



Planting Substrate Mixture Impacts Yield and Size of Nuclear Potato Tubers

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Abstract

Potato (*Solanum tuberosum*) is a vegetatively propagated crop for which certification programs seek to ensure cleanliness of seed supply and true-to-type cultivars. Seed potato planting stocks start from micropropagated plantlets transplanted to a greenhouse for nuclear tuber production. Tubers are then grown for multiple generations in the field before use in commercial ware potato production. Growers consider factors such as crop genetics and management to ensure productivity and quality. However, the impact of the media in which micropropagated potato plantlets are grown and the host plant's associated bacterial communities are often overlooked. We hypothesized that tubers from high yielding plants will have higher endophytic bacteria diversity, and these will continue to produce high yield in the subsequent field season. In this study, we conducted two sequential experiments involving potato plantlets of two cultivars ('Russet Norkotah' and 'Silverton Russet'). The first experiment was conducted in a greenhouse, where the plantlets were grown in four different planting substrates: peat-based media (a former standard now discontinued due to potential for pathogen contamination), and three alternate substrates (Perlite, 2:1 perlite and calcined clay, and 2:1 perlite and rice hulls). Dry aboveground biomass, tuber set, and yield at harvest were measured. The tubers harvested from the greenhouse were then used in a subsequent field experiment. In this second experiment, we measured canopy coverage, total tuber yield and tuber set. For both cultivars in both the greenhouse and field experiment, 2:1 perlite and calcined clay had similar high nuclear tuber yield to plants grown in peat-based media. In the field experiment, for both cultivars, larger planted tuber size resulted in increased measurements across all tuber metrics. Therefore the results indicated that 2:1 perlite and calcined clay mixture has the potential as a suitable alternative substrate for micropropagated potato plantlets. Alpha diversity indices revealed no significant differences in tuber bacterial richness and diversity between substrates and experiment repetitions. Beta diversity analyses demonstrated that experiment repetition had the strongest effect on the composition of the bacterial communities on tubers. This research highlights the need for greater understanding of planting substrate impact on potato microbiome, nuclear tuber productivity and product quality, which may lead to improved early generation seed potato production practices.

Keywords Potato production · Endophytes · Minituber · Russet norkotah · Silverton russet · Microbiome · Bacteria · Illumina sequencing · Yield · Planting substrate

Resumen

La papa (*Solanum tuberosum*) es un cultivo de propagación vegetativa para el cual los programas de certificación buscan garantizar la limpieza del suministro de semillas y cultivares fieles al tipo. Los suministros de siembra de papa para semilla comienzan a partir de plántulas micropropagadas trasplantadas a un invernadero para la producción de tubérculos

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nucleares. Luego, los tubérculos se cultivan durante varias generaciones en el campo antes de su uso en la producción comercial de papas. Los productores consideran factores como la genética y el manejo de los cultivos para garantizar la productividad y la calidad. Sin embargo, a menudo se pasa por alto el impacto de los sustratos en los que se cultivan las plántulas de papa micropropagadas y las comunidades bacterianas asociadas a la planta hospedante. Planteamos la hipótesis de que los tubérculos de plantas de alto rendimiento tendrán una mayor diversidad de bacterias endófitas, y estas continuarán produciendo un alto rendimiento en el ciclo de campo posterior. En este estudio, realizamos dos experimentos secuenciales con plántulas de papa de dos variedades ('Russet Norkotah' y 'Silverton Russet'). El primer experimento se llevó a cabo en un invernadero, donde las plántulas se cultivaron en cuatro sustratos de siembra diferentes: medios a base de turba (un estándar anterior ahora discontinuado debido a la posible contaminación por patógenos) y tres sustratos alternativos (perlita, perlita 2:1 y arcilla calcinada, y perlita 2:1 y cáscarilla de arroz). Se midió la biomasa seca del follaje, la tuberización y el rendimiento en la cosecha. Los tubérculos cosechados del invernadero se utilizaron en un experimento de campo posterior. En este segundo experimento, medimos la cobertura del dosel, el rendimiento total de tubérculos y la tuberización. Para ambas variedades, tanto en el invernadero como en el experimento de campo, la perlita 2:1 y la arcilla calcinada tuvieron un alto rendimiento de tubérculos nucleares similar al de las plantas cultivadas en medios a base de turba. En el experimento de campo, para ambas variedades, un mayor tamaño de tubérculo plantado resultó en un aumento de las mediciones en todas las métricas de tubérculos. Por lo tanto, los resultados indicaron que la mezcla de perlita y arcilla calcinada 2:1 tiene el potencial como sustrato alternativo adecuado para las plántulas de papa micropropagadas. Los índices de diversidad alfa no revelaron diferencias significativas en la riqueza bacteriana de los tubérculos y la diversidad entre sustratos y repeticiones de experimentos. Los análisis de diversidad beta demostraron que el tiempo de cosecha tuvo el efecto más fuerte en la composición de las comunidades bacterianas en los tubérculos. Esta investigación destaca la necesidad de una mayor comprensión del impacto del sustrato de siembra en el microbioma de la papa, la productividad del tubérculo nuclear y la calidad del producto, lo que puede conducir a mejores prácticas de producción de semilla de papa de generación temprana.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops that feeds the world (Devaux et al. 2021). The crop is widely researched due to the availability of a fully sequenced genome and its ability to grow in a variety of environmental conditions and propagation systems, making it a convenient and important subject for study (Zaheer and Akhtar 2016). For more than a century, clonal propagation has facilitated commercial production with techniques such as micropropagation. This fast method for vegetative propagation produces high quality, disease free plants (Butt et al. 2015). A wide range of horticultural crops benefit from this method, such as potato, and various fruit trees (Donnelly et al. 2003; Lal et al. 2022). While effective, these methods are considered expensive for resource-challenged producers due to high cost of consumables and media and need for well-equipped laboratories (Naik et al. 2020). Beyond these direct costs, current micropropagation practices often overlook the importance of investigating alternative substrates and impact of endophytes to optimize growth, yield, and health, potentially enhancing productivity and making micropropagation a more accessible and lucrative alternative to crop production.

High-quality, disease-free micropropagated potato plants are grown in vitro, acclimated to a greenhouse environment in a potted or hydroponic propagation system and eventually

grown for multiple generations in the field. Acclimatization is the process of transferring in vitro plantlets to a soil- or water-based system in a controlled environment to ensure that a sufficient number of plants grow vigorously, survive, and produce minitubers (Preece and Sutter 1991). Different types of solid substrates used include potting soil, peat, vermiculite, or sand (Cavallazzi et al. 2007; Goulart 1989; Vosátka and Gryndler 2000). Compared to crops grown in the field or soil-based substrates, microbial transmission is reduced when generating early-generation, clonally propagated crops in vitro because of the sterile environment in which propagation necessarily occurs in order to omit pathogenic microorganisms. However, these crops are subsequently grown in controlled greenhouse environments where they acquire microorganisms from the environment, including wind, dust, and growth substrates, whether soil-based or water-based (Catara and Bella 2020).

Plants are intrinsically associated with microbial communities that are crucial for their functioning and health (Berg et al. 2020). Different parts of the plant, such as the above-ground (phyllosphere), below-ground (rhizosphere) and internal (endosphere) tissues, can harbor endophytes of distinct taxa living as commensals, pathogens and beneficials (Turner et al. 2013). Schulz and Boyle (2006) define endophytes as those that can be detected not causing apparent harm within healthy plant host tissues. Some of the reported benefits of bacterial and fungal endophytes on their plant hosts include plant growth promotion, nitrogen fixation,

induction of systemic resistance, drought tolerance, reduced salt stress and antagonism towards plant pathogens (Ali et al. 2014; Bresson et al. 2013; Gond et al. 2015; Zhou et al. 2014). Endophytic bacteria and fungi are typically considered to be transmitted horizontally, by entering the plant from an environmental origin, such as water or the air, or vertically, from generation to generation via seed (Samreen et al. 2021).

