereika, M. F., Ibrahim, O. H., Alqubaie, A. I., & Abo-Elyousr, K. A. (2023). Effectiveness of Bacillus cereus in controlling potato bacterial wilt caused by Ralstonia solanacearum: greenhouse and field studies with insights into resistance-related enzymes in potatoes. *Journal of Plant Diseases and Protection*, 1-11.

Sha, S., Zhao, X., Li, Y., Li, C., Zhu, L., Wang, Y., … & Gao, Q. (2021). Nutrient expert system optimizes fertilizer management to improve potato productivity and tuber quality. Journal of the Science of Food and Agriculture, 102(3), 1233-1244. https://doi.org/10.1002/jsfa.11461

Shah, A. and Smith, D. L. (2020). Flavonoids in agriculture: chemistry and roles in, biotic and abiotic stress responses, and microbial associations. Agronomy, 10(8), 1209. https://doi.org/10.3390/agronomy10081209

Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., … & Smith, D. L. (2021). Pgpr in agriculture: a sustainable approach to increasing climate change resilience. Frontiers in Sustainable Food Systems, 5. https://doi.org/10.3389/fsufs.2021.667546

Shahwar, D., Mushtaq, Z., Mushtaq, H., Alqarawi, A. A., Park, Y., Alshahrani, T. S., & Faizan, S. (2023). Role of microbial inoculants as bio fertilizers for improving crop productivity: A review. *Heliyon*. https://doi.org/10.1016/j.heliyon.2023.e16134

Singh, B., Gupta, S., Azaizeh, H., Shilev, S., Sudre, D., Song, W., … & Mench, M. (2011). Safety of food crops on land contaminated with trace elements. Journal of the Science of Food and Agriculture, 91(8), 1349-1366. https://doi.org/10.1002/jsfa.4355

Singh, R., Kaur, S., Bhullar, S. S., Singh, H., & Sharma, L. K. (2023). Bacterial biostimulants for climate smart agriculture practices: Mode of action, effect on plant growth and roadmap for commercial products. *Journal of Sustainable Agriculture and Environment*. https://doi.org/10.1002/sae2.12085

Singh, V. P., & Maiti, R. K. (2022). A review on mineral nutrition of potato (Solanum tuberosum L.). *Farming and Management*, *7*(2), 93-109. https://doi.org/10.31830/2456-8724.2022.fm-116

Slimani, A., Raklami, A., Benmrid, B., Oufdou, K., & Meddich, A. (2023). Salt-tolerant plant growth-promoting rhizobacteria mitigate salinity in barley by improving photosynthetic capacity, antioxidant activity, and soil fertility. *Biologia*, *78*(12), 3367-3379. https://doi.org/10.1007/s11756-023-01541-0

Smith, D. L., Gravel, V., & Yergeau, E. (2017). Signaling in the Phytomicrobiome. *Frontiers in Plant Science*, *8*, 611. https://doi.org/10.3389/fpls.2017.00611

Smith, D. L., Praslickova, D., & Ilangumaran, G. (2015). Inter-organismal signaling and management of the phytomicrobiome. Frontiers in Plant Science, 6. https://doi.org/10.3389/fpls.2015.00722

Soares, N. R., Huguet-Tapia, J. C., Guan, D., Clark, C. A., Yang, K. T., Kluchka, O. R., ... & Pettis, G. S. (2023). Comparative genomics of the niche-specific plant pathogen Streptomyces ipomoeae reveal novel genome content and organization. *Applied and Environmental Microbiology*, *89*(12), e00308-23. https://doi.org/10.1128/aem.00308-23

Song, J., Kong, Z., Zhang, D., Chen, J., Dai, X., & Li, R. (2021). Rhizosphere microbiomes of potato cultivated under bacillus subtilis treatment influence the quality of potato tubers.

International Journal of Molecular Sciences, 22(21), 12065. https://doi.org/10.3390/ijms222112065

Soni, P. G., Basak, N., Rai, A. K., Sundha, P., Chandra, P., & Yadav, R. K. (2023). Occurrence of salinity and drought stresses: status, impact, and management. In *Salinity and Drought Tolerance in Plants: Physiological Perspectives* (pp. 1-28). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-4669-3_1

South, K., Nordstedt, N., & Jones, M. (2021). Identification of plant growth promoting rhizobacteria that improve the performance of greenhouse-grown petunias under low fertility conditions. Plants, 10(7), 1410. https://doi.org/10.3390/plants10071410

Svubure, O., Struik, P., Haverkort, A., & Steyn, J. (2016). A quantitative framework for evaluating the sustainability of irish potato cropping systems after the landmark agrarian reform in zimbabwe. Outlook on Agriculture, 45(1), 55-65. https://doi.org/10.5367/oa.2016.0228

Syed, A., Elgorban, A. M., Bahkali, A. H., Eswaramoorthy, R., Iqbal, R. K., & Danish, S. (2023). Metal-tolerant and siderophore producing pseudomonas fluorescence and trichoderma spp. improved the growth, biochemical features and yield attributes of chickpea by lowering cd uptake. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-31330-3