Previous studies looking at soil, endophytic and rhizospheric bacterial communities associated with potato plants have mainly focused on revealing the taxonomy and diversity (Barnett et al. 2015; Jeanne et al. 2019). As seen in studies with other crops, bacterial communities associated with the potato plant rhizosphere mainly vary with growth stage (Nahar et al. 2020). Researchers have attempted to decipher changes in bacterial community composition in soil after inoculation of biocontrol agents as well as plant pathogens (Roquigny et al. 2018; Shi et al. 2019). Changes in cultural practices also impact soil bacterial communities associated with potato plants (Akimbekov et al. 2020; Xue et al. 2018; Simmons et al. 2018). When Buchholz et al. (2019) studied the microbes transmitted into potato tubers over three generations, they found recruitment would vary by soil, not by variety. The authors also concluded that bacterial communities assembled in potato tubers consist mainly of bacteria vertically transmitted through seed tubers and to a lesser extent, bacteria recruited from the soil through the roots.

While growers traditionally focus on crop genetics and management practices to optimize productivity and quality, the crucial impact of beneficial plant-associated bacterial communities is often overlooked. These communities can confer beneficial effects, including growth promotion, nitrogen fixation, drought tolerance, and antagonism towards plant pathogens. The potato micropropagation pipeline, which involves initially sterile plantlets followed by growth in greenhouse substrates and subsequent field planting, represents a unique system for studying microbial recruitment and persistence. However, the influence of the propagation environment, particularly the planting media, on the endophytic microbial communities of minitubers and their subsequent effect on field productivity remains poorly understood. Despite clonally propagated crops being largely axenic, a reduced endophytic microbiome remains and few studies have explored the endophytic bacteria able to persist in these environments (Abreu-Tarazi et al. 2010; Esposito-Polesi et al. 2017; Lucero et al. 2011).

The present study aimed to explore the impact of different planting substrates used in nuclear production systems on plant productivity, product quality and bacterial community composition. The recent finding of *Spongospora subterranea* f. sp. *subterranea* in peat-based media prompted potato propagators to consider alternative substrates (Zeng et al.

2020). Preliminary results from trials conducted by the Wisconsin Seed Potato Certification Program or WSPCP identified three non-peat based media options that performed similarly to standard peat-based medium: perlite, a mixture of 2:1 perlite and rice hulls and a mixture of 2:1 perlite and calcined clay. This project examined these three planting substrates, that vary in amount of organic matter and moisture retention, as potential replacements for peat-based media. We analyzed the impact of substrates used during the acclimation process of seed potato tissue culture plantlets of two potato cultivars, ‘Russet Norkotah’ and ‘Silverton Russet’ using physiological measurements and endophytic bacterial communities of nuclear tubers. Tubers harvested from the alternative substrates were planted and grown in the field to compare greenhouse study results with physiological measurements of first-generation nuclear tubers.

Materials and Methods

Plant Material

Plant material was obtained from the WSPCP Tissue Culture Lab at the University of Wisconsin in Madison, WI. Three batches of 40 sterile micropropagated potato (*Solanum tuberosum*) plantlets, each corresponding to a repetition of the experiment, were generated by the WSPCP Tissue Culture Lab. Plantlets were grown in Murashige and Skoog media (PhytoTech Labs, Lenexa, Kansas, USA) in 16 h light period followed by a 8 h dark period at 17 °C for four weeks then transplanted as outlined below in the Walnut Street Greenhouse at the University of Wisconsin-Madison, Madison, WI, USA. The two commonly grown commercial cultivars of interest were ‘Russet Norkotah’ and ‘Silverton Russet’. ‘Russet Norkotah’ is classified as an early to mid-season maturing potato, whereas ‘Silverton Russet’ is a mid-season to late-maturing cultivar (Wisconsin Seed Potato Certification Program 2018; 2019).

Propagation System and Experimental Design

Four planting substrate mixtures were prepared using four media: Standard peat-based medium (PRO-MIX LP15, Premier Tech Horticulture, Cromwell, MN, USA), perlite (Therm-O-Perl, New Eagle, PA, USA and Horticulture Perlite, Midwest Perlite, Appleton WI, USA.), 2:1 perlite and rice hulls (PBH Nature’s Media, Riceland, Stuttgart, AR, USA) and 2:1 perlite and calcined clay (OIL DRI Premium Absorbent, OIL DRI, Chicago, IL, USA). To prepare the planting substrate mixtures, an 18-gallon plastic tub was used to mix substrates and water was added to reach field capacity, an optimal moisture level after saturation and

gravitational drainage. Each repetition of the experiment consisted of 40 plants: five of each of the two cultivars within the four planting substrate mixtures. Before adding the planting substrate mixture, two paper towels were placed at the bottom of the pots to prevent substrates from flowing out. Six liters of each planting substrate mixture were used to fill one 3-gallon pot and pots were shaken to allow the substrate to settle. Randomized, numbered plastic tags were placed in the substrate to label each pot and reduce bias during sampling. Once each pot was filled, the sterile micropropagated potato plantlets were removed from their plastic propagation boxes. The roots were gently rinsed with greenhouse tap water to remove excess solid media and plantlets were transplanted to the prepared pots.

Growth Period and Harvesting

After plantlets were transplanted, a 32-oz clear plastic container was inverted over each plantlet to retain moisture for the first five days. No water was added to the pots while the container was in place. After five days, plastic containers were removed, and plants were watered with Peters' Professional 20-10-20 with Micronutrient fertilizer (ICL, Tel-Aviv). The plants were maintained in a greenhouse with a 12-h photoperiod at 1000 watts using High Pressure Sodium lights and temperature ranged from 14 to 26 °C during the day and 12–24 °C at night. For the first two months, plants were watered every other day until soil was saturated and fertilized once a week. In the last month of the study, plants were watered every day. At approximately six weeks, all potato plants were growing beyond the pot and vines were staked. Each of the three repetitions of the experiment was planted four weeks apart. The first trial repetition was initiated on 26 July 2021, the second on 24 August 2021 and the third on 21 September 2021. Pots were placed in a randomized complete block design, with each block occupying a different space in the greenhouse. Each experimental repetition was terminated and harvested after 12 weeks. Harvests for each repetition took place on 18 October 2021, 16–17 November 2021, and 14–15 December 2021.

Plant Harvest and Sample Processing

At harvest, plant tissues were destructively sampled. After cutting the base of the plant using garden shears, the aboveground plant tissue was stored in paper bags and dried for a week in an oven at 45 °C. Each pot was sifted to separate the tubers from the planting substrate. Aboveground plant tissue was dried and weighed, and tubers were measured for tuber set (total tuber number) and total tuber yield (mass indicator). Tubers were then stored in plastic mesh bags in a 6 °C cold room until further use.

Genomic DNA Extraction of Tubers

For DNA extraction, three tubers were selected per each cultivar, treatment, and repetition of the experiment, for a total of 72 samples. Surface sterilization of tubers involved submerging them for one minute each in a series of solutions: 70% ethanol, 1:10 0.5% sodium hypochlorite mixed with Tween 20, 70% ethanol and three times in sterile water. Following the rinse in sterile water, tubers were pierced from end-to-end with a 20 mm cork borer (Avantor, Radnor Township, PA, USA). Each core was cut into 1.3 cm disks, disposing of ends. Two tuber disks were placed into a sterile bag filled with 1 ml of 1:10 tryptic soy broth (TSB; Neogen, Lansing, MI, USA) and macerated with a hammer. DNA extraction of tubers was performed using FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions.