Tabassum, B., Khan, A., Tariq, M., Ramzan, M., Khan, M. S. I., Shahid, N., & Aaliya, K. (2017). Bottlenecks in commercialisation and future prospects of PGPR. *Applied Soil Ecology*, *121*, 102-117. https://doi.org/10.1016/j.apsoil.2017.09.030

Takishita, Y., Charron, J. B., & Smith, D. L. (2018). Biocontrol rhizobacterium Pseudomonas sp. 23S induces systemic resistance in tomato (Solanum lycopersicum L.) against bacterial canker Clavibacter michiganensis subsp. michiganensis. *Frontiers in microbiology*, *9*, 2119. https://doi.org/10.3389/fmicb.2018.02119

Thanh, D. T., Tarn, L. T. T., Hanh, N. T., Tuyen, N. H., Srinivasan, B., Lee, S. Y., & Park, K. S. (2009). Biological control of soil-borne diseases on tomato, potato and black pepper by selected PGPR in the greenhouse and field in Vietnam. *The Plant Pathology Journal*, *25*(3), 263-269. https://doi.org/10.5423/ppj.2009.25.3.263

Tiwari, S., Prasad, V., Chauhan, P. S., & Lata, C. (2017). Bacillus amyloliquefaciens confers tolerance to various abiotic stresses and modulates plant response to phytohormones through osmoprotection and gene expression regulation in rice. *Frontiers in Plant Science*, *8*, 1510. https://doi.org/10.3389/fpls.2017.01510

Triwidodo, H. and Listihani, L. (2020). High impact of pgpr on biostatistic of aphis craccivora (hemiptera: aphididae) on yardlong bean. Biodiversitas Journal of Biological Diversity, 21(9). https://doi.org/10.13057/biodiv/d210912

Tsegaye, Z., Alemu, T., Desta, F., & Assefa, F. (2022). Plant growth-promoting rhizobacterial inoculation to improve growth, yield, and grain nutrient uptake of teff varieties. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.896770

Türkmen, A., Yavuz, C., Dangol, S., Tarim, C., Demirel, U., & Çalişkan, M. (2017). Evaluation of micro tuberization performances of different genotypes. Turkish Journal of Agriculture - Food Science and Technology, 5(4), 353. https://doi.org/10.24925/turjaf.v5i4.353-357.1203

Unel, N. M., Baloglu, M. C., & Altunoglu, Y. Ç. (2023). Comprehensive investigation of cucumber heat shock proteins under abiotic stress conditions: A multi-omics survey. *Journal of Biotechnology*, *374*, 49-69. https://doi.org/10.1016/j.jbiotec.2023.07.010

Vacheron, J., Desbrosses, G., Bouffaud, M., Touraine, B., Moënne-Loccoz, Y., Müller, D., … & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00356

Van Oort, P. A. J., Timmermans, B. G. H., Meinke, H., & Van Ittersum, M. K. (2012). Key weather extremes affecting potato production in The Netherlands. *European Journal of Agronomy*, *37*(1), 11-22. https://doi.org/10.1016/j.eja.2011.09.002

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules*, 21(5), 573. https://doi.org/10.3390/molecules21050573

Velivelli, S. L. S., Kromann, P., Lojan, P., Rojas, M., Franco, J., Suárez, J. P., … & Prestwich, B. D. (2014). Identification of mvocs from andean rhizobacteria and field evaluation of bacterial and mycorrhizal inoculants on growth of potato in its center of origin. Microbial Ecology, 69(3), 652-667. https://doi.org/10.1007/s00248-014-0514-2

Velivelli, S. L., Lojan, P., Cranenbrouck, S., de Boulois, H. D., Suarez, J. P., Declerck, S., ... & Prestwich, B. D. (2015). The induction of Ethylene response factor 3 (ERF3) in potato as a result of co-inoculation with Pseudomonas sp. R41805 and Rhizophagus irregularis MUCL 41833–a possible role in plant defense. *Plant signaling & behavior*, *10*(2), e988076. https://doi.org/10.4161/15592324.2014.988076

Velivelli, S. L., Sessitsch, A., & Prestwich, B. D. (2014). The role of microbial inoculants in integrated crop management systems. *Potato Research*, *57*, 291-309. https://doi.org/10.1007/s11540-014-9278-9

Vishwakarma, S. K., Ilyas, T., Shahid, M., Malviya, D., Kumar, S., Singh, S., ... & Singh, H. V. (2024). Bacillus spp.: Nature's Gift to Agriculture and Humankind. In *Applications of Bacillus and Bacillus Derived Genera in Agriculture, Biotechnology and Beyond* (pp. 1-36). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-8195-3_1

Vitorino, L. and Bessa, L. (2017). Technological microbiology: development and applications. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.00827

Wadas, W. and Dziugieł, T. (2020). Quality of new potatoes (solanum tuberosum l.) in response to plant biostimulants application. Agriculture, 10(7), 265. https://doi.org/10.3390/agriculture10070265

Wang, C., Zhao, D., Qi, G., Mao, Z., Hu, X., Du, B., ... & Ding, Y. (2020). Effects of Bacillus velezensis FKM10 for Promoting the Growth of Malus hupehensis Rehd. and Inhibiting Fusarium verticillioides. *Frontiers in microbiology*, *10*, 2889. https://doi.org/10.3389/fmicb.2019.02889

Wang, D., Chen, X., Tang, Z., Liu, M., Jin, R., Zhang, A., & Zhao, P. (2022). Application of humic acid compound fertilizer for increasing sweet potato yield and improving the soil fertility. *Journal of Plant Nutrition*, *45*(13), 1933-1941. https://doi.org/10.1080/01904167.2022.2046064

Wang, N., Xing, Y., & Wang, X. (2019). Exploring options for improving potato productivity through reducing crop yield gap in loess plateau of china based on grey correlation analysis. Sustainability, 11(20), 5621. https://doi.org/10.3390/su11205621

Wardati, I., Irawan, T., Erawati, D., Rahmawati, N., & Arifiana, N. (2023). Response of oil palm plant seed growth to the application of plant growth promoting rhizobacteria (PGPR) and trichoderma sp. Iop Conference Series Earth and Environmental Science, 1168(1), 012017. https://doi.org/10.1088/1755-1315/1168/1/012017

Wozniak, E., Blaszczak, A., Wiatrak, P., & Canady, M. (2020). Biostimulant Mode of Action: Impact of Biostimulant on Whole‐Plant Level. *The Chemical Biology of Plant Biostimulants*, 205-227. https://doi.org/10.1002/9781119357254.ch8

Xie, S., Tran, H. T., Pu, M., & Zhang, T. (2023). Transformation characteristics of organic matter and phosphorus in composting processes of agricultural organic waste: research trends. *Materials Science for Energy Technologies*. https://doi.org/10.1016/j.mset.2023.02.006

Xing, Y., Niu, X., Wang, N., Jiang, W., Gao, Y., & Wang, X. (2020). The correlation between soil nutrient and potato quality in loess plateau of china based on plsr. Sustainability, 12(4), 1588. https://doi.org/10.3390/su1204158

Yasmin, F., Othman, R., & Maziz, M. N. H. (2020). Yield and nutrient content of sweet potato in response of plant growth-promoting rhizobacteria (PGPR) inoculation and N fertilization. *Biological*, *13*(1), 1117. https://doi.org/10.1016/j.scienta.2007.04.013

Zaheer, K. and Akhtar, M. H. (2014). Potato production, usage, and nutrition—a review. Critical Reviews in Food Science and Nutrition, 56(5), 711-721. https://doi.org/10.1080/10408398.2012.724479

Zarzecka, K., Gugała, M., Mystkowska, I., & Sikorska, A. (2020). Total and true protein content in potato tubers depending on herbicides and biostimulants. Agronomy, 10(8), 1106. https://doi.org/10.3390/agronomy10081106

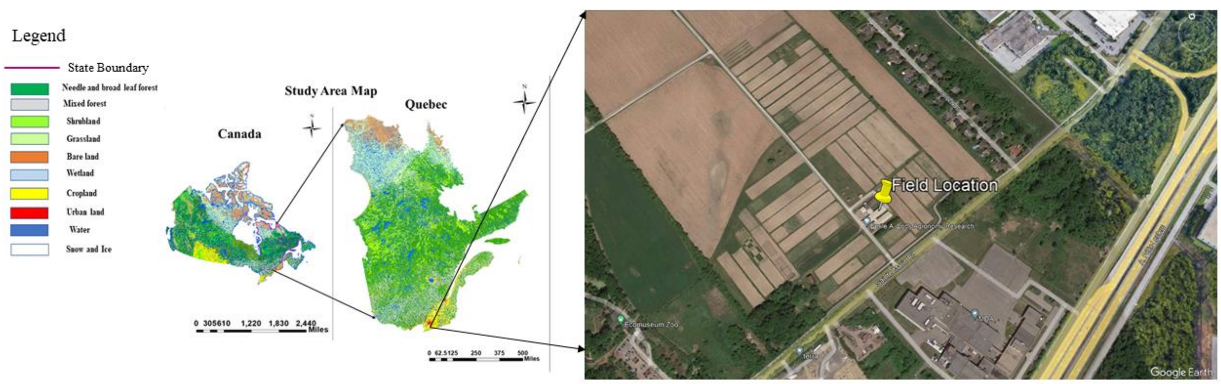
Zarzecka, K., Gugała, M., Sikorska, A., Grzywacz, K., & Niewęgłowski, M. (2020). Marketable yield of potato and its quantitative parameters after application of herbicides and biostimulants. Agriculture, 10(2), 49. https://doi.org/10.3390/agriculture10020049

Zebelo, S., Song, Y., Kloepper, J., & Fadamiro, H. (2016). Rhizobacteria activates (+)-δ-cadinene synthase genes and induces systemic resistance in cotton against beet armyworm (spodoptera exigua). Plant Cell & Environment, 39(4), 935-943. https://doi.org/10.1111/pce.12704