16S rRNA Gene Fragment Amplicon Library Preparation and Sequencing

For the preparation of 16S rRNA gene amplicon libraries of seed potato tubers, a first round of PCR was performed using the primer pair 799f (5'-AACMGGATTAGATACCKG-3'), which excludes amplification of chloroplast 16 S, and 1392r (5'-ACGGGCGGTGTGTRC-3'), both carrying Illumina adapters, i5 or i7 index, for NexTera barcodes (Bulgarelli et al., 2012; Illumina 2024; Lundberg et al., 2012). The reaction conditions were as follows: 25 µl reaction volume that contained DNA, 1x KAPA HiFi GC Buffer (5x) with MgCl₂, 10 mM dNTPs, 10 µM of each primer and 0.5 units of KAPA HiFi DNA polymerase (Kapa Biosystems, Boston, MA, USA). This reaction was carried out with the following settings: 95 °C for 5 min, 25 cycles at 98 °C for 20 s, 55 °C for 15 s, 72 °C for 1 min and final extension at 72 °C for 5 min. To visualize amplicons, 25 µl of PCR product were loaded onto a 2% agarose gel (w/v) in 1x TAE, and the bacterial 16 S rRNA gene amplicons (approximately 600 bp) were separated from eukaryotic 18 S rRNA gene amplicons (1000 bp) by electrophoresis for 50 min at 90 V and excised from the gel using Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) following manufacturer's instructions. For the second round of PCR, 5 µl of the eluate was used with the to attach the NexTera i5 forward primer and i7 reverse primer for sample recognition in sequencing. Illumina sequencing (MiSeq 2 × 300 bp) was performed at University of Wisconsin Biotechnology Center, Madison, Wisconsin, USA.

Field Experiment Design

On 21 April 2022, two weeks before planting, the tubers of a given cultivar harvested from the greenhouse experiment

were pooled by each of the four different planting substrate mixtures and each of the three experiment repetitions. These pooled tubers were then placed in plastic mesh bags and dipped in a five-gallon bucket containing a solution of five gallons of tap water, 7.5 ml of ProGibb (Valent Biosciences, Libertyville, Illinois, USA), 60 ml of rubbing alcohol, and 5 ml of dishwashing liquid, to promote sprouting. Tubers were subsequently set out in plastic trays and dried at room temperature in low lighting. Two days before planting, tubers were categorized by three weight classes: small (less than 28 g), medium (28 g to 255 g), and large (greater than 255 g). On 6 May 2022, whole tubers were hand-planted at the University of Wisconsin Hancock Agricultural Research Station, Hancock, Wisconsin, USA (44.116680039397835, -89.55236386313643).

The field experiment was structured as a Split-Plot design arranged within a Randomized Complete Block design framework. The main field plot was divided in half, dedicating one side to each cultivar ('Russet Norkotah' and 'Silverton Russet'). Within each cultivar's area, the design was a split-plot with three main plot treatment levels based on tuber weight class (small, medium, and large), and each of these weight classes was replicated three times, resulting in a total of nine complete blocks per cultivar (3 weight classes $\times$ 3 replicates). The experimental units were individual plots, each containing three whole tubers of a given cultivar from the same weight class. These tubers originated from one of the four greenhouse planting substrates and one of the three greenhouse experiment repetitions, resulting in 12 unique combinations of substrate and greenhouse repetition. These 12 treatment combinations were then randomized within each of the nine complete blocks (which were themselves defined by weight class). However, due to inherent variability in tuber production from the greenhouse and limitations in the availability of sufficient tubers within specific weight classes for all necessary plots, unavoidable substitutions were made during planting. If insufficient tubers were available for a plot within its designated weight block, tubers from the immediately adjacent larger available weight class were primarily used to complete that plot. These instances were carefully documented during planting, and the actual distribution of planted tubers by cultivar, field block, and actual tuber weight class is provided in Supplementary Table 1. This design resulted in 103 and 108 experimental plots for Russet Norkotah and Silverton Russet, respectively, totaling 211 experimental plots across both cultivars. The physical layout of the field comprised 36-foot-long rows spaced three feet apart, with each row containing 14 plots. To mitigate potential edge effects and prevent plot mixing during harvest, the red potato cultivar 'Dark Red Norland' was planted at each plot-end. The potatoes were irrigated at an average three times per week with

overhead sprinklers and grown using conventional management practices typical for commercial production for our growing region including fungicides for foliar disease management, herbicides for weed management, and insecticides for insect pest management. A foliar desiccant Diquat 2L (Nufarm, Melbourne, Australia) was utilized on 19 September 2022 to hasten vine-kill necessary for periderm maturation prior to harvest. One month after planting, emergence was recorded every third day until all plants had emerged. Plants were considered to have emerged when green foliage was seen protruding through the soil surface. To record and measure canopy coverage, overhead pictures were taken of each plot and uploaded to the Canopeo phone application on 10, 16, and 21 June 2022 (Patrignani et al., 2015). On 6 October 2022, potato tubers were harvested using a lifter and hand harvested to keep treatments separate. A week later, potatoes were graded using a potato sorting machine (AgRay Vision, California, USA) where they were measured for total tuber yield and tuber set.

Data Analysis

Since 'Silverton Russet' and 'Russet Norkotah' differ in time to maturity, the agronomic data were not analyzed together. The tuber set was determined by the total number of tubers. Dry, above ground biomass, tuber set and tuber yield differences between treatments, greenhouse blocks and experiment repetitions within cultivars were tested for normality and homogeneity with Shapiro-Wilk's test and Levene's test using the 'stats' and 'car' packages in R (Fox et al. 2007; R Core Team 2021), respectively. Physiological data that met parametric assumptions were analyzed using analysis of variance and Tukey's HSD test with significance set at 0.05 using the 'stats' and 'agricolae' package, respectively (Dinno and Dinno 2017; de Mendiburu 2020). Physiological data that did not meet parametric assumptions were analyzed using Kruskal-Wallis tests and Dunn's test of multiple comparisons Using Rank Sums, adjusted using the Holm-Bonferroni method with significance set at 0.05 and performed using the 'stats' and 'dunn.test' package, respectively (Dinno and Dinno 2017; Fox et al. 2007). The percent canopy cover output from Canopeo was used to generate the mean percent growth of the canopy cover over time.

Sequencing reads were processed with QIIME 2 (Bolyen et al., 2019). Amplicon sequence variants (ASVs) were generated from the 4,874,269 high-quality non chimeric sequences, based on 97% sequence similarity, and taxonomy assignments against the SILVA_138–99 database (Yilmaz et al. 2014). OTUs with plant chloroplast and mitochondrial taxonomic assignments were removed. A phylogenetic neighbor-joining tree was generated and used for alpha and beta diversity metrics (Price et al. 2010). Processed

sequence data were imported into the R statistical environment. To ensure sufficient diversity by maintaining an adequate sequencing depth, samples with a read number below 0 were excluded from the analysis. Alpha diversity metrics, Chao1 richness, Shannon's diversity index and Simpson's index of diversity (1-D), and pairwise UniFrac distances between samples were calculated using the 'phyloseq' package (McMurdie et al., 2013). Richness and diversity values were compared between the planting substrates and experiment repetitions using Kruskal-Wallis tests 'stats' package (Fox et al. 2007). For beta diversity analysis, a normalization method based on cumulative sum scaling (CSS) was used to remove biases in the count data with the 'metagenomeSeq Bioconductor' package (Paulson et al. 2013). The differences between bacterial communities were investigated using the weighted UniFrac distance metric using the 'phyloseq' package (McMurdie et al., 2013). Effects of each factor on the community composition (beta diversity) were tested by permutational multivariate analysis of variance (PERMANOVA) using the 'vegan' package (Oksanen et al. 2019). Multivariate analysis of community structure and diversity was performed using principal coordinate analysis (PCoA) and plotted using the 'phyloseq' package (McMurdie et al., 2013). To identify the differentially abundant taxa, 'DESeq2' package was used to analyze between planting substrates and experiment repetitions (Love et al. 2014).

The field experiment data were analyzed to assess the impact of greenhouse planting substrate and greenhouse experiment repetition on key potato physiological measurements. For continuous response variables including total tuber yield, and tuber set, a linear mixed model (LMM) approach was utilized. This framework was chosen to appropriately account for the hierarchical and blocked nature of the experimental design, as well as the variability introduced by unavoidable tuber substitutions. For each continuous response variable, a separate LMM was fitted and it included fixed effects for planting substrate (categorical, four levels: Standard Peat, Standard Perlite, 2:1 Perlite & Rice Hulls, 2:1 Perlite & Calcined Clay) and greenhouse experiment repetition (categorical, three levels: 1, 2, 3) the tubers originated from. To account for the inherent variability in initial tuber size and the unavoidable substitutions made during planting (Supplementary Table 1), the log-transformed total tuber weight before field planting was included as a continuous covariate in the fixed effects structure. This approach statistically controlled for the actual initial weight of planted tubers, improving the precision of estimated effects for the other fixed factors. The random effect structure included a field block (categorical, 18 levels across both cultivars, 9 levels within a single cultivar analysis) as a random intercept term. This accounted for the variability between the randomized complete blocks in the field, which

inherently incorporated the intended tuber weight class groupings and spatial variation. The significance of fixed effects was determined using F-tests with Satterthwaite's method for approximating degrees of freedom. P-values for these overall F-tests are reported without adjustment for multiple comparisons. When a fixed effect was found to be statistically significant, post-hoc pairwise comparisons of estimated marginal means were performed. Tukey's method was applied to adjust p-values for these multiple pairwise comparisons. Significant differences were indicated by letters (e.g., A, B) on figures, where groups sharing a letter were not statistically different. All statistical modeling and model selection procedures were performed in RStudio (version 2024.12.1.563) using R and various packages including lme4, lmerTest, emmeans (Bates et al. 2015; Kuznetsova et al. 2017; Lenth 2024; R Core Team 2021). All figures were constructed using R.

Results

Physiological Data from Greenhouse Experiment

According to the ANOVA and Kruskal-Wallis tests, in both 'Silverton Russet' and 'Russet Norkotah' plants, tuber yield and dry aboveground biomass were significantly different across planting substrates (Table 1). There were significant differences in tuber set between planting substrates only for 'Silverton Russet'. Experiment repetition did not significantly affect biomass in 'Russet Norkotah', but 'Silverton Russet' showed a significant effect, with the first repetition yielding higher median biomass than the third repetition. Greenhouse block did not significantly affect tuber sets in 'Russet Norkotah' and showed significant effect in 'Silverton Russet' (Table 1).

For both 'Silverton Russet' and 'Russet Norkotah', 2:1 perlite and calcined clay, was similar in high nuclear tuber yield when compared to plants grown in peat-based media (Fig. 1). The 2:1 perlite and rice hulls mixture supported the lowest yield and dry aboveground biomass for both cultivars. Although not as prominent, a similar pattern was observed for 'Silverton Russet' tuber set distribution where plants grown in both 2:1 perlite and calcined clay and peat-based media, had more tubers than those grown in the other two substrates.

Bacterial Community Profiling

A total of 48 samples were analyzed for bacterial diversity, following the exclusion of 24 samples that yielded no sequencing reads from the initial set of 72. The sequencing of 16 S rRNA gene amplicons of tubers yielded 7974 reads

Table 1 Analysis of variance (ANOVA) and kruskal-wallis (P values) showing statistically significant differences in the physiological measurements of harvested plant tissue from the greenhouse study, separated by cultivar. Kruskal-wallis tests were utilized for tuber yield and aboveground biomass measurements. ANOVA was applied to tuber set measurements. Tuber set data for ‘russet norkotah’ were log-transformed for this analysis

Measurements	Fixed effect	Cultivars	
		‘Russet Norkotah’	‘Silverton Russet’
Tuber yield	Planting substrate	<0.001	<0.001
	Experiment repetition	0.55	0.35
	Greenhouse block	0.72	0.17
Aboveground biomass	Planting substrate	<0.001	<0.001
	Experiment repetition	0.76	0.02
	Greenhouse block	0.8	0.09
Tuber set	Planting substrate	0.11	0.01
	Experiment repetition	0.07	<0.001
	Greenhouse block	0.36	0.01

for ‘Russet Norkotah’ and 47,089 reads for ‘Silverton Russet’, corresponding to an average of 379 and 1744 reads per sample, respectively. Sequencing reads from all 48 samples were clustered into operational taxonomic units (OTUs), which resulted in 1214 OTUs with an average of 45 OTUs per sample.

Variability in richness and diversity was evaluated using three alpha diversity indices, Chao1 richness, Shannon’s Diversity Index and Simpson’s Index of Diversity. The Kruskal-Wallis tests for Chao1 richness, Shannon’s index and Simpson’s index had no significant differences in the bacterial community of tubers between planting substrates and experiment repetitions (Table 2). To further evaluate differences between bacterial communities between repetitions of the experiment, weighted normalized UniFrac principal coordinate analysis (PCoA) was performed. The PCoA plot generated from weighted UniFrac distances showed that bacterial communities were distinct between repetitions of the experiment only (Fig. 2). Based on a PERMANOVA test, the tuber samples were overall significantly different between experiment repetitions (PERMANOVA, p value=0.0201). On the other hand, samples clustered less due to potato cultivar and planting substrate mixtures.

The bacterial composition in the initial tuber samples showed a high relative abundance of sequences assigned to the Proteobacteria phyla across samples originating from all planting substrate mixtures (Fig. 3). The most abundant classes found in tuber bacterial communities for both cultivars were Gammaproteobacteria, Actinobacteria and Alphaproteobacteria (data not shown).

Physiological Data from the Field

To compare the impact of nuclear tuber planting substrate on agronomic features of field-grown seed tubers, nuclear tubers harvested from the greenhouse substrate experiment were separated into one of three weight classes and planted in the field. Both ‘Russet Norkotah’ and ‘Silverton Russet’ produced tubers in the small and medium weight classes across all four substrate treatments. However, only ‘Russet Norkotah’ produced tubers in the large weight class, specifically originating from the peat and 2:1 perlite and calcined clay treatments. At the end of the 22-week growing cycle for potato plants from all treatment combinations, all potato plants were harvested. No plants were lost in the process. Canopy growth was greater for both cultivars in large and medium weight classes when generated from tubers harvested from peat and 2:1 perlite clay treatments (Figs. 4 and 5).

Effects of Starting Seed Tuber Size on Tuber Yield at Harvests

The analysis of tuber yield and tuber set revealed distinct patterns of influence from planting substrates, greenhouse repetitions, and initial tuber weight (Table 3). For ‘Russet Norkotah’, tuber yield was additionally significantly influenced by the original planting substrate and the greenhouse repetition from which tubers originated. Post-hoc comparisons for planting substrate indicated that the standard peat substrate resulted in significantly higher tuber yield compared to the standard perlite, 2:1 perlite & rice hulls, and 2:1 perlite & calcined clay mixtures (Fig. 6). Similarly, tubers from the first greenhouse repetition produced significantly higher yields than those from the second and third repetitions, which did not statistically differ from each other. In contrast, for ‘Silverton Russet’, neither the original planting substrate nor the greenhouse repetition significantly influenced tuber yield. For ‘Russet Norkotah’, the tuber set was also significantly affected by the original planting substrate and the greenhouse repetition. Post-hoc comparisons for planting substrate revealed that standard peat substrate resulted in significantly higher tuber set than standard perlite (Fig. 6). Regarding greenhouse repetition, tubers from the first repetition developed significantly more tuber sets than those from the second and third repetitions, which were not statistically different from each other. For ‘Silverton Russet’, the tuber set was significantly influenced by both the original planting substrate and the greenhouse repetition. Post-hoc comparisons for planting substrate showed that 2:1 perlite & calcined clay and 2:1 perlite & rice hulls yielded significantly higher tuber sets than standard peat. Standard perlite formed an intermediate group, not significantly different from any other substrate (Fig. 6). Notably, despite the