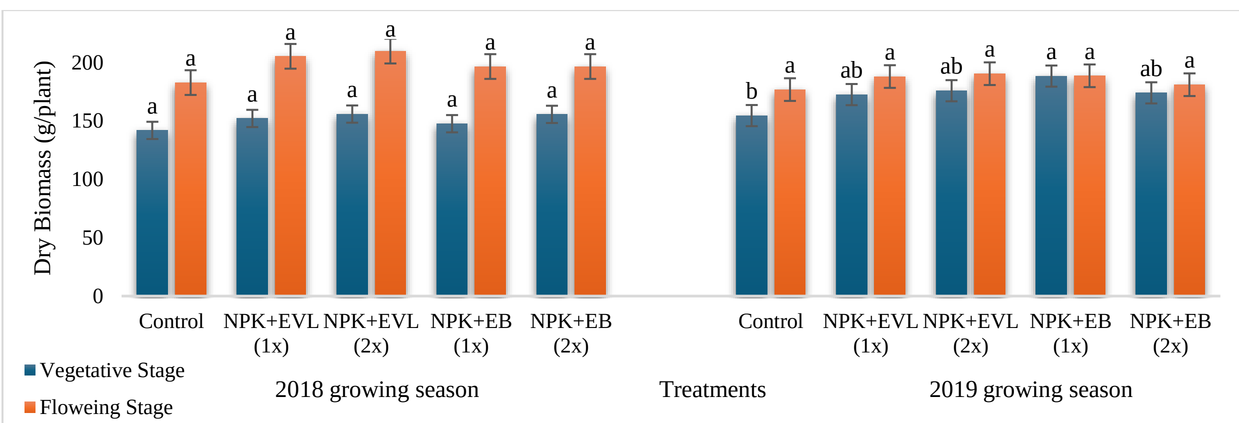
Zhang, J., Huang, X., Yang, S., Huang, A., Ren, J., Luo, X., … & Dong, P. (2023). Endophytic bacillus subtilis h17-16 effectively inhibits phytophthora infestans, the pathogen of potato late blight, and its potential application. Pest Management Science, 79(12), 5073-5086. https://doi.org/10.1002/ps.7717

Zhang, Y., Li, C., Yao, T., Li, M., Lan, X., & Wang, Z. (2023). Plant growth–promoting Rhizobacteria enhance salt tolerance in oat by upregulating the antioxidant system and promoting root growth. *Journal of Plant Growth Regulation*, *42*(6), 3568-3581. https://doi.org/10.1007/s00344-022-10821-z

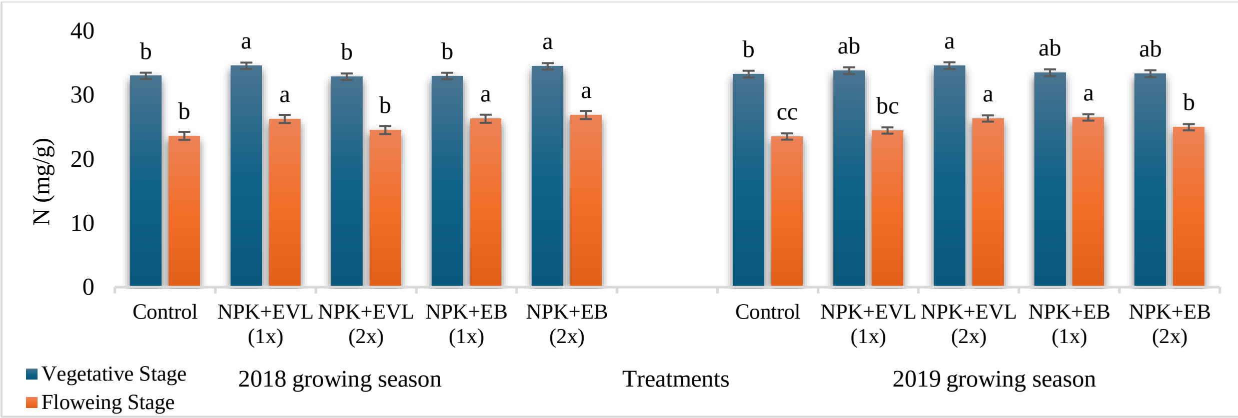
## Appendix (Chapters 3 and 4)