Fig. 1 Tuber yield, dry aboveground biomass, and tuber set for each cultivar ('russet norkotah' and 'silverton russet') across all experimental repetitions, categorized by planting substrate mixture. Statistically significant differences were determined using kruskal-wallis tests followed by dunn's post-hoc tests for tuber yield and dry aboveground biomass. For the tuber set, analysis of variance (ANOVA) was performed, followed by Tukey's HSD post-hoc comparisons. Different letters above the boxplots indicate statistically significant differences within each cultivar and measurement. Uppercase letters correspond to 'russet norkotah,' while lowercase letters correspond to 'silverton russet.' The absence of letters above the boxplots indicates that no statistically significant differences were found among the substrate mixtures for that specific cultivar and measurement

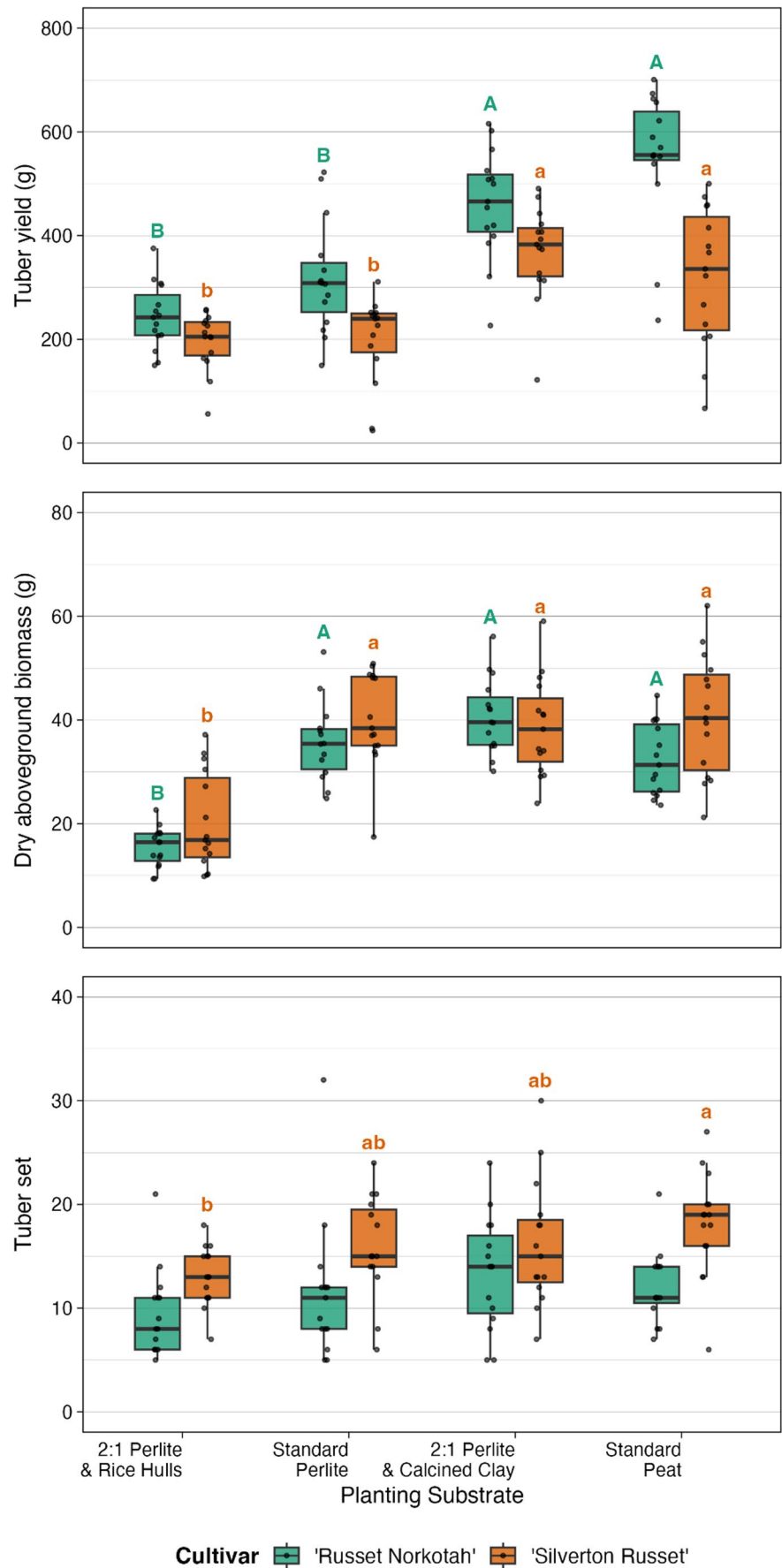


Table 2 Kruskal-wallis (P values) of alpha diversity values measured by chao1 richness, shannon's diversity index and simpson's index of diversity for 'russet norkotah' and 'silverton russet'

	Planting substrate		Experiment repetition	
	'Russet Norkotah'	'Silverton Russet'	'Russet Norkotah'	'Silverton Russet'
Chao1 richness	0.145	0.143	0.191	0.626
Shannon's diversity index	0.170	0.117	0.177	0.772
Simpson's index of diversity	0.183	0.118	0.171	0.733

overall significant effect of greenhouse repetition, post-hoc analysis found no significant pairwise differences among the three greenhouse repetitions for 'Silverton Russet' tuber set. In summary, increasing initial tuber weight consistently led to higher yields and tuber set in both cultivars.

Discussion

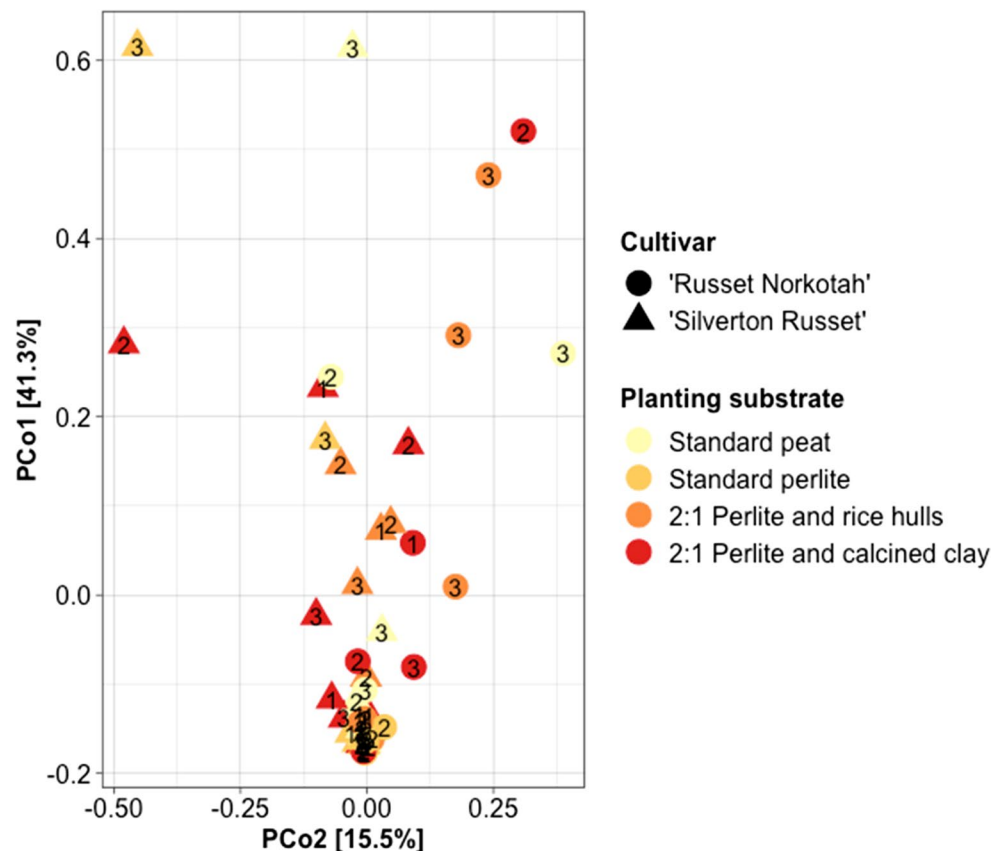
Our results indicated that the planting substrate mixture used during greenhouse acclimation has an impact on plant physiology and minitubers that persists in the field. Endophytic bacterial communities were not affected by substrate

variation. This impact on plant physiology and minitubers might be closely related to the tuber size since there was a positive correlation between planted tuber size and other measurements collected in this study. It is known that large minitubers possess a high amount of energy reserves, which result in early leaf and root development. However, small tubers bear fewer reserves and produce a small root system, which leads to delayed and irregular emergence as well as low tuber yield (Fulladolsa et al. 2018).