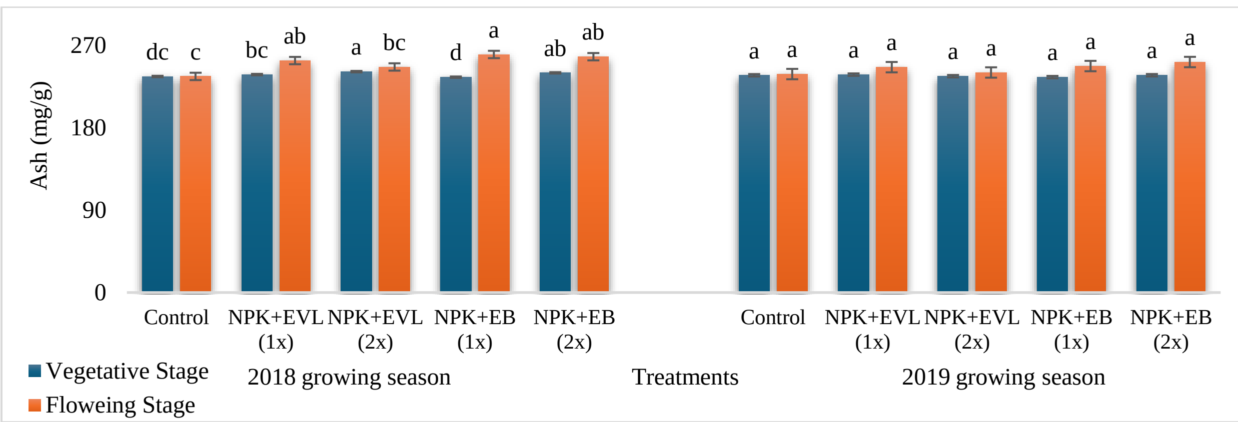

*Appendix 3-1: The location of the research center where the experiments were undertaken.*



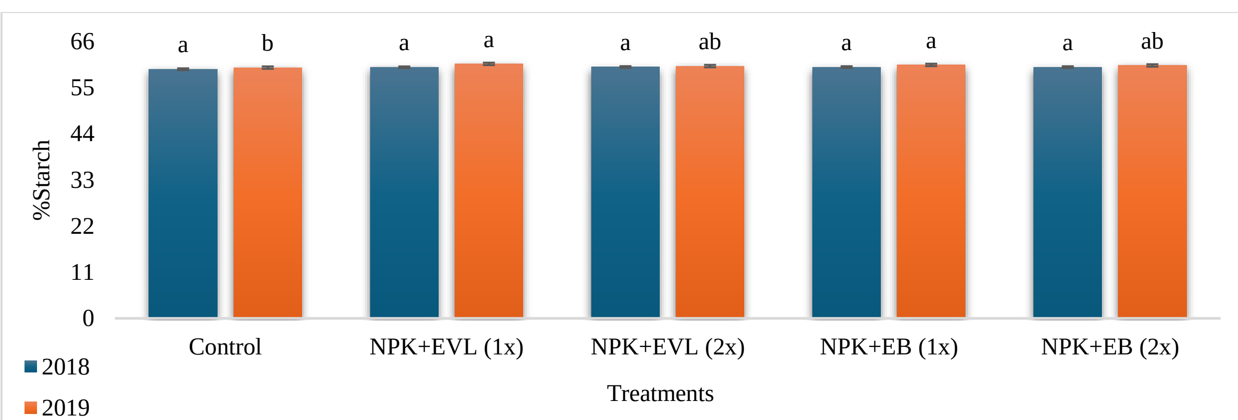

*Appendix 3-2: Potato dry biomass at the vegetative and flowering stages in 2018 and 2019 in response to the MCF treatments.*



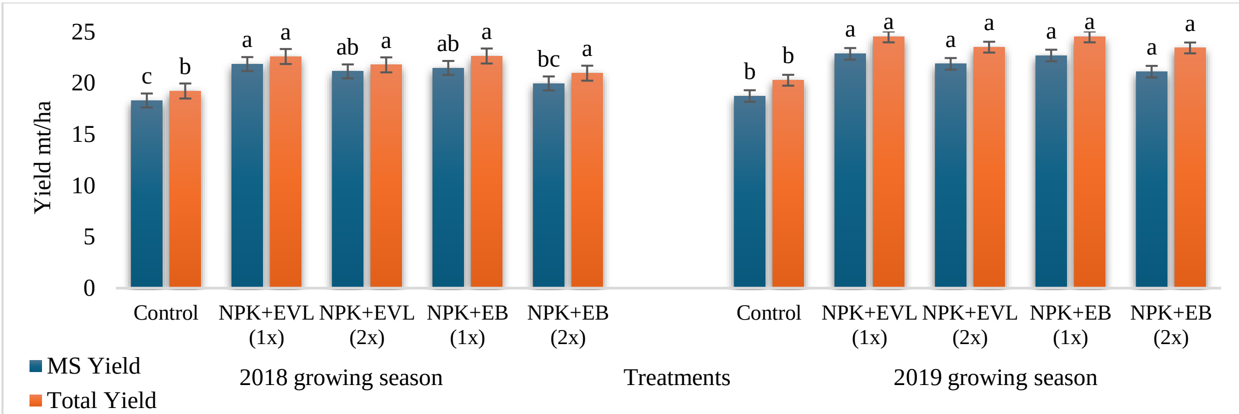

*Appendix 3-3: Potato plant tissue nitrogen concentration at the vegetative and flowering stages in 2018 and 2019 in response to the MCF treatments.*



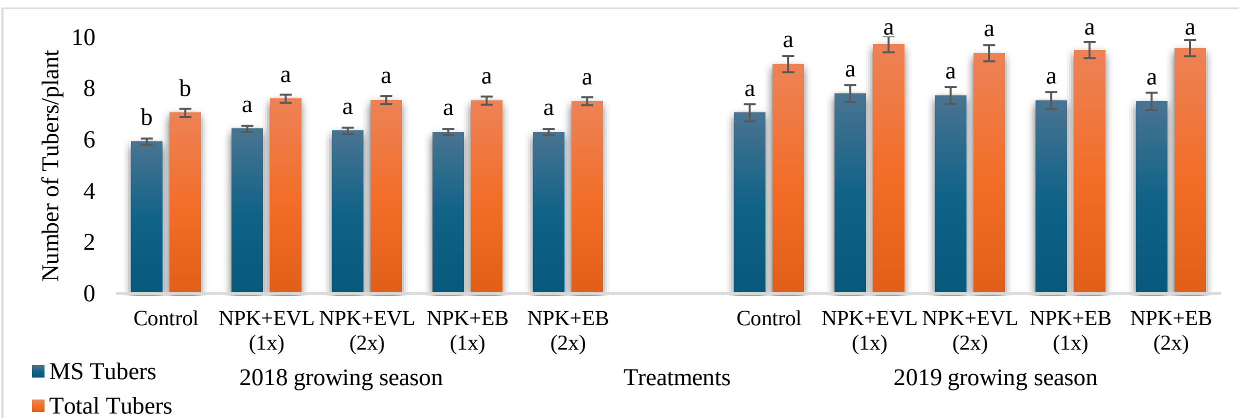

*Appendix 3-4: Potato plant tissue ash content at the vegetative and flowering stages in 2018 and 2019 in response to the MCF treatments.*



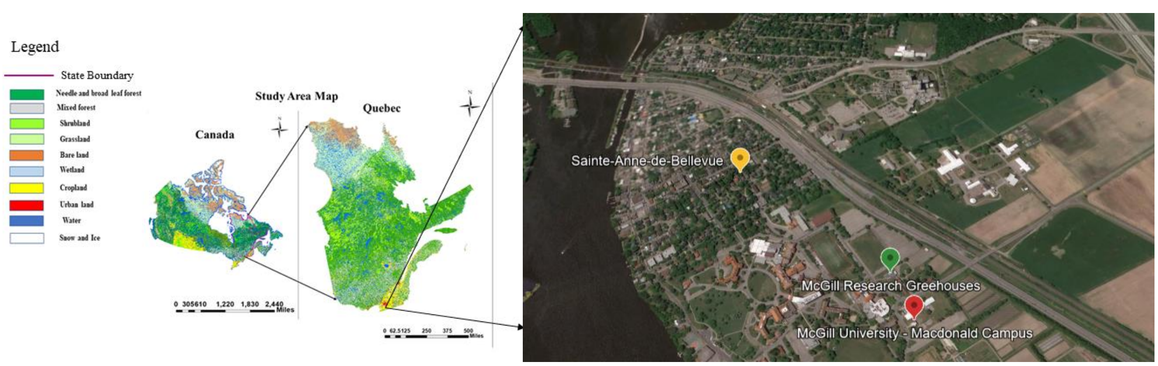

*Appendix 3-5: Total starch in potato tubers in 2018 and 2019 in response to the MCF treatments.*