This study addresses alternative substrates for minituber production and investigates the bacterial outcomes, plant productivity and tuber yield stemming from the novel growth media types in two potato cultivars. In early generation seed potato production, greenhouse-grown plantlets are cultivated in substrate mixtures including peat for the production of minitubers (Hajiaghahi Kamrani et al. 2019). In recent years, peat-based media was discovered to be a source of *Spongospora subterranea* f. sp. *subterranea*, the causal agent of powdery scab in potato, a highly concerning disease impacting yield and quality (Alaryan et al. 2023; Zeng et al. 2020). The exploration of media alternatives that are free from potato pathogens is critical for optimizing high-valued minituber production.

In the greenhouse experiment, both 'Silverton Russet' and 'Russet Norkotah' exhibited higher tuber yield and dry

Fig. 2 Weighted normalized Uni-Frac principal coordinate analysis of bacterial communities of tuber samples from each planting substrate mixture and cultivar. Numbers 1, 2 and 3 represent the repetitions of the experiment. Each data point represents one sample. The greater the distance between two dots are, the less similar their bacterial communities are



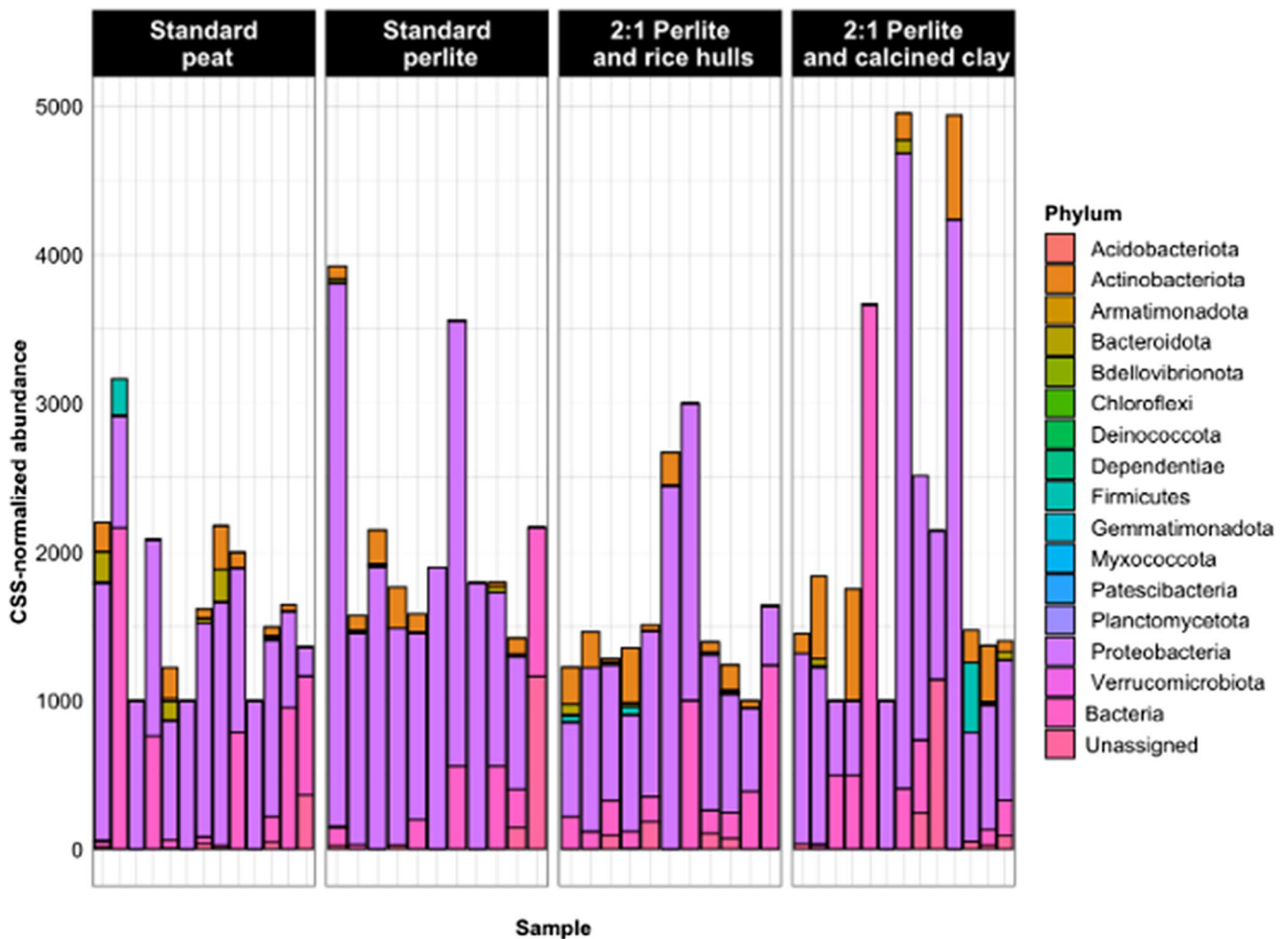


Fig. 3 Relative abundance of bacterial phyla of harvested tubers from the greenhouse from each planting substrate identified with amplicon sequencing data. The taxa abundance profile represents the proportion of each microbial taxon per sample. Data were normalized using

Cumulative Sum Scaling (CSS) normalization to adjust for compositional biases. Abundance values are scaled to reflect the percentage contribution of each taxon within the overall microbial community composition

aboveground biomass in 2:1 perlite: calcined clay and peat-based media, likely due to these substrates' superior water retention and capacity to effectively hold onto and supply essential plant nutrients. Organic amendments impact soil porosity, water infiltration, water holding capacity and soil organic matter (Lal 2020; Obi and Ebo 1995). Peat-based media is known for its high organic matter and has a high water retention capacity whereas perlite, an inorganic substrate exhibits a lower water retention capacity (Agra et al. 2018; Önemli 2004; Schmilewski 2008). However, Markoska et al. (2018) determined that both peat and perlite have favorable water retention capacity due to their high porosity resulting in fertilizer availability. Although perlite is an inorganic substrate, all plants received fertilizer. Perlite may have impacted fertilizer nutrient availability in differing substrates due to its retention capacity. Calcined clay, commonly known as Turface®, is used to retain moisture

and nutrients, increase drainage, and reduce compaction (Calonje et al. 2010). Rice hulls have been considered as an alternative to perlite due to their favorable water holding capacity. However, a study testing combinations of perlite or rice hulls with peat-based media showed that although 20% of rice hulls incorporated had high water capacity, media containing 30% or higher amounts of rice hulls decreased water capacity (Evans and Gachukia 2007). Rice hulls in this study had higher air-filled pore space which is inversely related to water-holding capacity.

Planting substrate had no significant influence on the endophytic bacterial communities of potato tubers. We hypothesized that different substrates might alter these communities, given that endophytic bacteria originate from the plant's environment, particularly the soil and rhizosphere (Petrushin et al. 2024). However, the harvested tubers predominantly contained Proteobacteria and Actinobacteria,

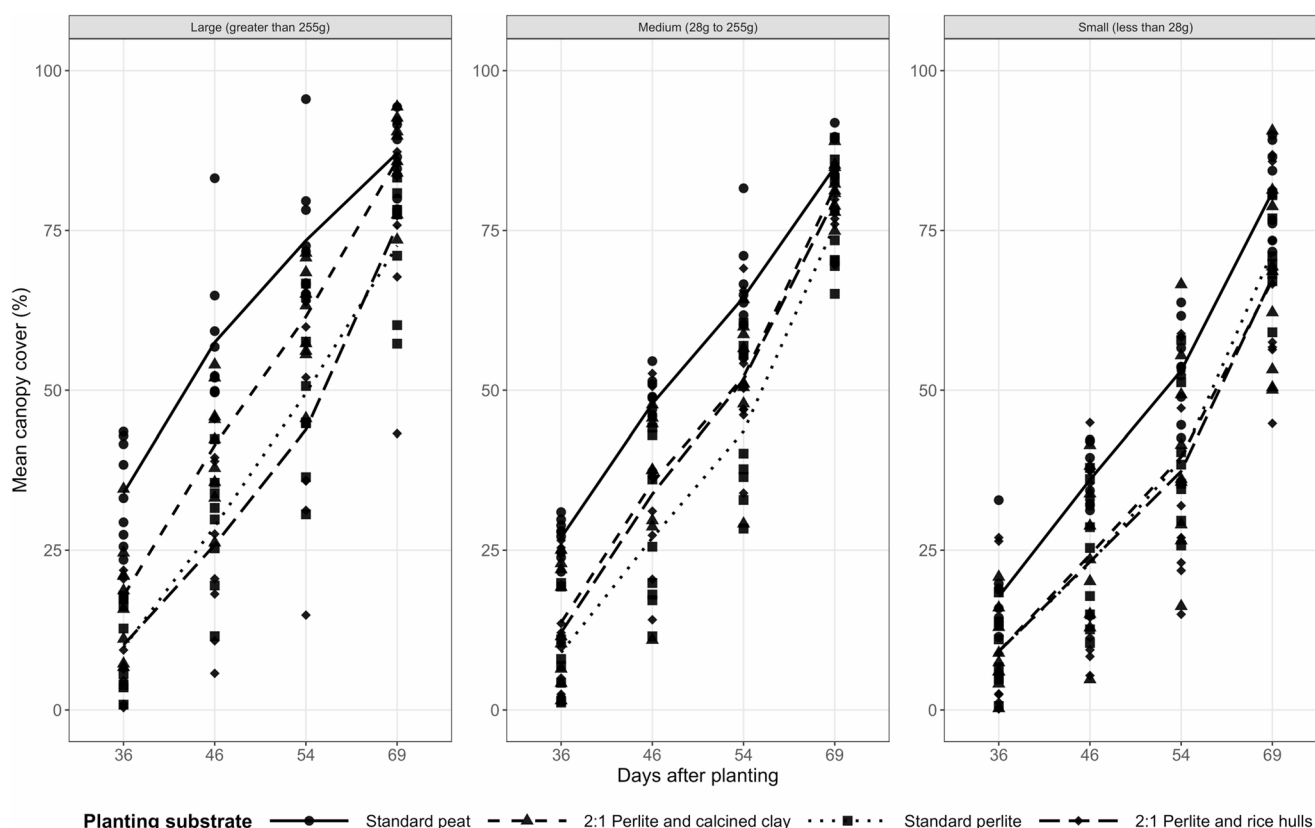


Fig. 4 Mean percentage of canopy growth of ‘russet norkotah’ plants measured over a four-week period. Data were grouped by tuber weight class at planting

which are commonly found across various plant species (Bulgarelli et al. 2013). The consistent presence of Gammaproteobacteria and Alphaproteobacteria, in both tubers and rhizosphere soil, further suggests a stable community, largely unaffected by substrate variation (Barnett et al. 2015; Buchholz et al. 2019). Our results showed no substantial differences in richness, diversity, or community composition across substrates, though beta diversity showed significant differences due to experimental repetition, indicating other external influences. Physiological differences between early- and late-maturing potato varieties, such as ‘Russet Norkotah’ and ‘Silverton Russet,’ might indirectly influence these communities. A previous field study found that delayed harvests resulted in decreased nitrate content in tubers, which could have implications for microbial dynamics (Ierna 2009). These findings highlight the complexity of endophytic bacterial communities. Future research should investigate how initial bacterial sources and plant developmental stages contribute to community structures, exploring relationships between nuclear tuber core microbiome, nutrient uptake and tuber yield, as Barnett et al. (2015) linked 123 bacterial operational taxonomic units to these factors. Additionally, understanding the rhizosphere microbiome’s

composition and functional capacity across different planting substrates can identify core bacterial taxa that persist in the field. Pfeiffer et al., found that the rhizosphere of potato plants cultivated under highly different conditions shares several core bacterial taxa tightly associated with potato, regardless of site and vegetation stage (2017).

This study can be used to develop recommendations for early generation seed potato growers to improve commercial management of seed potato and make economic evaluations of which substrates and mixtures are cost-effective for early nuclear tuber generation. Peat-based media is more cost-effective than perlite and calcined clay. Perlite and calcined clay are inorganic substrates that can be subjected to an extra sterilization step for pathogen removal, but this introduces logistical expenses involving sterilization, mixing, and the reliance on successful delivery of two separate products. When factoring in the potential risks associated with pathogen contamination in peat, as well as the potential losses in plant material and reduced water- and nutrient-use efficiency, investing in perlite and calcined clay may offer the most economically advantageous solution.

Understanding the impact of propagation systems on microbial communities and tuber yield in early generation