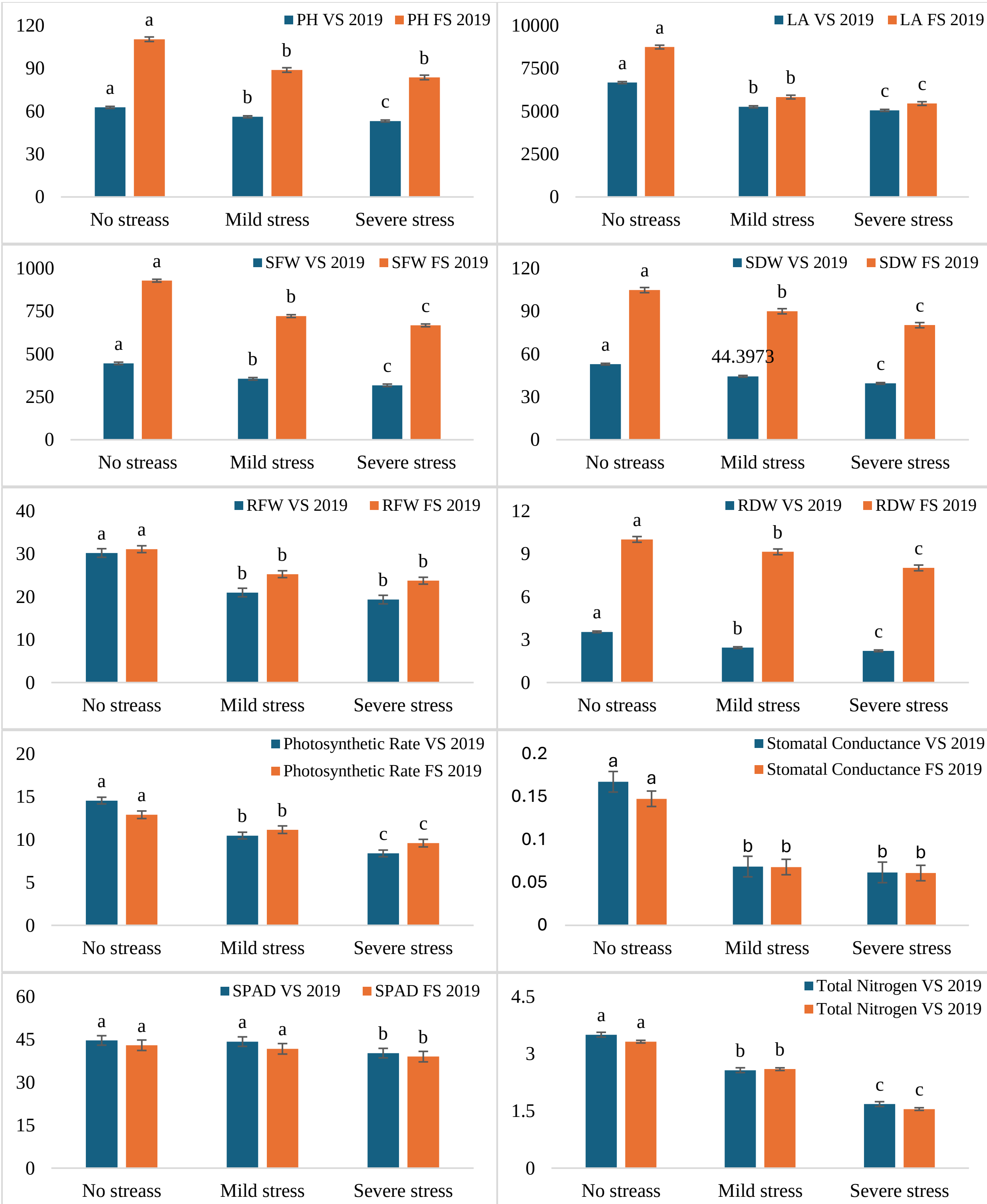

*Appendix 3-6: Marketable-size and total potato yield in 2018 and 2019 in response to the MCF treatments.*