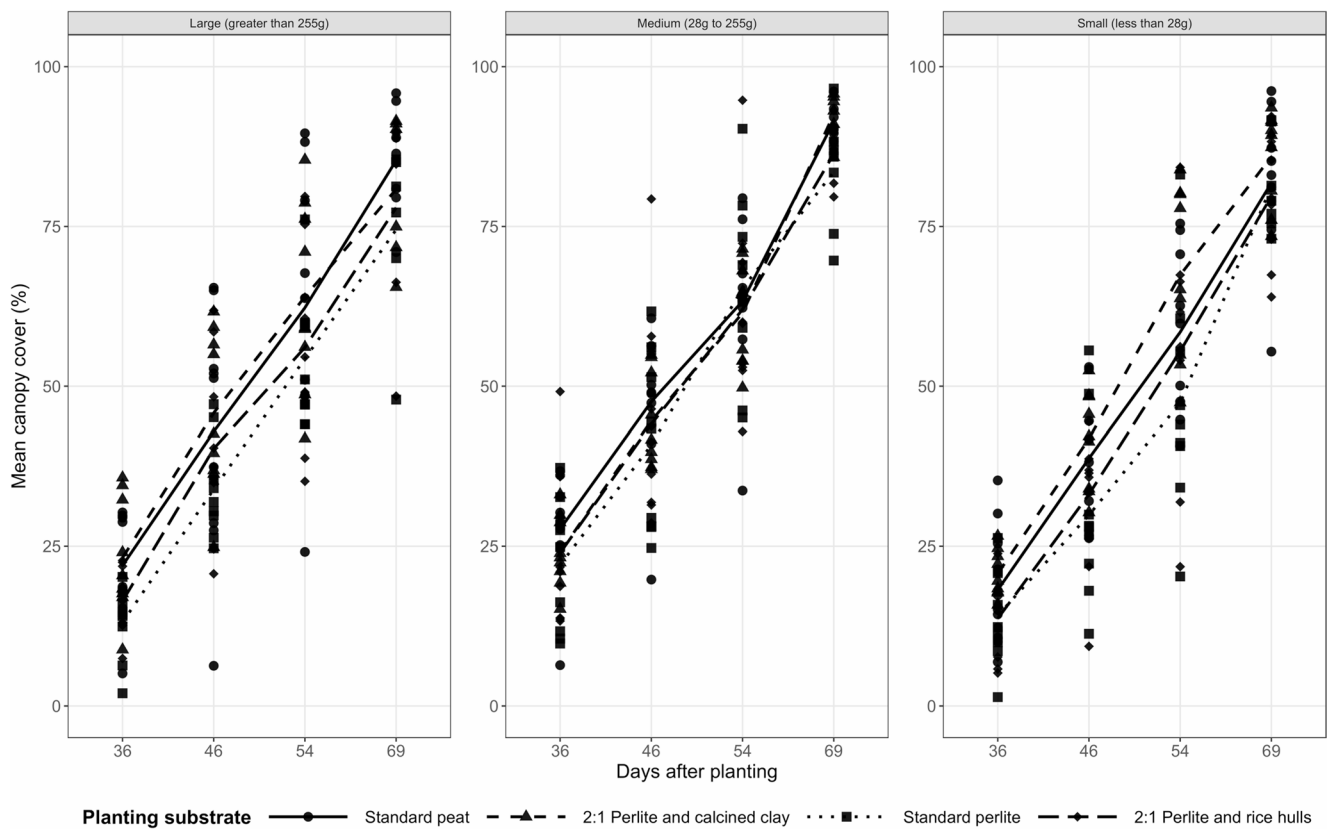


Fig. 5 Mean percentage of canopy growth of ‘silverton russet’ plants over the course of four weeks. Data is grouped by tuber size at planting

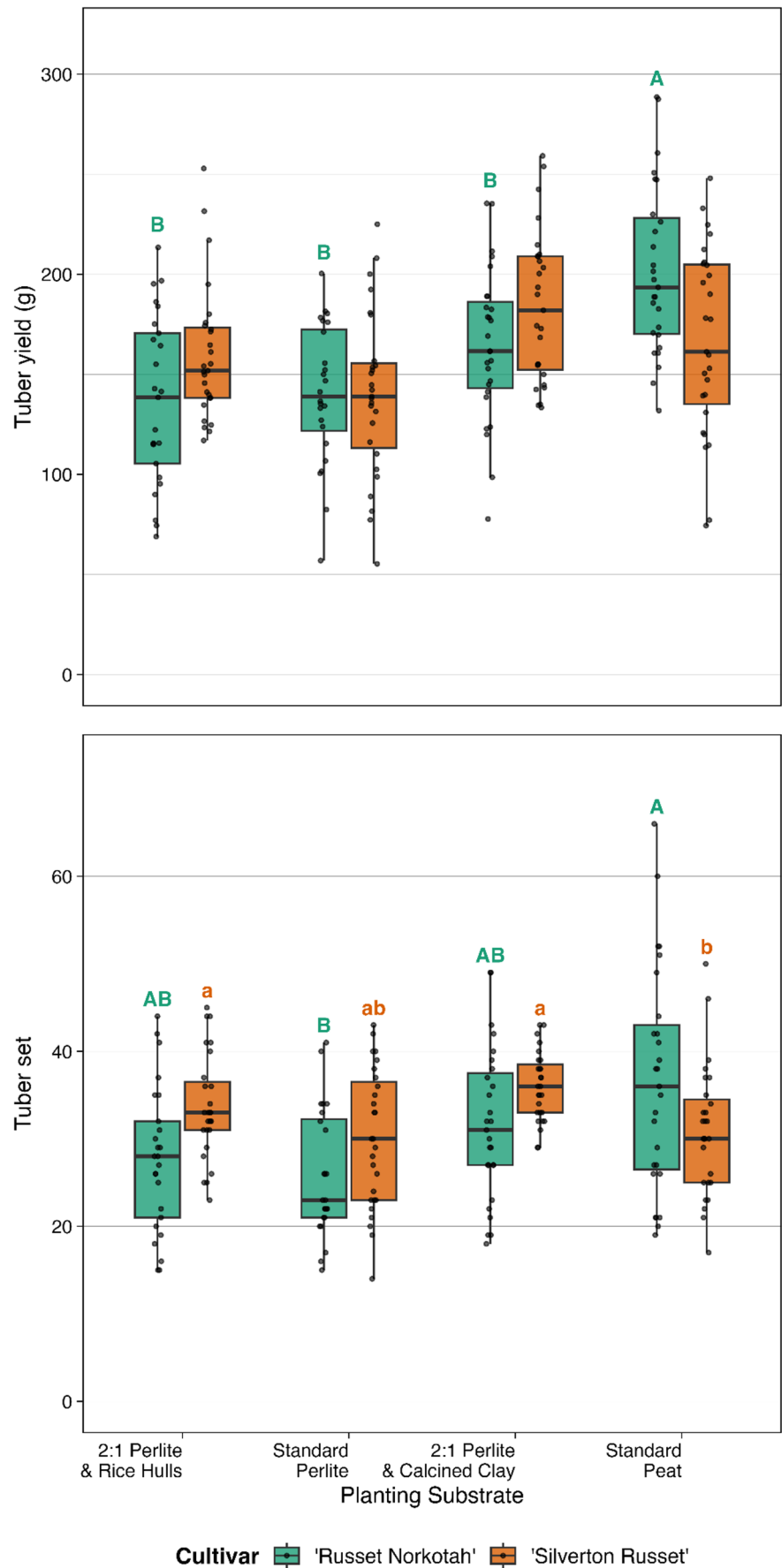
Table 3 Analysis of variance (ANOVA) for linear mixed models showing statistically significant differences in the physiological measurements of harvested tubers from the field study, separated by cultivar. Total tuber weight before field planting was log-transformed and included as a continuous covariate in the fixed effects structure

Measurements	Fixed Effect	Degrees of freedom	‘Russet Norkotah’		‘Silverton Russet’	
			F Value	Pr(>F)	F Value	Pr(>F)
Tuber yield	Original planting substrate	3	14.75	<0.001	1.75	0.16
	Greenhouse repetition	2	12.24	<0.001	1.57	0.21
	Total tuber weight before field planting	1	63.96	<0.001	64.88	<0.001
	Original planting substrate: Greenhouse repetition	6	1.73	0.13	1.15	0.34
Tuber set	Original planting substrate	3	4.3	0.01	5.26	<0.001
	Greenhouse repetition	2	24.74	<0.001	3.24	0.04
	Total tuber weight before field planting	1	91.17	<0.001	22.78	<0.001
	Original planting substrate: Greenhouse repetition	6	0.59	0.74	1.72	0.13

seed potato planting stocks will help address downstream effects on potato plant health and productivity, as well as other vegetatively propagated and transplant crops, such as banana, cassava, and sugarcane. Some downstream effects include the improvement of crop growth and resilience to stressors through manipulation of the environment or inoculation of specific beneficial microbes. Subsequently, growers can be better supported by providing relevant information that can lead to greater profitability and sustainability, such as reduction in pesticide use and chemical fertilizers or

enhanced water- and nutrient-use efficiency. Foundational to the generation of potatoes, globally, is the early culture of plantlets to generate a tuber stage for initiating the chain of seed tuber production of this high value and nutritive crop. It is crucial to continually enhance seed potato production through innovation in the early culture approaches to sustainably and cleanly fulfill the requirements of growers from all markets of the US and global potato industry. Innovations help us to lower labor and resource expenses, enhancing capacity for disease-free seeds.

Fig. 6 Tuber yield and tuber set for each cultivar ('russet norkotah' and 'silvertown russet') harvested at the end of the field study, categorized by planting substrate mixture. Significant differences among groups were determined by estimated marginal means from Linear Mixed Models. Different letters above the boxplots indicate statistically significant differences within each cultivar and measurement. Uppercase letters correspond to 'russet norkotah,' while lowercase letters correspond to 'silvertown russet.'. The absence of letters above the boxplots indicates that no statistically significant differences were found among the substrate mixtures for that specific cultivar and measurement



Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12230-025-10021-x>.

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Data Availability Raw fastq amplicon sequence data are uploaded to the National Center for Biotechnology Information sequence read archive database under BioProject accession number PRJNA1145469.

Declarations

Conflicts of interest The authors declare that they have no known conflicts of interest.

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