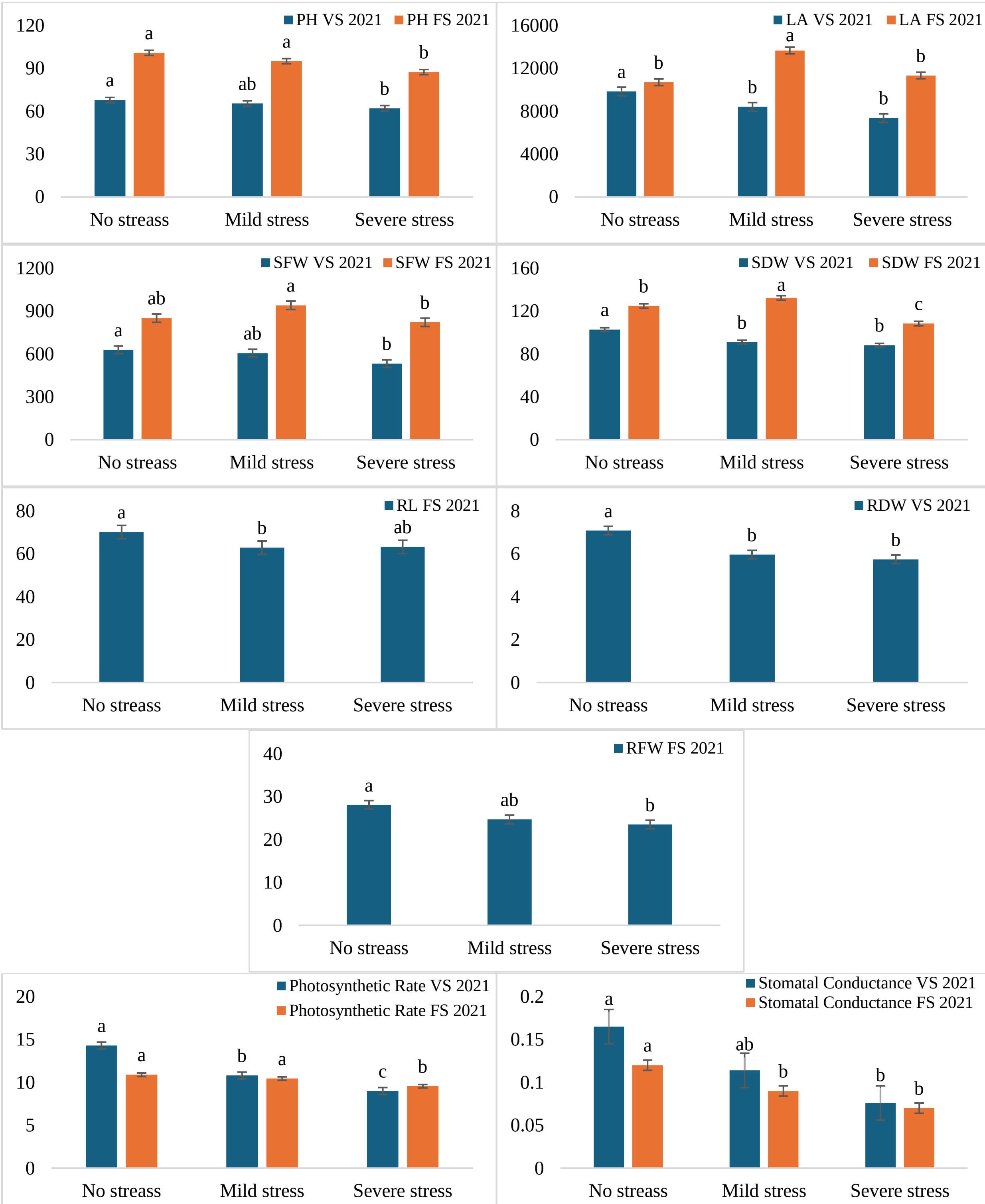

*Appendix 3-7: Marketable-size and total number of potato tubers in 2018 and 2019 in response to the MCF treatments.*

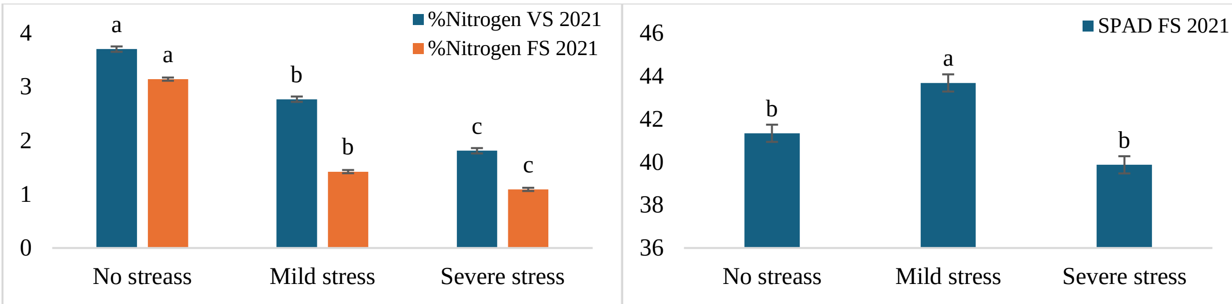



*Appendix 4-1: The location of the research greenhouse where the experiments were undertaken.*

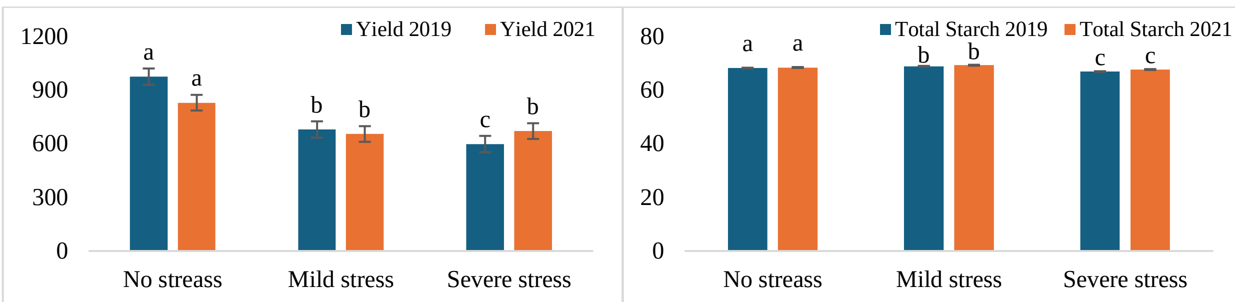



*Appendix 4-2: Response of all studied variables to water-deficit stress at the vegetative (VS) and flowering (FS) stages in Experiment 1 (2019).*


PH VS 2021
PH FS 2021
120
90
60
30
0
a
a
ab
a
b
b
No streass
Mild stress
Severe stress
LA VS 2021
LA FS 2021
16000
12000
8000
4000
0
a
b
b
a
b
b
No streass
Mild stress
Severe stress
SFW VS 2021
SFW FS 2021
1200
900
600
300
0
a
ab
ab
a
b
b
No streass
Mild stress
Severe stress
SDW VS 2021
SDW FS 2021
160
120
80
40
0
a
b
b
a
b
c
No streass
Mild stress
Severe stress
RL FS 2021
80
60
40
20
0
a
b
ab
No streass
Mild stress
Severe stress
RDW VS 2021
8
6
4
2
0
a
b
b
No streass
Mild stress
Severe stress
RFW FS 2021
40
30
20
10
0
a
ab
b
No streass
Mild stress
Severe stress
Photosynthetic Rate VS 2021
Photosynthetic Rate FS 2021
20
15
10
5
0
a
a
b
a
c
b
No streass
Mild stress
Severe stress
Stomatal Conductance VS 2021
Stomatal Conductance FS 2021
0.2
0.15
0.1
0.05
0
a
a
ab
b
b
b
No streass
Mild stress
Severe stress




*Appendix 4-3: Response of all studied variables to water stress at the vegetative (VS) and flowering (FS) stages in Experiment 2 (2021).*




*Appendix 4-4: Effect of water stress on yield and total starch content experiments 1 and 2 (2019 and 2021).*